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# THE EFFECT OF CIMATEROL ON MYOFIBRES AND METABOLIC ASPECTS IN DIFFERENT STRAINS OF BELGIAN WHITE-BLUE BULLS

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## INTRODUCTION

Earlier experiments showed that most organoleptical meat quality parameters were not altered in beef cattle by ß-agonists, except shear force value which was increased (Hambly *et al.*, 1986; Miller *et al.*, 1988; Fiems *et al.*, 1990a).

ß-agonists are also known to affect metabolism. Blood urea is decreased, nitrogen retention is improved and non-esterified fatty acids and hormone concentration are also affected (Williams *et al.*, 1987; Quirke *et al.*, 1988; Fiems *et al.*, 1989; 1990b). These effects may promote muscle hypertrophy.

Holmes and Ashmore (1972) found that hypertrophied animals of genetic origin have more and larger white fibres than normal animals. Therefore, it can be questioned if a ß-agonist-induced hypertrophy provokes a change in muscle fibre type towards the fibre pattern observed in DM animals, and if there is an effect of ß-agonists on muscle fibre in DM cattle.

## MATERIAL AND METHODS

Two experiments were conducted with 13 N bulls and 16 DM Belgian White-blue bulls respectively. Within each strain, eight animals were fed cimaterol during a finishing period of 140 days on average. Four ppm cimaterol in a concentrate diet were fed to N bulls. DM bulls were fed a diet of maize silage supplemented with concentrate at 1% of their live weight containing 6ppm cimaterol. Daily dose rate amounted to 70 and 60µg cimaterol per kg live weight for the treatment groups in N and DM bulls respectively.

Cold carcass weight amounted to 474±23 and 515±17kg for control and cimaterol-treated N bulls, and 495±29 and 516±27kg for control and cimaterol-treated DM bulls respectively. One meat sample per animal of about 1cm$^3$ was taken for histochemical analysis from the LD adjacent to the 8$^{th}$ rib within one hour, post-mortem. Samples were cooled in liquid nitrogen and stored at -30°C. Fibre types were determined on 10 microscopic fields from two transverse cryosections of 10µm thick, which were stained for myofibrillar ATPase activity, as described by Brooke and Kaiser (1970) and for succinic dehydrogenase (SDH) activity according to Nachlas et al. (1957). The method of Brooke and Kaiser (1970) was slightly modified for the pre-incubation pH and time. The best differentiation between the three fibre types was obtained at a pre-incubation pH of 4.3 instead of 4.6 during 90 seconds instead of 10 minutes. The occurrence and the area of the three myofibre types were determined by image analysis. Therefore, a Reichert Polyvar light microscope was directly coupled to a Cambridge Quantimet 970 computer with standardized light intensity and grey levels, provided with an appropriate program for measuring the three fibre types. Because of the deterioration of some

samples during storage, histochemical data were obtained from nine samples from N bulls: four control (CON) and five cimaterol-fed (CIM) animals, and 11 DM bulls: seven CON and four CIM.

The metabolic activity (Van Den Hende *et al.*, 1986) of the myofibres was also estimated, based on the biochemical determination of lactate dehydrogenase (LDH) and malate dehydrogenase (MDH) using diagnostic kits (Boehringer 124 885 and 124 940 respectively). Muscle pH was measured on the LD of the carcass one hour after slaughtering ($pH_{60}$).

Date are given as means with pooled standard errors. Differences were tested with a one-way analysis of variance.

## RESULTS AND DISCUSSION

Histochemical characteristics of N and DM bulls are shown in Table 1. Cimaterol reduced type IIA fibre number and increased type IIB fibres in N bulls. Concomitantly, the relative area of type I and type IIA fibres was reduced and type IIB fibre area was increased. The magnitude of these alterations was markedly smaller in DM bulls. Taking a different diet in both experiments into account, a significant type and strain interaction was found for the occurrence and the relative area of type IIA and IIB fibres and for type I fibre area.

The increased percentage area of IIB fibres in cimaterol-treated bulls was observed in both strains. This effect was not provoked by an enlarged IIB fibre area but by an increased frequency of IIB fibres, mainly at the cost of a reduced occurrence of IIB fibres. Furthermore, IIB fibres are the largest of the fibre types (Holmes and Ashmore, 1972).

Cimaterol and clenbuterol also affected myofibre characteristics in several experiments with lambs (Beermann *et al.*, 1987; Kim *et al.*, 1987), heifers (Coleman *et al.*, 1986; Miller *et al.*, 1988) and rats (Maltin *et al.*, 1986). However, there was not always a complete accordance among the results reported by these authors and our experiments. No significantly different percentage occurrence of type I fibres in LD muscles was reported by Beermann et al. (1987) and Kim et al. (1987). Maltin et al. (1986) found a reduced percentage occurrence of type I and type IIB fibres in the *extensor digitorum longus* muscle after four and 21 days clenbuterol feeding. However, percentage occurrence was not affected by clenbuterol in the *soleus* muscle. Also, our experiments showed a significant effect of cimaterol on the myofibre type pattern, but only in the N bulls. Most authors found larger fibres after ß-agonist treatment. Only type II fibre area was increased in experiments of Kim *et al.* (1987) and Miller *et al.* (1988). Coleman *et al.* (1986) reported an increased type IIB fibre area. Type I and type II fibre areas were increased in the experiment of Beermann *et al.* (1987). Maltin *et al.* (1986) found an increased type I fibre area in *soleus* and *extensor digitorum longus* muscles and an increased type IIB fibre area in the *soleus* muscle after four days clenbuterol feeding. Feeding clenbuterol for 21 days did not provoke a significant effect on fibre area. Beermann *et al.* (1987) also reported significant increased type II fibre areas for *semitendinosus* and *semimembranosus* muscles. The reduced type I fibre area provoked by cimaterol in N bulls in our experiment is an isolated case in the literature which cannot be easily explained. In some degree, our results indicated that cimaterol shifted this myofibre characteristic and also the myofibre pattern towards that of DM animals. But, it remains questionable why type II fibre area was not moved in the direction of the DM feature. The different effect of cimaterol treatment within strains, obtained for several parameters, was not completely unexpected. Indeed, the effects of cimaterol were smaller in DM bulls. DM animals are already characterized by more IIB fibres (Holmes and Ashmore, 1972).

Furthermore, the opposite effect of a ß-agonist treatment on percentage occurrence and area of myofibres, observed in the literature, may be related to effects of breed, strain, sex, ß-agonist, duration of treatment, dose level, muscle, or even others. Maltin *et al.* (1986) showed that some effects differed between *soleus* and *extensor digitorum longus* muscles. Maltin *et al.* (1986) also showed that effects that were significant after a four-day treatment disappeared after 21 days.

Metabolic characteristics were changed in N cimaterol-treated bulls. A high anaerobic fibre ration and a high metabolic capacity is a typical phenomenon for DM animals (Batjoens *et al.*, 1989). Cimaterol exerted no significant effect on these parameters in DM bulls, but this strain showed a faster pH fall after cimaterol treatment. An increased anaerobic

ratio (type II area/type I area) was also reported by Kim *et al.* (1987) for the *semitendinosus* as well as for the LD muscle in cimaterol-treated lambs.

The significantly decreased $pH_{60}$ in DM animals means that cimaterol may accelerate pH fall, while it is already faster in these animals in comparison with other genotypes (Batjoens *et al.*, 1989). Nevertheless, ultimate pH in N and DM animals of the White-blue breed was not significantly affected by cimaterol, while drip and cooking losses were rather reduced in cimaterol-fed animals (Fiems *et al.*, 1990a). The lower initial pH in cimaterol-treated DM animals would suggest that these animals are more prone to a PSE-like condition. Results of Batjoens *et al.* (1989) indicated a brighter colour in White-blue animals in comparison with animals of other genotypes, without any adverse effect on other meat quality parameters.

## CONCLUSION

In some degree, our results indicated that histochemical and metabolic characteristics of N bulls were shifted by cimaterol towards more type IIB fibres and a larger relative area of this fibre type. Consequently, these histochemical characteristics become similar to the properties of animals with muscle hypertrophy of genetic origin. These alterations were less pronounced in DM animals. However, we did not find a clear hypertrophy of muscle fibres due to the cimaterol treatment.

## REFERENCES


BATJOENS, P., Van HOOF, J., and VERBEECKE, D. 1989. The influence of the muscle fibre composition on some meat quality characteristics in young bulls. *Proc. 35th ICMST.* Copenhagen. Vol.III:1082-1087.

BEERMANN, D.H., BUTLER, W.R., HOGUE, D.E., FISHELL, V.K., DALRYMPLE, R.H., RICKS, C.A., and SCANNES, C.G. 1987. Cimaterol-induced muscle hypertrophy and altered endocrine status in lambs. *J. Anim. Sci.* 65:1514-1524.

BROOKE, M.H., and KAISER, K.K. 1970. Three "myosin adenosine triphosphatase" systems: The nature of their pH liability and sulphydryl dependence. *J. Histochem. Cytochem.* 18:670-672.

COLEMAN, M.E., EKEREN, P.A., LUNT, D.K.,and SMITH, S.B. 1986. Muscle and adipose tissue development in heifers fed the ß-agonist clenbuterol. *J. Anim. Sci.* 63 (Suppl 1):3(Abst).

FIEMS, L.O., BUTS, B., BOUCQUE, Ch.V., DEMEYER, D.I., and COTTYN, B.G. 1990a. Effect of a ß-agonist on meat quality and myofibrillar protein fragmentation in bulls. *Meat Sci.* 27:29-39.

FIEMS, L.O., COTTYN, B.G., Van EENAEME, C., and BOUCQUE, Ch.V. 1990b. Effet du cimatérol sur le métabolisme de l'azote chez des taurillona à l'engrais. *Repro. Nut. Develop.* 29 (suppl 2):243-244.

FIEMS, L.O., DECUYPERE, E., and BOUCQUE, Ch.V. 1989. A note on the effect of feeding cimaterol on some endrocrinological parameters and growth in different types of finishing beef bulls. *Anim. Pro.* 48:601-605.

HAMBY, P.L., STOUFFER, J.R., and SMITH, S.B. 1986. Muscle metabolism and realtime ultrasound measurement of muscle and subcutaneous adipose tissue growth in lambs fed diets containing a ß-agonist. *J. Anim. Sci.* 63:1410-1417

HOLMES, J.H.G., and ASHMORE, C.R. 1972. A histochemical study of development of muscle fibre type and size in normal and "double muscled" cattle. *Growth.* 36:351-372.

KIM, Y.S., LEE, Y.B., and DALRYMPLE, R.H. 1987. Effect of the repartitioning agent cimaterol on growth, carcass

and skeletal muscle characteristics in lambs. *J. Anim. Sci.* 65:1392-1399.

MALTIN, C.A., DELDAY, M.I., and REEDS, P.J. 1986. The effect of a growth promoting drug, clenbuterol, on fibre frequency and area in kind muscles from young male rats. *Bioscience Reports.* 6:293-299.

MILLER, M.F., GARCIA, D.K., COLEMAN, M.E., EKEREN, P.A., LUNT, D.K., WALKER, K.A., PROCKNOR, M., WELSH, T.B., and SMITH, S.B. 1988. Adipose tissue, longissimus muscle and anterior pituitary growth and function in clenbuterol-fed heifers. *J. Anim. Sci.* 66:12-20.

NACHLAS, M., TSON, K., De SONZA, E., CHENG, C., and SELIGMAN, A. 1957. The cytochemical demonstration of succinic dehydrogenase by the use of a new p-nitrophenyl substituted ditetrazole. *J. Histochem. Cytochem.* 5:420-436.

QUIRKE, J.F., ALLEN, P., MOLONEY, A.P., SOMMER, M., HANRAHAN, J.P., SEEHAN, W., and ROCHE, J.F. 1988. Effects of the ß-agonist cimaterol on blood metabolite and hormone concentrations, growth and carcass composition in finishing friesian steers. *J. Anim. Physio. Nutrit.* 60:128-136.

Van Der HENDE, C., MUYLLE, E., and OYAERT, W. 1968. Enzyme activities of liver, heart and skeletal muscles of the pig. *Zentralblatt für Veterinär Medizin.* 15:135-142.

WILLIAMS, P.E.V., PAGLIANI, L., INNES, G., PENNIE, K., HARRIS, C.I., and GARTHWAITE, P. 1987. Effects of a ß-agonist (clenbuterol) on growth, carcass composition, protein and energy metabolism of veal calves. *Brit. J. Nutrit.* 57:417-428.

WHEELER, T.L., and KOOHMARAIE, M. 1992. Effects of the ß-adrenergic agonist L644,969 on muscle protein turnover, endogenous proteinase activities and meat tenderness in steers. *J. Anim. Sci.* 70:3035-3043.

Table 1. Effect of cimaterol on histochemical characteristics.

| Fibre type | I | IIA | IIB |
|---|---|---|---|
| Normal bulls (Exp. 1): | | | |
| # Fibre, % | | | |
| Control | 32.0 ± 2.9 | 31.0 ± 7.0 | 37.0 ± 6.4 |
| Cimaterol | 29.4 ± 3.1 | 14.0** ± 3.0 | 56.6***± 3.2 |
| Cross-sectional area ($\mu m^2$) | | | |
| Control | 2751 ± 407 | 4023 ± 705 | 4976 ± 506 |
| Cimaterol | 2099* ± 471 | 3943 ± 892 | 5016 ± 1132 |
| Relative area (%) | | | |
| Control | 22.1 ± 2.4 | 31.7 ± 8.8 | 46.2 ± 6.9 |
| Cimaterol | 15.6** ± 2.8 | 13.5** ± 2.5 | 70.1***± 6.7 |
| Double-muscle bulls (Exp. 2): | | | |
| # Fibre, % | | | |
| Control | 29.0 ± 5.4 | 22.1 ± 3.4 | 48.9 ± 3.8 |
| Cimaterol | 27.5 ± 6.1 | 17.0 ± 6.7 | 55.5* ± 8.1 |
| Cross-sectional area ($\mu m^2$) | | | |
| Control | 1350 ± 300 | 2637 ± 769 | 4261 ± 1205 |
| Cimaterol | 1268 ± 186 | 2097 ± 486 | 4361 ± 884 |
| Relative area (%) | | | |
| Control | 13.0 ± 2.3 | 19.5 ± 4.7 | 67.5 ± 4.3 |
| Cimaterol | 11.2 ± 2.1 | 12.5* ± 5.9 | 76.6* ± 8.3 |

* $P<0.05$; ** $P<0.01$; *** $P<0.001$

Table 2. Effect of cimaterol on metabolic aspects.

| | Aerobic fibre ratio | Anaerobic fibre ratio | Metabolic capacity (Q) | $pH_{60}$ |
|---|---|---|---|---|
| Normal bulls (Exp.1): | | | | |
| Control | 1.2 ± 0.4 | 3.6 ± 0.5 | 5.9 ±0.5 | |
| Cimaterol | 0.4**± 0.1 | 5.5* ± 1.3 | 8.9***±1.2 | 6.77±0.13 |
| | | | | 6.76±0.11 |
| Double-muscle bulls (Exp.2): | | | | |
| Control | 0.5 ± 0.1 | 6.9 ± 1.2 | 8.6 ± 1.3 | |
| Cimaterol | 0.3 ± 0.1 | 8.2 ± 1.5 | 8.9 ± 2.7 | 6.68±0.17 |
| | | | | 6.43**±0.2 |

* P<0.05; ** P<0.01; *** P<0.001