ECT OF POSTNATAL FEEDING REGIME AND CASTRATION ON MUSCLE FIBER TYPES IN CATTLE.

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MARY : Twenty–four newborn Montbeliard calves were raised with milk replacer to achieve either a high or low rate of gain, 1 167 y and 658 g/day, respectively. At 146 kg, animals were weaned and 6 calves from each group were castrated. Subsequently, all calves e fed a diet of 70 % corn silage and 30 % concentrate that was calculated for a predetermined body weight gain of 1 100 g/day. At 300 after which puberty was well established, a biopsy of the semitendinosus muscle was taken. Fiber types were determined using ochemistry (ATPase and SDH activity) and immunocytochemistry with monoclonal antibodies against slow and fast myosin heavy in isoforms. This trial shows that a significant number of type IIc cells (with both fast ans slow myosin isoforms) remain in these tively old cattle, regardless of sex and initial rate of gain. The main effect of restriction of food intake is a change in fiber types ease in type I and decrease in type IIb), and an overall decrease in fiber size, animals being compared at same body weight. No ificant differences are observed between sexual types four months after castration.

RODUCTION : With the recent reduction of per capita meat consumption, it is important to improve the quality of products. ever, improvements cannot occur until a better knowledge of the biological mechanisms involved in the production of meat are known. well documented that muscle characteristics such as : fiber types, collagen and lipids affect the transformation of muscle to meat.

Muscle cell replication and differentiation occur during fetal development which is characterized by an important cellular iplication and an intense protein synthesis. Although, the number of cells is fixed at birth, muscle fiber size increases during postnatal wth (Robelin et al., 1991). Muscle cell lineages appear during fetal development, and give rise to adult fiber type I and fiber types IIA, and IIC (Robelin et al., 1992). The IIC fibers which express simultaneously fast and slow myosin heavy chain isoforms are not pletely differentiated at birth because proportion of the type IIC fibers decreases after birth, they may constitute a marker of the end of ferentiation.

Since muscle fiber differentiation is not terminated at birth, it may be possible to alter this maturation event by different factors. eral studies have shown that energy level in the diet during the perinatal period affects muscle fiber types, both size (Bedi et al., 1982 ; ideman and Crouse , 1986) and relative proportion (Haltia et al., 1978 ; Johnston et al., 1975, 1981). In addition, sex affects muscle racteristics (Dreyer et al., 1977 ; Young and Bass, 1984 ; Clancy et al., 1986 ; Seideman et al., 1986 ; Raj et al., 1991). Therefore, to plete these results, the objectives of this research are to determine the effect of castration and restricted nutrition during perinatal period muscle fiber differentiation and distribution in calves.

TERIAL AND METHODS : Twenty four newborn Montbeliard calves were allocated, by weight, weight at birth and age, to two ps (I and II), of twelve each. All the animals of the two groups were weaned at the same weight, 146 kg. Six calves from each group e castrated at five months of age.

During the milk feeding period, group I received a diet of reconstituted milk *ad libitum* to achieve a high rate of gain, 1167 g/day, ereas animals of the group II received a restricted diet of reconstituted milk to achieve a low rate of gain, 658 g/day. In the post weaning iod, all animals were fed a diet of 70% corn silage and 30% concentrate calculated for a predeterminated body weight gain of 1100 day. During these two periods all animals were weighed weekly.

At 300 kg, after which puberty was well established, a biopsy of the Semitendinosus muscle was taken from the animals of the groups. The samples were frozen in isopentane, in liquid nitrogen and stored at – 80 °C.

Circulating testosterone concentration was measured using a radioimmunoassay, just prior to castration, and after castration, at and nine months.

Frozen 10 μm cross sections of muscle were made perpendicular to muscle fibers with a microtome. Trichrome Hematox ATPase activity (Guth and Samaha 1970), SDH activity and myosins isoform quantification were performed on serial sections. immunological method used was the immunofluorescence technique described by Pons et al. (1986). Two monoclonal antibodies used, one specific for slow myosin heavy chain, and the other specific for fast myosin heavy chains (MHC IIA and IIB) (Robelin et 1992). Muscle sections were analysed for both number and area of each fiber type using a Visilog image analyser.

Data were analysed according to a variance analysis model, with effects of sex and diet, using G.L.M. module of SAS pac (1985).

RESULTS AND DISCUSSION :

1 – Testosterone :

Mean plasma testosterone concentrations at five months was 1.19 ng/ml (Standard Deviation, S.D.= 0.67). This shows testosterone synthesis has begun at this stage. Mean values obtained at nine months were 1.2 ng/ml (S.D.= 1.14) for bulls and 0.02 ng (S.D.=0.02) for steers. Lacroix et al.(1979) showed a peak of testosterone at five months with a concentration of 2.32 ng/ml (0.71 S. D.) six months the concentration was 1.19 ng/ml (0.19 S. D.), it was the same at seven months and it decreased after with some fluctuatio However, Butterfield (1963a) showed that secondary sex characteristics, like muscles of the neck which are more developed in b appeared later. This suggests that receptors to testosterone may be not present at this early time and that testosterone has no effect at stage. Further, these results verify reduced testosterone production in steers.

2 – Castration effects :

No differences were observed in the number of each fiber type : I, IIA, IIC between bulls and steers (Figure 1). The abundance IIB fibers tended to be greater for steers then bulls. Fiber size was not different between bulls and steers, compared at the same w (Figure 2).

Several authors have found that castraction affects size and type of fibers. All fiber types were larger in bulls than in steers steers had more fiber type IIB and less fiber type IIA than bulls (Dreyer et al., 1977 ; Ockerman et al., 1984 ; Young and Bass, 198 Seideman et al., 1986 and Raj et al., 1991). In bulls, androgen may slow the transformation of IIA to IIB fibers. This study suggests testosterone has little effect on fiber type. However, fiber types were mesured four months only after castration and it is possible that period was two short to elicit the effect of testoterone on fiber type.

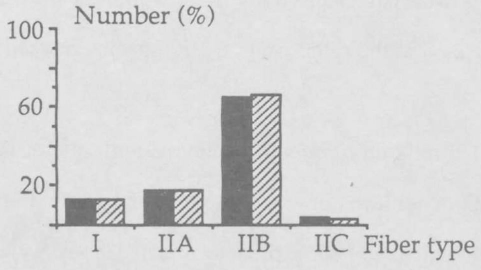

FIGURE 1 :
Comparison of the proportion, in percent, of the different fiber types I, IIA, IIB,IIC in bulls and in steers.

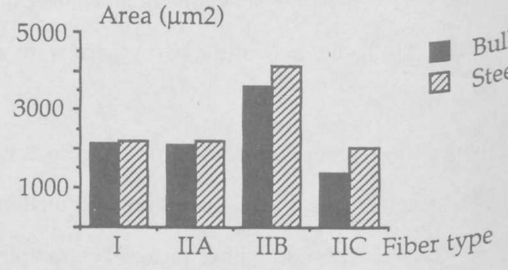

FIGURE 2 :
Comparison of the size in μm2 of the different fiber types in bulls and in steers.

3 - Diet effects :

Comparing animals on a similar muscle weight basis, group II, perinataly restricted, had less type I fibers and more type IIB fibers animals of the group I, ad libitum fed (Figure 3). The number of type IIA and IIC fibers was not significantly different. The size of all fibers, exept type IIC, was smaller for restricted animals than for animals fed *ad libitum* during perinatal period (Figure 4).

These results desagreeded with those reported by Johnston et al.(1981) and Seideman and Crouse (1986). This difference is bly due to the fact that diet levels energy concerned post weaning period instead of perinatal period. However, Haltia et al.(1978) and ely et al. (1991) showed that perinatal undernutrition in rats caused a decrease in the relative number of type I fibers with a ponding increase in type IIB fibers. These differences were not observed if undernutrition occurred after weaning (Dobling et al., ; Bedi et al., 1982). Haltia et al. (1978) also found decreases in mean fiber cross sectional area of I, IIA and IIB fiber types. These ors hypothesized that undernutrition during early postnatal development would delay the normal disappearance of immature myosin forms. They further speculated that alterations in myosin isoform transitions are induced by hypothyroidism that is often associated with ernutrition.

It is surprising to detect an effect on fiber type repartition four months after the application of the two different diets. In fact, ely et al., (1991) observed that the effects of diet on rat skeletal muscle after weaning can be reversed whereas undernutrition before ning causes irreversible damage. Restrictited nutrient intake may accelerate the normal development of fibers, increase type IIB and ease types I and IIA. The restricted animals reached the same weight (300 kg) at a greater chronolycal age than animals fed *ad libitum* (e months versus six months). These data suggest that the fiber type partition is more related to chronological age rather than to the ght of animal.

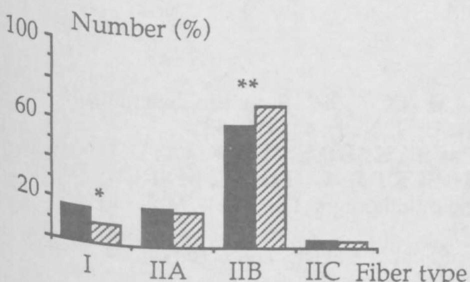

FIGURE 3 :
Comparison of the proportion, in percent, of the different fiber types, in the animals fed *ad libitum* and limited.

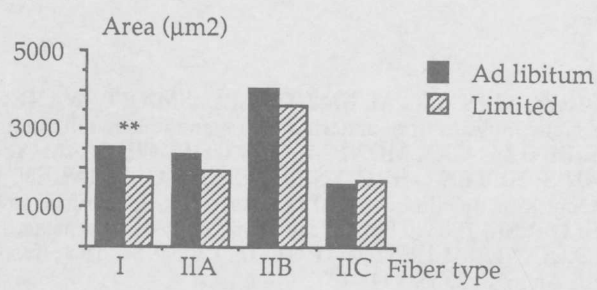

FIGURE 4 :
Comparison of the size of fiber types in animals fed *ad libitum* and restricted.

4 - Fiber type IIC :

Type IIC fibers, which express fast and slow myosin, were present at an average of 3.5%, which is surprising in relatively old animals (300 kg). At birth, in the same muscle, the proportion of these fibers was 4% (Robelin et al., 1992). It shows that the differentiation of these fibers is relatively slow in this muscle. Results in the litterature showed that these fibers disappeared after birth. In fact, it is difficult to identify these fibers with classical histology. In this study they were identified by the sensitive immunohistology technique with monoclonal antibodies specific to fast myosin heavy chains (MHC IIA and IIB) and to slow myosin heavy chain (MHC I). Cells recognized by the two antibodies (figure 5) were classified as IIC fibers.

The presence of this fiber type shows that differentiation, in term of myosin isoforms, is not ended at nine months (300kg).

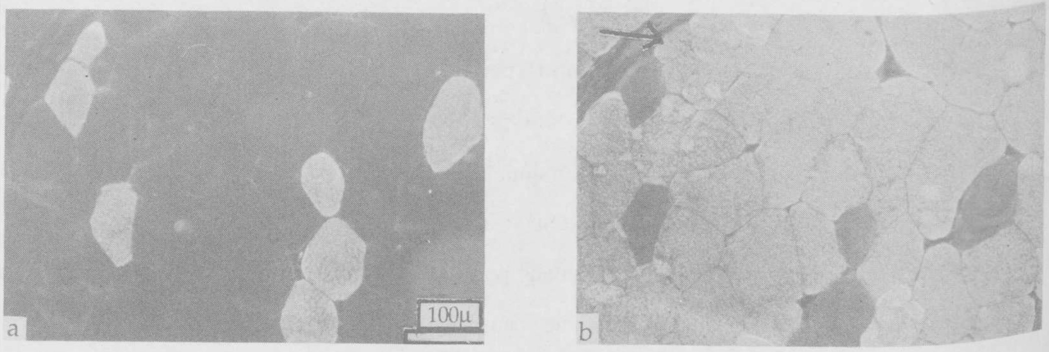

FIGURE 5 :
Identification of IIC fiber type by immunohistological technique.
(a) White fibers are those containing slow myosin heavy chain of type I.
(b) White fibers are those containing fast myosin heavy chains of types IIa and IIb.
Cell in white on the two pictures are IIC fibers, containing myosin heavy chains of type I, IIa and IIb.

CONCLUSION :

The results of this study show that the end of muscle fiber differentiation progresses relatively slowly after birth, 3.5% of fiber ty IIC being still present at nine months of age. This may allow modification of the postnatal evolution of fiber types by different factors.

The effect of castration was not visible four months after castration ; it certainly will be detectable in the older stage. Undernutri during perinatal period induced a reduction of growth of fiber size, an increase of type I and a decrease of type IIB fibers. This eff persists four months after the different diets. It shows the possibility of modification of the evolution of fiber type after birth to fovor development of biological characterics that are related to meat quality.

REFERENCES

BEDI K.S., BIRZGALIS A.R., M. MAHON, J.L. SMART, WAREHAM A.C., 1982. Early life undernutrition in rats : 1. Quantitativ histology of skeletal muscles from underfed young and refed adult animals. Br. J. Nutr., 47, 417–431.
BEVERLY S. BROZANSKI, MONICA J. DAOOD, WILLIAM A. LaFRAMBOISE, JON F. WATCHKO, THOMAS P. FOLEY J.R., GILLIAN S; BUTLER-BROWNE, ROBERT G. WHALEN, ROBERT D. GUTHRIE, MARCIA ONTELL, 1991. Effects of perinatal undernutrition on elimination of immature myosin isoforms in the rat diapragm. Lung Cell. Mol. Physiol., 5, 49–54.
BUTTERFIELD R.M., 1963 a. PhD Thesis, University of Queensland.
CLANCY M.J., JANET M.LESTER, ROCHE J.F., 1986. The effects of anabolic agents and breed on the fibers of Longissimus mus of male cattle.J. Anim. Sci., 63, 411–427.
DREYER J.H., NAUDE R.T., HENNING J.W.N., ROSSOUW W., 1977. The influence of breed, castration and age on muscle fi type and diameter in Friesland and Afrikaner cattle S Afr. J. Anim.Sci., 7, 171–180.
GUTH L., SAMAHA F.J., 1970. Procedure for the histochemical demonstration of actomyosin ATPase. Exp. Neurol. ,28, 365-367.
HALTIA M., BERLIN O., SCHUGHT H., SOURANDER P., 1978. Postnatal differentiation and growth of skeletal muscle fibers normal and undernourished rats. A histochemical and morphometric study. J. Neurol., Sci., 36, 25–39.
JOHNSTON M.D., MOODY W.G., BOLING J.A., BRADLEY N.W.,1981. Influence of breed type, sex, feeding systems, and mus bundle size on bovine fiber type characteristics. J. of Food Sci., 46, 1760–1765.
JOHNSTON M.D., STEWART D.F., MOODY W.G., BOLING J., KEMP J.D., 1975. Effect of breed and time on feed on the size an distribution of beef muscle fiber types. J. of Anim. Sci., 40, 613–620.
LACROIX A., PELLETIER J., 1979. Short-term variations in plasma LH and testosterone in bull calves from birth to 1 year of age. Reprod. Fert., 55, 81–85.
OCKERMAN H.W;, JAWOREK D., VAN STAVERN B., PARRETT N., PIERSON C.J., 1984. Castration and sire effects on carcas traits, meat palatability and muscle fibre characteristics in Angus cattle. J. Anim. Sci., 59, 981–990.
PONS F. ,LEGER J.O.C., CHEVALLAY M., TOME F.M.S., FARDEAU M., LEGER J.J., 1986. Immunocytochemical analysis myosin heavy chains in human fetal skeletal muscles. J. Neurol. Sci., 76, 151–163.
RAJ MOHAN A.B., MOSS B.W., McCAUGHEY WS.J., McLAUCHLAN W., McGAUGHEY S.J., KENNEDY S., 1991. Effects of surgical and immunocastration of beef cattle on meat colour, post-mortem glycolytic metabolites and fibre type distribution. J. Sci. Fo Agric., 54, 111–126.
ROBELIN J., LACOURT A., BECHET D., FERRARA M., BRIAND Y., GEAY Y., 1991. Muscle Differentiation in the Bovine Fet : A Histological and Histochemical Approach. Growth, Dev. and Aging, 55, 151–160.
ROBELIN J., PICARD B., LISTRAT A., JURIE C., BARBOIRON C., PONS F., GEAY Y., 1992. Myosin transitions in Semitendinosus muscle during fetal development of cattle : immunocytochemical an electrophoretic analyses. Reproduction, Nutritio Development, Submited
SEIDEMAN S.C., CROUSE J.D., 1986. The effects of sex condition, genotype and diet on bovine muscle fiber characteristics. Meat Sci. 17, 55–72.
YOUNG O.A., BASS J.J, 1984. Effect of castration on bovine muscle composition. Meat Sci., 11, 139–156.