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Expression of β, β-carotene 15,15' oxygenase in bovines*

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ABSTRACT

Bovines might ingest large quantities of carotenoids in the diet. Not all the ingested and absorbed ß-carotene is transformed into vitamin A, the surplus is mainly deposited in the adipose tissue; with the aim of study the expression of β,β-carotene 15,15'oxygenase in the duodenal and hepatic tissues of animals with or without yellow fat, *in situ* hybridization was performed. Sense and antisense 744 bases RNA digoxigenin labeled probes were used. Results showed expression in epithelial cells of crypts and villi on duodenum. Apparent differences were found in duodenum between pigmented and non-pigmented animals. However, in the case of the liver large differences were found.

KEY WORDS: ß-carotene, bovines, yellow fat, *in situ* hybridization

INTRODUCTION

In Mexico most bovines are finished on pasture, and the adipose tissue of those animals shows a yellow pigmentation which results in an important economic loss for the producer, because the downgrading or even rejection of resulting carcasses (Mora and Shimada, 2001).

Yellowness of fat is caused by excessive carotenoids present in the diet, of which β-carotene is predominant (Morgan et al., 1969). Because β-carotene is not cleaved in the rumen (Van Soest, 1994), it is transported to the small intestine, where it is absorbed. β,β-carotene 15,15'oxygenase (β-oxy) is the enzyme that cleaves the β-carotene into two molecules of vitamin A (Glover, 1960), but because cattle can absorb large quantities of β-carotene from forages, not all the absorbed β-carotene is transformed into vitamin A. The vitamin A and the excess of β-carotene are transported to the liver where the latter could be cleaved by

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β-oxy and stored or packed with another lipid compounds and carried to different tissues (Gottesman et al., 2001) including the adipose (Yang et al., 1992).

The aim of this study was to determine the expression pattern of β-oxy in duodenal mucosal and hepatic cells of grass-fed compared with grain-fed cattle.

MATERIAL AND METHODS

Sample collection

All biological samples were taken at Querétaro's Municipal abattoir from bovines at slaughter. For total RNA assays, intestinal mucosae was taken in Trizol reagent (Gibco BRL cat. 15595-026), homogenized and transported on ice to the laboratory. For *in situ* hybridization duodenal and hepatic tissue samples were taken and fixed on 3.5% paraformaldehyde.

Preparation of RNA labeled probes

Total RNA from duodenal bovine mucosae was isolated with Trizol reagent. Antisense for 744 nucleotides was used for RT-PCR amplification for β-oxy cDNA. This PCR product was inserted into TOPO vector 4.0 (Invitrogen cat. K457501) and cloned into *E. coli* bacteria (Invitrogen TOPO cloning reaction).

Sense and antisense probes of β-oxy were derived from TOPO vector, using a partial digestion with the restriction enzymes Not I and Spe I, and then synthesized digoxygenin labeled probes with T3 and T7 RNA polymerases. Probes served for the hybridization of duodenal and hepatic cryosections of 15 μm from four pigmented and four non-pigmented animals. The hybridization conditions were a four-hour pre-hybridization at 65°C and an overnight hybridization at the same temperature. The slides were washed and incubated overnight at 4°C with a digoxygenin/alkaline phosphatase antibody. The slides were washed and incubated with alkaline phosphatase-nitro blue tetrazolim/5-bromo-4-cloro-3-indoly-phosphate on darkness overnight at room temperature (Varela-Echavarria et al., 1996).

RESULTS AND DISCUSSION

Results from *in situ* hybridization show the location within a tissue. The hybridization signal on duodenum was observed in the epithelial cells of crypts and villi; similar results have been reported in the chicken (Wyss et al., 2001); in this site, the β-carotene is absorbed, cleaved and/or packed to be carried to the liver (Laksman and Okoh, 1993). Apparently there were no differences in duodenum *in situ* hybridizations between pigmented and non-pigmented animals. This could mean that the expression levels at this site were probably not affected by the diet β-carotene.

However there were large differences in liver hybridizations between pigmented and non-pigmented animals. These hybridizations in the pigmented animals showed a higher level of expression of β-oxy near the portal space, and a lower level of expression at the central vein in the liver lobule, but in non-pigmented animals the expression level was either very low or not detected. This could mean that as grass-fed animals (yellow adipose tissue) consume high quantities of β-carotene and its cleavage is insufficient at the intestinal level, then the compound is carried to the liver where its cleavage is continued, stimulating a higher expression of β-oxy. At this point, there are contradictory opinions on the relationship between the level of dietary β-carotene and the activity of β-diox (Mora et al., 2000; Parvin and Sivakumar, 2000).

CONCLUSIONS

Future studies are required to confirm that liver β-oxy might play a central role in the metabolism of β-carotene in bovines.

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