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CHARACTERISTICS OF HEART FATTY ACID-BINDING PROTEIN (H-FABP)

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Fatty acid binding proteins (FABP) play a role in fatty acid metabolism, especially in the uptake and intracellular transport of fatty acids to β-oxidation sites and to sites of triacylglycerol and phospholipid synthesis. Heart fatty acid-binding protein (H-FABP) is the most widely distributed protein of the FABP family. A search for polymorphisms in the H-FABP gene locus led to the identification in pigs of substitution in coding and non-coding sequences, but did not conclusively show their relationship with meat parameters.

Key words: PIG, PROTEINS, FATTY ACID-BINDING PROTEIN GENES

Lipid binding proteins (LBP)

Lipid binding and transporting proteins (LBP) are commonly present in extra- and intracellular fluids of all organisms (Veerkamp and Maatman 1995). Some LBP proteins are characterized by high specificity, while others bind different hydrophobic ligands. In general three major lipid binding protein families are distinguished:

- ✓ Plasma albumin — the main transporter of free fatty acids in the blood, α-fetoprotein and vitamin D plasma binding protein (Calvo and Ena 1989; Subbiah 1991). All proteins from this group act intracelullarly and have a similar size of 50–70 kDa.
- ✓ Extracellular lipocalins. The only representative of this family is lactoglobulin, which has a high affinity for fatty acids (Puyol et al. 1991). The structure of these proteins contains a β-barrel of 8 peptide chains. Molecular weight ranges from 18 to 20 kDa (Jones et al. 1988).
- ✓ Fatty acid binding proteins (FABP) are involved in the regulation of many cell processes (Veerkamp et al. 1993). This family also includes cellular retinol binding proteins (CRBP) and cellular retinoic acid binding proteins (CRABP) (Veerkamp and Maatman 1995).

Fatty acid binding proteins (FABP)

The existence of small cytoplasmic proteins that bind fatty acids was questioned for almost 30 years until research on *FABP* gene in knockout mice provided evidence that FABP proteins are involved in the transport and absorption of long-chain fatty acids (Zimmerman and Veerkamp 2002).

FABP proteins can be divided into two groups: plasma membrane fatty acid binding proteins ($FABP_{PM}$) and cytoplasmic (intracellular) fatty acid binding proteins ($FABP_C$) (Glatz and Van der Vusse 1996). The most common proteins ($FABP_C$) are a family of at least 9 different cytoplasmic proteins of 14–15 kDa that contain 126–137 amino acids with tissue-specific distribution (Chmurzyńska 2006; Glatz and Van der Vusse 1996). Large differences in the primary structure made it possible to divide FABP proteins into groups reflecting their capacity to bind different compounds. This division also reflects their evolutionary origin, because the ancestor of all LBP

proteins may have encoded a universal hydrophobic ligand binding protein. With time, many duplications gave rise to what is now known as FABP (Schlap et al. 2002).

FABP proteins have 22–73 % amino acid sequence similarity and share a tertiary structure despite the differences (Xu et al. 1993; Zimmerman and Veerkamp 2002). Structurally, every FABP protein is composed of two α-helices and 10 antiparallel β-strands that form a β-barrel capped by a helix-turn-helix motif (Banaszak et al. 1994). Ligand binds to the molecule because of interaction with specific amino acids in the binding pocket (Banaszak et al. 1994).

The essential role of all FABP proteins is fatty acid metabolism, especially absorption and intracellular transport to β-oxidation sites and to triacylglycerol and phospholipid synthesis sites (Veerkamp and Maatman 1995). Cytoplasmic proteins of the FABP family may act in two ways: by increasing the dissociation of fatty acids from plasma membranes as a result of increased solubility (Vork et al. 1993) or by increasing transport efficiency to target organelles (Hsu and Storch 1996).

Fatty acids are thought to passively pass through the plasma membrane by simple diffusion. Another stage involves binding to cytoplasmic proteins (FABP) and transport to appropriate cell organelles (Veerkamp et al. 1991). It was also found that FABP proteins are connected with the plasma membrane, where they carry fatty acids to the membrane, thus regulating their intracellular concentration. In addition to regulating lipid metabolism, FABP proteins are involved in modulation of cell growth and proliferation.

Characteristics of H-FABP protein

Heart fatty acid binding protein (H-FABP) is the most widely distributed protein of the FABP family. It has a molecular weight of 15 kDa and occurs in many tissues characterized by a high fatty acid requirement. It can be found in the cardiac, smooth and skeletal muscles, breast gland epithelial cells, aorta, kidneys, lungs, brain, placenta and ovary (Zimmerman and Veerkamp 2002).

In pigs, the composition of H-FABP amino acid chain is largely homologous with that of humans (90 %), cattle (92 %), mice (87 %) and rats (86 %) (Billich et al. 1988; Binas et al. 1992; Claffey et al. 1987; Peeters et al. 1991).

The creation of transgenic mice in 1999 was a major contribution to explaining the physiological role of some FABP proteins. Mice lacking the *H-FABP* gene showed defects in the metabolism of long-chain fatty acids. Reduced palmitic and oleic acid utilization and increased glucose oxidation in cardiac muscle were observed. Lack of H-FABP also caused exercise intolerance and localized vascular hypertrophy (Binas et al. 1999; Schaap et al. 1999). H-FABP also regulates cardiomyocyte growth and differentiation in neonatal mouse hearts and stimulates the growth of cell surface area, leading to cardiomyocyte hypertrophy (Tang et al. 2004). Together, these studies confirm a significant role of H-FABP in fatty acid metabolism and body homeostasis. It was also shown that mammary derived growth inhibitor (MDGI), a protein that inhibits tumor cell growth, is a mixture of H-FABP and A-FABP (Yang et al. 1994).

H-FABP encoding gene

The heart fatty acid binding protein (*H-FABP*) gene belongs to a family of 9 FABP genes that have common names depending on the site of expression (Chmurzyńska 2006). Chromosomal mapping of the *FABP* genes shows that they are dispersed in the genome, but some loci create a synteny group (Chmurzyńska 2006). Expression of *FABP* genes is tissue specific and the levels of protein accumulation depend on transcription level (Glatz and Van der Vusse 1996).

The overall structure of this gene family is identical. All the genes consist of four exons separated by three introns. Intron length varies between organisms. All the *FABP* genes contain a canonical TATA box located about 23–30 nucleotides upstream of the transcription start site (Sweetser et al. 1987).

H-FABP is encoded by a gene located on chromosome 4 in mice, chromosome 5 in rats, chromosome 1 in humans, and chromosome 2 in cattle (Roy et al. 2003; Troxler et al. 1993; Zhang et al. 1999). In pigs, the *H-FABP* gene locus is localized on chromosome 6q 21–26.

Exons of the *H-FABP* gene encode 24, 58, 34 and 17 amino acids, respectively, and are separated by three introns of 4,2, 2,5 and 1,5 kb (Gerbens et al. 1997). The *H-FABP* gene is expressed in many body tissues such as cardiac muscle, skeletal muscle, brain, kidney and blastocyst. The *H-FABP* gene in pigs shares considerable homology with humans (91 %), cattle (92 %), mice (84 %) and rats (85 %) (Billich et al. 1988; Binas et al. 1992; Claffey et al. 1987; Peeters et al. 1991). Three different pseudogene sequences were detected within the porcine *H-FABP* gene, each containing an internal 27-bp duplication (Gerbens et al. 1997). Similar pseudogene sequences were localized on chromosomes 8, 10 and 17 in mice and chromosome 13 in humans (Heuckeroth et al. 1987; Veerkamp and Maatman 1995).

Polymorphism of the *H-FABP* gene locus

Because of the growing overweight and obesity problem in humans, increasing attention has been given to the genetic background of these diseases. Therefore polymorphism of genes involved in lipid metabolism acquires significance. Fat absorption was found to be a major factor in the development of metabolic (insulin resistance) syndrome (Chmurzyńska 2006). As a result, research on the polymorphism of the *I-FABP* gene revealed the presence of several polymorphisms that influence changes in body weight (Damcott et al. 2003). Ala54Thr missense mutation was implicated in insulin resistance because of almost two-fold greater affinity of threonine-containing protein for fatty acids (Levy et al. 2001).

A search for polymorphisms in the *H-FABP* gene locus of farm animals led to the identification in pigs of substitution in coding and non-coding sequences:

- ✓ T → C at position 1324 (5' regulator region) using the *HinfI* enzyme (Gerbens et al. 1997)
- ✓ Mutation in intron 2 using *HaeIII* and *MspI* enzymes (Gerbens et al. 1997)
- ✓ G[T_6] in intron 2 (Gerbens et al. 1998)

The mutations were identified using Restriction Fragment Length Polymorphism (RFLP). The same method was also employed to detect G7516C and G7713C polymorphisms in the *H-FABP* gene locus in cattle and their effect on meat parameters was determined (Michal et al. 2006; Cho et al. 2008).

It was hypothesized that the *H-FABP* gene has an effect on intramuscular fat content in muscle tissue and on fatness in pigs. Research with Duroc pigs proved that polymorphism in the *H-FABP* gene locus has a direct influence on pig fatness parameters such as IMF and BFT (Gerbens et al. 1997, 1999). A study with a group of Large White and Landrace pigs did not show the effect of *H-FABP* genotype on the analysed parameters of meat quality, but the effect on intramuscular fat content was almost significant (Urban et al. 2002). Analysis of genetic structure performed in pig breeds raised in Austria (Pietrain, Large White and Landrace) failed to confirm the relationship between polymorphism at the loci of *H-FABP* and *A-FABP* genes and the intramuscular fat content of meat. A marked correlation was observed between genotype and other meat quality traits such as colour and pH (Nechtelberger et al. 2001).

The polymorphisms at the locus of the *H-FABP* gene that have been identified to date confirm or exclude their relationship with production traits in pigs. However, research results do not conclusively show whether the polymorphism influences fatness or muscling traits. Clear between-breed differences were found in the frequency of different *H-FABP* gene variants and it is appropriate to conduct further research on variation at the *H-FABP* gene locus.

Conclusions

This paper discusses the function of fatty acid binding proteins (FABP), in particular heart fatty acid binding proteins (H-FABP) and shows the relationship between the identified

polymorphisms of the *H-FABP* gene locus and production traits in pigs. The present findings confirm or exclude their relationship with meat quality parameters in pigs and prevent the classification of the above SNPs as markers of fatness or muscling traits in pigs.

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ХАРАКТЕРИСТИКА СЕРЦЕВОГО БІЛКА, ЯКИЙ ЗВ'ЯЗУЄ ЖИРНІ КИСЛОТИ (H-FABP)

Р е з ю м е

Білки, які зв'язують жирні кислоти (H-FABP), відіграють роль у метаболізмі жирних кислот у поглинанні та міжкліклітинному транспортуванні жирних кислот до β-окислювальних ділянок і ділянок синтезу триацилгліцеролів та фосфоліпідів. H-FABP білка найбільше виробляється у сімействі білків, які зв'язують жирні кислоти. Пошук поліморфізму у місцезнаходженні гену H-FABP допоміг ідентифікувати заміни у закодованих і незакодованих послідовностях у свиней, але у результаті не показав їх співвідношення з м'ясними параметрами.

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ХАРАКТЕРИСТИКА СЕРДЕЧНОГО БЕЛКА СВЯЗЫВАЮЩЕГО ЖИРНЫЕ КИСЛОТЫ (H-FABP)

А н н о т а ц и я

Белки, которые связывают жирные кислоты (H-FABP) играют роль в метаболизме жирных кислот в поглощении и межклеточной транспортировке жирных кислот к β-окислительным участкам и участкам синтеза триацилглицерола и фосфолипидов. H-FABP белка больше всего производится в семействе белков, которые связывают жирные кислоты. Поиск полиморфизма в местонахождении гена H-FABP помог идентифицировать замены в закодированных и незакодированных последовательностях у свиней, но в итоге не показал их соотношения с мясными параметрами.

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