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IMPORTANCE OF LEPTIN GENE POLYMORPHISM IN CATTLE

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SUMMARY: Leptin is a protein hormone with a key role in feed intake regulation and energy expenditure. Leptin expression and secretion is highly correlated with body fat mass and adipocyte size. Leptin is thought to be a metabolic signal that regulates nutritional status effects on reproductive function. Leptin receptors are found in the ventromedial and arcuate regions of the hypothalamus and are thus positioned anatomically in regions associated with the control of appetite and reproductive neuroendocrine function. It is interesting that neuropeptid Y (NPY) is present in the hypothalamic regions involved in neuroendocrine control of feed intake. In contrast to leptin, NPY is a powerfull stimulator of feed intake and inhibitor of gonadotropin secretion. Leptin receptors have been localized on hypothalamic NPY neurons, and it has been revealed that leptin downregulates NPY expression. Investigations of leptin gene single nucleotide polymorphism (SNP) revealed that certain nucleotide supstitutions may lead to changes in leptin expression. Gene-specific single nucleotide polymorphisms in the regulatory region (promoter) of the bovine leptin gene were detected that show associations with serum leptin concentration, growth rate, body weight, feed intake, feeding behaviour, and measures of carcass merit. The polymorphisms may also be predictive of other important traits in cattle such as milk yield and composition. However, further efforts are required to validate these findings in other bovine populations before their application in marker-assisted selection.

***Key words**: Leptin, single nucleotide polymorphism, economically important traits, cattle.*

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INTRODUCTION

Leptin (from Greek "leptos" meaning thin) is a 16kDa protein hormone which regulates energy intake and expenditure, appetite and metabolic rate. Leptin is one of the most important adipose tissue derived hormones (Brennan and Mantzoros, 2006), although leptin can also be produced in small amounts in various tissues such as brown adipose tissue, placenta, ovaries, skeletal muscle, stomach (lower parts of fundic glands), mammary epithelial cells, bone marrow, pituitary and liver (Margetic et al., 2002). The gene for leptin (locus *Ob* or *Lep*, *Ob* for obese, *Lep* for leptin) is located on chromosome 7 in humans (GreGreen et al., 1995), and on chromosome 4 in cattle (Stone et al., 1996).

The effects of leptin were observed by studying mutant obese mice that arose at random within a mouse colony at the Jackson Laboratory in 1950 (Ingalls et al., 1950). Namely, these mice were massively obese and hyperphagic. The leptin itself was discovered by Friedman et al. in 1994. by studing these mice at the Rockefeller University in the United States (Zhang i sar., 1994). In last couple of decades it has been realised that leptin has important roles not only in regulation of food intake and energy expenditure, but also in regulation of neuroendocrine and immune functions and in modulation of glucose and fat metabolism (Bluher and Mantzoros, 2009).

Obesity is a major health issue in much of the human population. In the United States it is estimated that over 30% of the population is overweight, and this proportion is increasing. Most attempts to treat obesity to date, except for the several types of surgical removal of the tissue, have failed to result in a sustained reduction of obesity (Houseknecht et al., 1998).

Although the obesity is not a major health problem in animal agriculture, changes in the fat composition in animal bodies with an aim to provide more meat and better productive efficacy are one of the main goals in modern zootechnique (Maratos-Flier, 2008). In addition, regulation of feed intake and energy balance in the bodies of domestic animals are important for optimization of growth, reproduction, lactation and overall health and well-being. Thus, understanding the basic mechanisms that regulate adiposity, feed intake and energy metabolism in livestock may lead to new technologies that will futrher enhance animal performance and health. (Houseknecht et al., 1998).

ROLE OF LEPTIN IN THE REGULATION OF REPRODUCTION

Literature data concerning role of leptin in the control of reproduction are rapidly developing. At this time, the majority of information in this area has been obtained form research with human subjects and laboratory rodents. The importance of adequate nutritional intake in maintaining reproductive function is well-established (Asdell, 1949; Armstrong and Britt, 1987; Peters et al., 2008). Inadequate nutrition delays or prevents the onset of puberty and interferes with normal cyclicity (Armstrong i Britt, 1987). In males, undernutrition is accompanied by hypogonadism and infertility. Analysis of various species revealed that undernutrition results in decreased gonadotropin secretion (Cameron i sar., 1993; Faria Tda et al., 2008).

The mechanisms responsible for communicating nutritional status to the repro-

ductive system have been sought for many years. A commonly held beleif at one time was that the amount of body fat was a controling factor in the onset of puberty and the maintenance of adult reproduction. More recent research has shown that metabolic and/or nutritionally induced changes in reproductive function can occur without changes in body fat (Beltranena et al., 1993). A variety of hormones may act as possible signals of nutritional status to the reproductive system. In addition, metabolites can exert potent effects on endocrine systems and have been implicated in the control gonadotropin secretion. It is beleived that leptin could represent indicator of nutritional status that allows reproductive processes to proceed. Leptin is produced mainly in adipose tissue, which actively responds to nutritional and metabolic changes. The production of leptin increases with feeding and body fat content (Hamann i Matthaei, 1996). Leptin receptors are found in the ventromedial and arcuate regions of the hypothalamus and are thus positioned anatomically in regions associated with the control of appetite and reproductive neuroendocrine function (Dyer i sar., 1997; Cottrell et al., 2009). Thus, leptin could provide an accurate, circulating signal of nutritional status.

Much of the evidence for the role of leptin as a reproductive hormone has been derived from *ob/ob* mice, which do not produce a functional leptin ptotein. An *ob/ob* females mouse is sterile and remains essentially in a constant prepubertal state (Tortoriello et al., 2007). Ovarian and uterine weights, sex steroid concentrations, and pituitary gonadotropin secretion are depressed in these animals. Administration of recombinant leptin to *ob/ob* female mice completely restores gonadotropin secretion, secondary sex organ weight and function, and fertility (Barash et al., 1996).

Similar evidence has been obtained in *ob/ob* male mice. (Farooqi, 2002). Male *ob/ob* mice demonstrate very low levels of fertility, low gonadotropin secretion and are hypogonadal (Mounzih et al., 1997). Semeniferous tubules contain few mature sperm, and Leydig cells are severely atrophied. As in female *ob/ob* mice, leptin administration to the male mice restores fertility (Mounzih et al., 1997).

Undernutrition results in a condition analogous to the *ob/ob* genotype, with inhibited leptin secretion and reproductive function (Yura et al., 2008). The effects of undernutrition on reproduction in non-obese animals also can be ameliorated by leptin treatment. The starvation-induced delay in ovulation in non-obese female mice is prevented by leptin treatment (Ahima et al., 1996). Similarly, serum LH and testosterone levels are increased by leptin administration in fasted male mice (Ahima et al., 1996). Restricting nutrition to 80% of ad libitum feed intake causes a >50% reduction in ovarian and uterine weiths that is completely prevented by twice-daily injections of leptin (Cheung et al., 1997).

The finding that leptin treatment allows reproductive maturation in malnutrition and normally fed animals, raises the question has leptin any role in the normal onset of the puberty? It has been found that serum concentrations of leptin are elevated at the onset of puberty in mice (Chehab et al., 1996). Also, before the onset of puberty there is a transient increse of leptin secretion in boys (Mantzoros et al., 1997). These observations suggest that there is a causal link between increasing leptin secretion and sexual maturation.

A logical first step in understanding the mechanisms by which leptin influences reproduction is to determine the location of functional receptors for this hormone (Grze-

gorzewska et al., 2008). Leptin receptor mRNA has been localized in ventromedial and arcuate hypothalamic nuclei and in anterior pituitary tissue of sheep (Dyer et al., 1997). In rats, leptin receptor mRNA can readily be detected in the ovary, testis, uterus, hypothalamus and pituitary gland (Zamorano et al., 1997). Localization of the receptor in reproductive tissues likely occurs across species, because leptin receptor mRNA also is found in human ovaries and testes (Cioffi i sar., 1996).

Thus, leptin could act at multiple sites in the reproductive system. Leptin treatment enhances gonadotropin secretion (Barash i sar., 1996; Kosior-Korzecka et al., 2006). Increased uterine weight in leptin-treated *ob/ob* mice seems to occur as a result of proliferative responses to increased ovarian estrogen production (Barash i sar., 1996). Similarly, trophic responses in seminal vesicles are likely a result of increased testosterone production (Barash i sar., 1996). Although the most accepted hypothesis is that the overall leptin-induced stimulation of reproductive function occurs secondarily to increased gonadotropin secretion, the possibility of direct effects can not be discounted.

It is interesting that neuropeptid Y (NPY) is present in the hypothalamic regions involved in neuroendocrine control of feed intake. In contrast to leptin, NPY is a powerfull stimulator of feed intake and inhibitor of gonadotropin secretion (Kalra i Kalra, 1996; Gupta et al., 2009). Undernutrition elevates the experssion of the gene for NPY in nucleus arcuatus of the hypothalamus and leads to the elevated concentration of NPY in the cerebrospinal fluid (Kaye i sar., 1990). The application of leptin decreases NPY experssion in n. arcuatus, possibly due to removing the inhibition of GnRH release. These data have led to the speculation that receptors for leptin may exist on NPY neurons. In fact, leptin receptors have been localized on hypothalamic NPY neurons in mice and sheep (Houseknecht i sar., 1998). In addition, *ob/ob* mice that also are homozygous for a recessive mutant NPY allele have been generated (Erickson i sar., 1996). These animals lack leptin and NPY and are less obese and more fertile than *ob/ob* mice with intact NPY production.

APPLICATIONS OF LEPTIN IN ANIMAL AGRICULTURE

Although obesity per se is not a major problem in animal agriculture, improvement of productive efficiency, carcass composition, and animal health and well-being are important goals. If leptin biology is similar for livestock species and human and rodent species, it is clear that leptin has myriad effects on tissues and endocrine system that ultimately lead to the coordination of whole-body energy metabolism. Therefore, leptin could have important influence the performance ane well-being of livestock species. (Farooqi and O'Rahilly, 2009). Leptin may be classified as a „metabolism modifier"; thus we can predict that the manipulation of leptin expression and/or action will be of interest to scientists and pharmaceutical companies wishing to improve productive performance of animals. Furthermore, if leptin is involved in animal responses to diesease or stress, the manipulation of leptin action may become an important therapy in the treatment of animal disease. Successful enhancement of reproductive function or manipulation of nutrient partitioning are more likely to be achieved through regulation of leptin production or sensitivity to leptin through regulation of leptin production or sensitivity to leptin through nutritional or metabolic manipulation. Genetic selection

also could be used to this end. Expressed (Matteri et al., 1998) and intronic (Sasaki et al., 1996) polymprphisms in the leptin gene that may be useful in RFLP-based selection have been discovered.

POLYMORPHISM OF THE LEPTIN GENE IN CATTLE

As mentioned, the leptin gene has been mapped to bovine chromosome 4 (Stone et al., 1996). Polymorphisms in the coding regions of the leptin gene in cattle have been associated with serum leptin concentrations (Leifers et al., 2003), feed intake (Leifers et al., 2002), milk yield (Leifers et al., 2002) and body fatness (Nkrumah et al., 2004). Although previous studies have focused on associations between polymorphisms in the coding regions of the leptin gene and economically important traits, studies in humans and other species have shown that polymorphisms in the leptin promoter may be of major importance (Nkrumah i sar., 2005). This is because such polymorphisms are generally associated with the sequence elements and factors regulationg gene expression and may completely abolish the inducibility of promoter (Miller i sar., 1996) or decrease its activity significantly (Mason i sar., 1998). The bovine leptin promoter has been sequenced by Taniguchi et al. (2002).

Nkrumah et al. (2005) investigated association between single nucleotide polymorphisms (SNP) in the leptin gene promoter in cattle and economically important phenotipic charateristics (serum leptin concentration, growth, feed intake, feeding behaviour and measures of carcass merit). They detected three bi-alelic single nucleotide substitutions, namely UASMS1, UASMS2 i UASMS3, in the bovine leptin promoter located at nucleotide positions 207, 528 i 1759. UASMS1 and UASMS2 are substitutions of cytosine with thymine (C→T), whereas UASMS3 is a substitution of cytosine with guanine (C→G). The analysis revealed significant linkage disequilibrium between UASMS2 and UASMS3, and both SNP separately show associations with performance, feed intake, and feeding behaviour in the experimental cattle population. In addition, the UASMS2 SNP shows significant associations with serum leptin concentration and measures of body fatness.

With respect to UASMS2 the results presented showed that serum leptin concentrations, body weight, marbling score and ultrasound backfat thickness were higher in animals with genotype TT compared to animals with genotypes CC or CT. Also, analysis of UASMS3 showed that animals with the genotype GG have higher growth rate and body mass, increased feed intake and higher feeding duration. (Nkrumah i sar., 2005).

The significant linkage disequilibrium between the two polymorphisms is not surprising as the two SNP are only 1231 bp apart, and it suggests that the effect of one of the SNP may reflect an indirect effect of the other. However, because UASMS2 shows stronger associations with serum leptin concentration and body fatness, it may be speculated that UASMS2 may be more functiionally significant. (Nkrumah et al., 2005). Significantly higher growth rates, body weight, and body fatness of the animals with higher serum leptin concentration and genotiype TT for UASMS2 is consistent with previous evidence in humans (Larsson et al., 1998) and cattle (Leifers et al., 2003), showing that serum leptin concentration is positively related to body weight and body fatness.

Nonetheless, the observed higher daily feed consumption of animals with higher

serum leptin concentration and genotype TT for UASMS2 is surprising and in contrast to evidence in humans (Larsson et al., 1998). Namely, high concentration of leptin in humans is associated with lower habitual food consumption.

There is a considerable interest in the application of molecular technologies in the form of specific DNA markers that are associated with various quantitative trait loci, to promote more efficient and relatifvely easy selection and breeding of farm animals with an advantage for an inheritable trait of growth rate, body weight, carcass merit, feed intake, and milk yield and composition. Several quantitative trait loci for performance and meat production in cattle have been identified, and a number of potential candidate genes have been identified and selected for analysis based on a known relationship with physiological or biochemical processes and production traits. It is seldom reported that one particular polymorphism in a candidate gene would influence several traits of economic importance in livesock at the same time. The leptin gene, however, seems to be one of the exceptions, as its involvement in the regulation of several biologically important processes in the body makes it, perhaps, one of the best physiological gauges for energy balance, body weight, and body fat content in mammals. Several studies have been conducted to characterize the relationship of circulating leptin with traits of economic importance in beef cattle. For example, Ehrhardt et al. (2000) and Delavaud et al. (2002) showed that circulating leptin levels are correlated with body weight, food intake, nutritional status, and adipose tissue mass. Circulating leptin concentrations are also correlated with the regional distribution of body fat (Yamada et al., 2003) and could be used as a predictor of carcass merit in cattle (Geary et al., 2003). Several polymorphisms have been described in the bovine leptin gene (Haegeman et al., 2000). Fitzsimmons et al. (1998) reported a positive association between a microsatellite marker (*BM1500*; located approximately 3.6 kb away from the leptin gene) and body fatness in cattle. Polymorphisms have also been reported in the coding regions of the leptin gene that show considerable associations with feed intake (Oprzadek et al., 2003), carcass merit (Buchanan et al., 2002), milk quantity and quality (Buchanan et al., 2003), and serum leptin (Liefers et al., 2003) in cattle.

The exact molecular and physiological mechanisms underlying the association of the polymorphisms with the variety of traits reported in the present study are unknown. It is assumed that SNP, partricularly UASMS2 in the regulatory region of the leptin gene contributes to the expression of leptin gene in cattle, or otherwise it serves as surrogate for causative SNP that are yet to be detected. Several putative Sp1, CCAAT/enhancer binding protein (C/EBP), and TATA box binding sequences were detected in the vicinity of the above mentioned SNP (Taniguchi i sar., 2002). Studies in humans have shown that mutations in the C/EBP-α region of the leptin promoter abolished the inducibility of the promoter by C/EBP-α (Miller et al., 1996). Mason et al. (1998) showed that mutations in the C/EBP-α and TATA motifs, as well as in a consensus Sp1 site of leptin gene, decreased promoter activity by 10-, 10-, and 2.5-fold, respectively, and abolished binding of these factors.

As for the polymorphisms in the coding regions of the bovine leptin gene, it should be mentioned that C→T transition in exon 2 of leptin that encodes an Arg25Cys substitution is associated with body fat deposition in beef cattle (Buchanan et al., 2002). In addition, it has been established that TT genotype has a great influence to milk produc-

tion, especially in early lactation. Namely, cows with the genotype TT have increased milk production and increased milk protein content without changes in yield of milk fat (Buchanan et al., 2003). These characteristics of milk have economic importance.

On the other hand, selection of cows for increased milk production has a negative impact on their fertility (Pryce i sar., 2000). Diary cows have small, but sometimes serious negative energy balance during early lactation, which influences duration of anoestrus period after calving. If there is a correlation between polymorphism of the leptin gene and milk yield, body weight, feed intake and fertility, it could be used not only for a discoveries of leptin mechanisms of action, but also in future programmes of cow breeding (Leifers i sar., 2002).

It should be mentioned that despite numerous literature data about biochemical and physiological effects of leptin, little is known about possible genotoxic and mutagenic effects of leptin. Namely, some hormones act as endogenous mutagens (Djelić, 2001; Djelić and Djelić, 2002, Djelić and Anderson, 2003; Djelić et al., 2005; Djelić et al., 2006; Djelić et al., 2007), therefore it would be interesting to examine the ability of leptin to induce mutagenic effects or to modulate mutagenic effects of hormones or environmental pollutants (Djelić and Djelić, 2000; Stanimirović et al., 2005; Stanimirović et al., 2007; Stevanović et al., 2008).

In conclusion, gene-specific single nucleotide polymorphisms in the regulatory region (promoter) of the bovine leptin gene were detected that show associations with serum leptin concentration, growth rate, body weight, feed intake, feeding behaviour, and measures of carcass merit. The polymorphisms may also be predictive of other important traits in cattle such as milk yield and composition. However, further efforts are required to validate these findings in other bovine populations before their application in marker-assisted selection.

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ZNAČAJ POLIMORFIZMA GENA ZA LEPTIN KOD GOVEDA

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Izvod

Leptin je proteinski hormon koji ima ključne uloge u regulacji unosa hrane i potrošnje energije. Ekspresija i sekrecija leptina su veoma povezani sa količinom masnog tkiva i veličinom adipocita. Smatra se da je leptin metabolički signal koji reguliše efekte nutritivnog statusa na reproduktivnu funkciju. Receptori za leptin pronađeni su u ventromedijalnom i arkuatnm regionu hipotalamusa, tako da su anatomski pozicionirani u regionima uključenim u kontrolu apetita i reproduktivne neuroendokrine funkcije. Interesanto je da se u hipotalamusu nalazi neuropeptid Y (NPY) u regionima uključenim u neuroendokrinu kontrolu unosa hrane. Nasuprot leptinu, NPY je moćan stimulator unosa hrane i inhibitor sekrecije gonadotropina. Receptori za leptin su lokalizovani na NPY neuronima hipotalamusa i pokazano je da leptin umanjuje eksperesiju NPY. Istraživanja polimorfizna pojedinačnih nukleotida (SNP) u okviru gena za leptin otkrila su da određene supstitucije nukleotida mogu voditi ka promenama u ekspresiji leptina. Detektovani su gen-specifični polimorfizmi pojedinačnih nukleotida u regulatornom regionu (promotoru) gena za leptin goveda koji su udruženi sa koncentracijama leptina u serumu, stopom rasta, telesnom težinom, unosom hrane, hranidbenim ponašanjem i karakteristikama trupa. Polimorfizmi bi mogli biti od značaja za predviđanje drugih važnih fenotipskih karakteristika kod goveda kao što je prinos i sastav mleka. Međutim, potrebni su dodatni napori da se dobijeni nalazi potvrde u drugim populacijama goveda pre njihove primene u selekciji na osnovu specifičnih genskih markera.

Ključne reči: Leptin, polimorfizam pojedinačnih nukleotida, ekonomski važne osobine, goveda

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