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COMPARISON AND ASSESSMENT OF LEPTIN RECEPTOR EXPRESSION BY THE FOLLOWING ORIGAMI AETHEROLEUM STUDY AT BROILER CHICKENS COBB 500

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ABSTRACT

The recently discovered protein, leptin, is a 16 kD protein consisting of 146 amino acids which is synthesized primarily by adipose tissue and is secreted into the bloodstream after cleavage of the 21 amino acids signal peptide. Leptin impacts feed intake, the neuroendocrine-axis, metabolism and immunological processes. Leptin was first identified as the gene product found deficient in the obese ob/ob mouse. The hypothalamus appears to be the primary site of action, since leptin receptors are located within hypothalamic areas associated with control of appetite, reproduction and growth. Using herbs and essential oils depends on their antimicrobial activity. Most plants have favorable multifunctional properties, which are the specific content of bioactive components. Some authors characterize phytogenic substance such as natural substances plant origin, which leave no residues in animal products and is not necessary to keep the trade period before slaughter animals. Analyzes suggest that the structural function of the receptor exists as a dimer constructively in the plasma membrane. Each receptor dimer pair is reversibly bound to one molecule of leptin. When bound, signaling pathways are responsible for beginning the activation receptor associated Janus kinase 2 (JAK2) and tyrosine phosphorylation of two key residues in the intracellular part of receptor. The aim of our experiment was to optimize the methodology for monitoring the expression of the leptin receptor extracellular avian model. We used samples of internal organs and abdominal fat chickens that were fed spirit and also fat and organ samples from broiler chickens from the control group. In heard tissue, spleen, liver at a relatively high concentration of total cDNA in the sample length leptin receptor extracellular fragment located in the expected quantities.

Keywords: leptin receptor, gene expression, broiler chickens, origami aetheroleum

INTRODUCTION

Since its discovery in mammals **(Zhang et al., 1994)** leptin has been established as a regulator of multiple physiological functions ranging from its effects as a hormone in the coordination of energy balance, metabolism and neuroendocrine pathways **(Ahima et al., 2000)**, to its role as a cytokine in the regulation of immune responses **(Matarese et al., 2005)**. Expresion of the ob gene and circulating leptin concentracions are highly correlated with percentage of body fat in rodents **(Soukas et al., 2000)** and the degree of obesity in humans **(Auwerx and Staels, 1998)**. The leptin receptor (LEPR) was identified soon after leptin itself by expression cloning from mouse choroid plexus **(Tartaglia et al., 1995)**.

It belongs to the class I. cytokine receptor superfamily that includes the receptors for interleukin 6, leukemia inhibitory factor, granulocyte-colony stimulating factor and glycoprotein 130. The LEPR is expressed in multiple isoforms derived from one gene that contains 17 common coding exons and several alternatively spliced exons **(Wang et al., 1996)**.

Weight loss and weight gain, which result in changes in the amount of adipose tissue, alter *LEP* mRNA in adipocytes and serum leptin levels. Increase in adipose tissue mass with weight gain results in a significant increase in circulating leptin while a decrease in fat mass with weight loss reduces serum leptin **(Rosenblum et al., 1996)**.

These observations thus support the concept that leptin provides a signal to the central nervous system of the size of energy stores in the body. However, extreme changes in energy intake such as fasting reduce serum leptin, suggesting a role for the hormone in coordinating the neuroendocrine response to caloric deprivation **(Vaisee et al., 1996)**.

Such a response would include initiation of food seeking behavior to increase energy intake, and activation of processes to reduce energy expenditure, both to insure survival should the fast be prolonged. Serum leptin levels are rapidly decreased with short-term fasting (24-72 h) in both animals and humans **(O'Rourke et al., 2002)**.

Leptin is also involved in reproductive function. Indeed, it has been shown in mice that leptin directly enhances insulin - and gonadotropin - stimulated ovarian steroid genesis **(Kunová, 2011)**.

The rapid fall in leptin with fasting is disproportionately greater than the small reduction in adipose tissue mass that occurs over the same time period. Thus it is reasonable to

suggest that serum leptin during fasting serves as a peripheral signal to the central nervous system that caloric restriction is occurring, rather than as a signal of current energy stores in the body. A chicken gene has been cloned that shows 60 % overall nucleotide sequence identity with mammalian LEPRs and contains the predicted exon boundaries and conserved motifs found in the long isoform, Rb, of the mammalian receptor **(Horev et al., 2000)**.

A turkey LEPR has also recently been characterized that shares 94 % nucleotide sequence identity with the chicken sequence **(Horev et al., 2000)**.

The structural conservation of the avian LEPR gene, together with its mapping to a chromosomal region synthetic with its mammalian counterpart **(Dunn et al., 2000)** suggests that the physiological role of leptin and its receptor in birds and mammals may be similar. However, there is uncertainty about the identity of the ligand for the avian LEPR because reports that a cDNA encoding chicken leptin had been cloned **(Liu et al., 2006)** have not been confirmed **(Lee et al., 1996)**.

In turn, polymorphismus in the corresponding gene have been proposed as predictors of relative differences among individuals for those traits **(Schenkel et al., 2005)**.

The focus of this study was to detect extracellular expression of leptin receptor on the analysis of cDNA for the type of final fattening broiler chickens Cobb 500. Another aim of study was to focus on the tissue distribution of leptin receptor expression in selected organs broiler chickens.

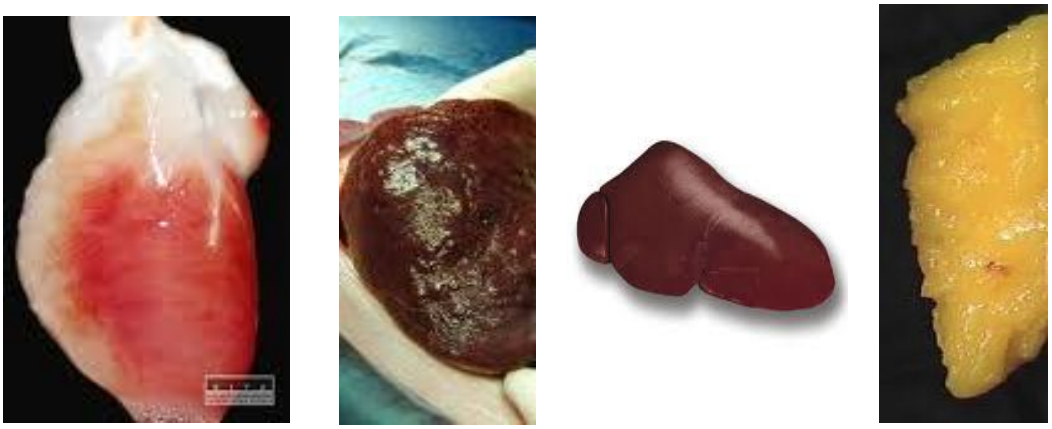
MATERIAL AND METHODOLOGY

Analysis was performed on samples which were obtained from the group experiment with broiler chickens. This experiment was located on poultry a farm with a hall designed for holding pieces 24000 broiler chickens for meat production. To detect the leptin receptor, we used tissue samples of internal organs and abdominal fat broiler chickens Cobb 500. Chickens were kept in the hall on deep litter with the recommended conditions and needs of farming. The experiment has been using his technology, feeding and watering. The experimental group was composed of 100 pieces of final fattening by the Cobb 500, from which we chose 10 pieces of equal body weight 1800 g on end fattening 40 days.

These broiler chickens were used as representative samples from which we used the internal organs and abdominal fat for analysis. The RNA we isolated crushed in mortar tissue sections of heart, spleen, liver and abdominal fat using the SV Total RNA Isolation System Trial Size kit (Promega, Madison USA).

The RNA was transcribed using reverse transcriptase – IMPROM-II™ Reverse Transcription System kit (Promega, Madison USA) with random hexamers as primers. The reaction mixture contained RNA, InPromII 5x buffer, MgCl2, dNTP mix, RNAsin MIX 40pmol.ul-1 and InPromII RT. Reaction mixture was complete by redestinated water to required volume. Reaction carried out in thermal cycler (PTC-150™ MiniCycler, Research, Watertown USA). Reaction was following by heating the RNA and Random Hex at 70 °C for 5 minutes. Cycle of reverse transcription started at 25 °C for 5 minutes. Cycles comprised at 42 °C for 50 minutes. This was followed 70 °C for 15 minutes and then the temperature was lowered to 20 °C for 1 second.

This reaction mixture for PCR contained cDNA, 1.80 mM MgCl2, 0.25 mM dNTPs mix. Primers were used, which we analyzed the identification of the extracellular receptor 0.20 pmol.µl-1 Cassy-F 5'-marker GTC CAC GAG ATT CAT CCC AG-3'and 0.20 pmol.µl-1 Cassy-R marker 5'-CCT GAG ATG CAG AGA TGC TC-3'(Cassy et al., 2004). Further primers were used for positive control and quality of isolated RNA during reverse transcription of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) GAPDH-F-marker 5'-GTGTTATCATCTCAGCTCC-CTCAG marker and GAPDH-R-marker 5'-AAAGGTGG-AAGAATGGCTGTCACC **(Liu et al., 2006)**. Other components were 0.80 U HotStart Polymerase GoTaq, redestinated water up to volume of 30 ml. Mixture contained buffer GoTaq green 5x buffer (Promega, Madison USA) and amplification was carried out in thermal cycler (PTC-150™ MiniCycler, Research, Watertown USA). PCR reaction procedure was following: PCR cycle started with pre-denaturation at 95 °C for 3 minutes. Subsequently, repeated 40 cycles comprising denaturation at 95 °C for 30 seconds and extension at 72 °C for 1 minute. The final extension fragments were at 72 °C for 5 minutes.



HEART **SPLEEN** **LIVER** **ABDOMINAL FAT**

Fig 1: heart, spleen, liver and abdominal fat

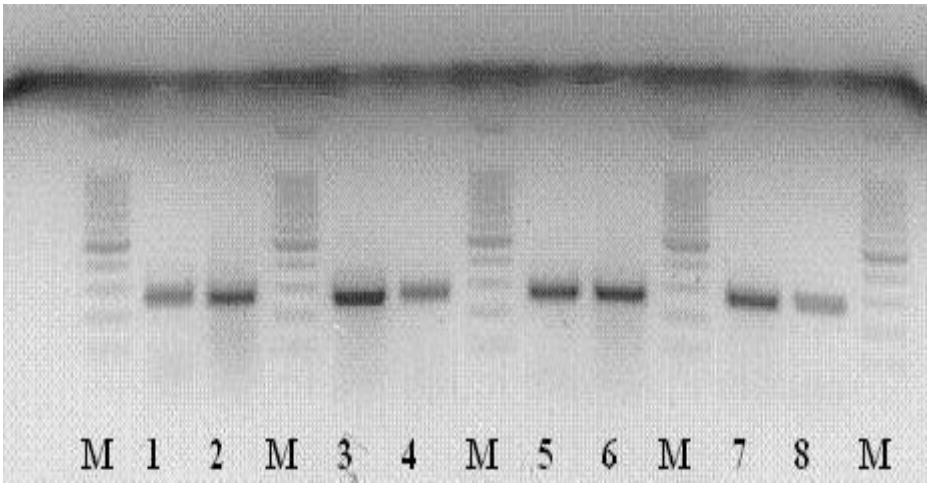
RESULTS AND DISCUSSION

The aim of our experiment was to optimize the methodology for study the expression of the leptin receptor extracellular avian model. We used samples of internal organs and abdominal fat of the broiler chickens that were fed spirit and also fat and organ samples from chickens from the control group. During the experiment, we succeeded isolate RNA from the heart, spleen, liver and inside of abdominal fat. The obtained total RNA after reverse transcription to cDNA was the template that we used to identify selected leptin receptor. In our experiment, we used weight marker 100 bp in size.

As shown in figure 2, in all organs analyzed we were able to isolated total RNA, which in the subsequent reverse transcription and PCR reaction using primer pairs Cassy-F and Cassy-R gave rise to fragments 1, 2, 3, 4, 5, 6 , 7 and 8 As indicated by the track number 4, the lowest content of isolated RNA was achieved abdominal fat, which we obtained from broiler chickens fed with origami aetheroleum, and track number 8, which we obtained from broiler chickens which we took from the control group. M is marker for the identification of extracellular leptin receptor. Interesting results offer track number 4, which should indicate the presence of leptin receptor in the national abdominal fat. This track was located fragment, which did not correspond to its length expectancy fragment bounded designed primers (Cassy-F and Cassy-R - 273bp), but was less than control GAPDH fragment length of 533bp **(Liu et al., 2006)**.

Unlike **Kunová et al. (2011)** that followed leptin in other species of animals for meat production, we have research on leptin receptor in chickens for meat production. The material tracking leptin receptor, we selected internal orfans such as heart, liver, spleen and abdominal fat.

It follows that, using primers and procedures described in the methodology of work can be studied extracellular expression of leptin receptor at broiler chickens Cobb 500. In heard tissue, spleen and liver is studied in leptin receptor expressed in the expected quantities. The tissue abdominal fat appears to be truncated RNA, which could cause alternative splicing molecules (splicing). As the final fattening type Cobb 500 has long focused on reducing the fat abdominal fat and increasing the volume of the breast muscle, we will give this issue more.



M - Weight marker, pathways 1, 5 - heart tissue, 2, 6 spleen tissue, 3, 7 liver tissue, 4, 8 fat tissue, pathways 1, 2, 3, 4 tissues, organs and fat of chickens fed Origami aetheroleum, pathways 5, 6, 7, 8 organs and fat tissue control, fragment 1, 2, 3, 4, 5, 6, 7, 8 fragment CASSY

Fig 2: Expression of leptin receptor in internal organs and abdominal fat broiler chickens

CONCLUSION

In our experiment, we succeeded isolated total RNA from tissues of internal organs such as heart, spleen, liver, and abdominal fat. In heard tissue, spleen, liver at a relatively high concentration of total cDNA in the sample length leptin receptor fragment located in the expected quantities. Abdominal fat for the presence of leptin receptor fragment showed that its length did not correspond to the expected length of the fragment bounded by the proposed ceasefire. Furthermore, we found that using a primer and the procedures described can be observed extracellular expression of leptin receptor in broiler chickens Cobb 500. In body fat tissue is RNA found in abbreviated form. The resulting was determined by UV-spectrophotometric quantification of DNA. This issue we will continue to pay.

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