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Research Article

Investigation of serum leptin, ghrelin, irisin, insulin levels and their correlations in cattle with subclinical ketosis

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ABSTRACT

In this study, it was aimed to investigate the correlations with leptin, ghrelin, irisin and insulin levels in the blood serum of cattle with subclinical ketosis. For this purpose, 10 healthy and 10 Holstein cattle with subclinical ketosis obtained from farms in Burdur region were used. A diagnosis of subclinical ketosis was made according to the Rothera test performed in milk, by performing a general clinical examination of the animals. Blood samples were taken from Vena jugularis into tubes without anticoagulant. Serum leptin, ghrelin, irisin and insulin levels were measured in the obtained sera using commercial ELISA kits. In cattle with subclinical ketosis, serum ghrelin, leptin, irisin and insulin values were increased compared to the control group ($p<0.05$). In the correlation findings, a highly and quite significant positive correlation was found between serum ghrelin and irisin values ($r= 0.802$; $p<0.001$). A moderately and quite significant positive correlation was found between serum ghrelin value and insulin value ($r=0.673$; $p=0.001$). A moderately and significant positive correlation was determined between serum ghrelin value and leptin value, between serum irisin value and leptin value, and between irisin value and insulin value ($r=0.623$; $p=0.003$, $r=0.474$; $p= 0.035$, $r=0.558$; $p=0.011$). In conclusion, in this study, correlations were observed between serum levels of leptin, irisin, ghrelin and insulin hormones in animals with subclinical ketosis. However, it is thought that leptin, ghrelin, insulin and irisin hormones, which are associated with lipid and carbohydrate metabolism, can be used as important biomarkers in the diagnosis of subclinical ketosis and in the follow-up of its prognosis.

INTRODUCTION

Ketosis is caused in ruminants when there is a disruption in the metabolism of carbohydrates as well as volatile fatty acids. It is defined as a metabolic disorder that is characterized by a decrease in the amount of blood glucose, the depletion of the amount of glycogen and glucose stored in the liver, and the presence of ketone bodies in the blood, urine, and milk as a result of fatty degeneration in the liver (Gül, 2012). It is possible for either the clinical or the subclinical form of ketosis to develop, but the subclinical form is more prevalent (Detilleux et al., 1994).

Subclinical ketosis is characterized by a persistent deficit in energy balance, and as a result, there are high levels of ketone bodies in the blood, urine, and milk, but there are no clinical signs (Herdt, 2000). It not only reduces the amount of milk that animals produce and their reproductive performance, but it also raises the risk of other metabolic and immunological illnesses that can be noticed in the postpartum period, which results in significant financial losses (Duffield, 2000). It can be occured in cases of nutritional deficits or in cows who have been fed meals low in energy (Youssef and El-Ashker, 2017). According to several studies, the predisposing factors of subclinical ketosis are the number of births, body condition score and season. (Duffield, 2000). It is reported that cattle whose body condition scores are higher than 4.0 have a greater risk of developing subclinical ketosis in the postpartum period compared to cattle whose body condition scores are lower (Duffield et al., 2003). There are numbered studies stating that hormonal control also plays an effective role in the etiology of ketosis and subclinical ketosis (Roh et al., 2016). It is common knowledge that hormones involved in the lipid and carbohydrate metabolism play an important part in the pathophysiology of metabolic illnesses such as ketosis (Roh et al., 2016).

Leptin is a hormone that plays a crucial role in maintaining metabolic balance. It can reduce the amount of food consumed while simultaneously increasing the amount of energy expended. The regulation of energy expenditure is just one of the many functions performed by the hormone leptin, which is released by adipocytes. In addition to the function it serves, it also possesses pleotropic effects, which manifest themselves in a variety of physiological pathways (He et al., 2018). Circulating leptin concentrations in dairy cattle have been the subject of a significant number of investigations (Vargová et al., 2015; Danicke et al., 2018; He et al., 2018).

Since the discovery of the leptin hormone that is secreted by adipocytes till the present day, numerous peptides and cytokines that are responsible for both favorably and negatively regulating the metabolic process have also been found. There are a variety of adipokines, hepatokines, and myokines that all perform the function of cytokines, and the roles that these proteins play in both people and animals are still being researched (Roh et al., 2016).

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The irisin hormone, which is formed as a result of the breakdown of the transmembrane protein containing fibronectin type III domain 5 (FNDC5), has recently been identified as a myokine. The irisin hormone provides the formation of brown adipose tissue from white adipose tissue, contributes to the loss of body weight and improves glucose metabolism. The irisin hormone is usually regulated along with exercise (Boström et al., 2012).

A study was conducted to evaluate the level of bovine FNDC5 protein. The FNDC5 protein from bovines, mice, and humans were all analyzed and compared in this particular study (Komolka et al., 2014). As a consequence of this study, it has been found that the transcript level at the protein locus in cattle is characterized by a greater degree of variability. Additionally, it has been proven that mice and cattle have fundamentally different approaches to the hormonal regulation of the FNDC5 protein and irisin (Komolka et al., 2014). It has been discovered that cold, physical activity, and leptin are among the different factors that modify the quantity of circulating irisin, while circulating irisin is positively connected with muscle mass and body mass index (Huh et al., 2012; Crujeiras et al., 2014; Arhire et al., 2019). On the other hand, contrary to what has been stated, irisin hormone has been shown to have a negative connection with both age and insulin levels (Park et al., 2013; Wen et al., 2013).

Insulin is a polypeptide hormone that is released from β cells in the pancreas, which are located in the centre of the islets of Langerhans (Greenspan and Gardner, 2004). The insulin hormone's primary role is to facilitate the transport of glucose into the cell, which is its primary function. Cows that have hepatic lipidosis and ketosis acquire insulin resistance in the peripheral tissues of their bodies over time (Senoh et al., 2019).

In ruminants, the ghrelin hormone is a peptide that is composed of 27 different amino acids. It is also referred to as the hunger hormone. In ruminants, the abomasum, the small intestine, and the pancreas are responsible for the production of the hormone ghrelin. Acylation only occurs in a minute portion of the hormone ghrelin. This causes both acyl and non-acyl ghrelin hormones to take place in the circulation. Both acylated and non-acylated versions of ghrelin contribute to the regulation of energy homeostasis in the body (Thidar Myint et al., 2006).

In this study, it was aimed to investigate the relationship between insulin, leptin, ghrelin and irisin hormones, which are involved in energy balance, and each other in subclinical ketosis, which occurs as a result of the deterioration of negative energy balance.

MATERIALS and METHODS

This research was conducted with the approval of the Burdur Mehmet Akif Ersoy University Local Ethics Committee for Animal Experiments. (Ethics committee approval number: 992/108-2022)

Animals

In this study, 10 healthy and 10 Holstein cattle with subclinical ketosis obtained from Burdur region farms were used as animal material. It was learned that the animals included in the study were fed with fabricated feed as concentrate feed and with straw, corn silage and fresh beet pulp as roughage. A diagnosis of subclinical ketosis was made according to the Rothera test performed in milk, by performing a general clinical examination of the animals. Blood samples were taken from *Vena jugularis* into tubes without anticoagulant. Sera obtained from blood samples were stored at -20 ºC until analysis.

Analysis of serum hormone levels

Serum leptin, ghrelin, irisin and insulin hormone analyzes were performed using commercial bovine ELISA hormone kits (BT-Lab, China. Catalog numbers of the kits: EA0007Bo, E0262Bo, E2318Bo, E0015Bo), in accordance with kit procedures. ELISA reader device (Biotek EPOCH, USA) was used for measurement.

Statistical analysis

The data from the study were analyzed with the help of the IBM SPSS 22.0 for Windows package program. The Shapiro-Wilk test was utilized in order to determine whether or not the groups in the analyses followed a normal distribution. For the purpose of making comparisons between measurements, a paired t-test was utilized because the data followed a normal distribution. Using the Pearson Correlation analysis, we were able to ascertain the nature of the link between the variables.

RESULTS

The levels of ghrelin, irisin, leptin, and insulin in the serum of cattle with subclinical ketosis were found to be higher than those found in the control group, and a statistically significant difference was found between the two groups ($p< 0.001$), (Table 1).

In correlation findings, a high and very significant positive correlation was found between serum ghrelin value and serum irisin value ($r= 0.802$; $p<0.001$). A moderate and highly significant positive correlation was found between serum ghrelin value and serum insulin value ($r=0.673$; $p=0.001$). A moderate and significant positive correlation was determined between serum ghrelin value and serum leptin value, serum irisin value and serum leptin value, and serum irisin value and serum insulin value respectively ($r=0.623$; $p=0.003$; $r=0.474$; $p=0.035$; $r=0.558$; $p=0.011$), (Table 2).

DISCUSSION

In dairy cattle, the term 'transition phase' refers to the time span that spans the first three weeks before birth and the first three weeks after birth (Grummer, 1995). Changes in hormone levels and a reduction in feed consumption have an impact on metabolism throughout the later stages of pregnancy, resulting in a predominantly negative energy balance (Drackley et al., 1999). Ketosis can occur in animals if their energy needs increase and they develop a negative energy balance (NED), which is especially common in high-yielding dairy cattle in the postpartum period (Guliński, 2021).

In addition to the effects that the insulin hormone has on

Table 1. Serum hormone levels in healthy cattle and cattle with subclinical ketosis

Parameter	Subclinical ketosis (n=10) x̄±ss	Healthy (n=10) x̄±ss	p
Ghrelin (ng/L)	346,26±130,75	117,20±72,07	<0,001
Irisin (ng/mL)	4,63±,1,25	2,00±0,62	<0,001
Insulin (mIU/L)	5,38±2,62	3,41±1,02	0,048
Leptin (ng/mL)	2,42±,46	1,73±,26	0,001

Table 2. Correlation findings of hormone values

		Ghrelin	Irisin	Leptin	Insulin
Ghrelin	Pearson Correlation	1	,802**	,623**	,673**
	Sig. (2-tailed)		,000	,003	,001
Irisin	Pearson Correlation	,802**	1	,474*	,558*
	Sig. (2-tailed)	,000		,035	,011
Leptin	Pearson Correlation	,623**	,474*	1	,319
	Sig. (2-tailed)	,003	,035		,170
Insulin	Pearson Correlation	,673**	,558*	,319	1
	Sig. (2-tailed)	,001	,011	,170	

**The correlation is significant at the 0.01 level (2-tailed).
*The correlation is significant at the 0.05 level (2-tailed).

the way glucose is metabolized, there is evidence to suggest that it also plays a part in ensuring that the body is able to adapt physiologically, particularly at the time of calving. Compared to humans, ruminants have a glucose metabolism that is distinct, characterized by low levels of glucose in the periphery and a diminished insulin response from the periphery's tissues (Zachut et al., 2013). Cows go through a brief period of insulin resistance after giving birth to their young in order to acquire insulin-independent glucose uptake by the mammary gland, which is necessary to maintain milk supply after the calving process. As a result, maintaining blood glucose levels within acceptable physiological ranges during the transition phase is of the utmost importance (De Koster and Opsomer, 2013). In the current study, higher serum insulin levels were found in cows with subclinical ketosis compared to the control group ($p<0.05$). Contrary to the results obtained in this study, some authors reported hypoinsulinemia and hypoglycemia in cows with subclinical ketosis (Tehrani-Sharif et al., 2012) and cows with clinical ketosis (Kerestes et al., 2009; Xu et al., 2014). However, in a study, statistically significantly higher serum insulin levels were reported in cows with subclinical ketosis compared to controls (Mohamed Youssef et al., 2017). It has been determined that the periparturient period in dairy cows may be associated with changes in insulin action, especially in peripheral tissues, and the extent of prepartum insulin secretion is associated with higher insulin action (Zachut et al., 2013).

Insulin levels and leptin hormone levels in ruminants have been shown to have a favorable association. In most cases, elevated levels of insulin are accompanied by elevated levels of leptin. The amounts of circulating leptin have been shown to have a positive correlation with body fat, the amount of food consumed, certain nutrients and hormones (particularly insulin), and the luteinizing hormone pulse. In a nutshell, this hormone is responsible for the connection that exists between a person's nutritional state and their reproductive function (Kadokawa et al., 2006). Yang et al. (2010) reported in their study that blood plasma leptin levels of dairy cows in the subclinical ketosis group showed a lower frequency change within 8 weeks after birth. In the same study, it was reported that the interval of change in leptin level of dairy cows in the before birth term subclinical ketosis group was higher than in the control group. According to the findings of another study, having low serum leptin levels before birth is a significant risk factor for developing subclinical ketosis. On the other hand, it was observed that plasma leptin levels peak after birth (especially at the 7th week) in animals that have subclinical ketosis (He et al., 2018). In the current study, it was discovered that the serum leptin levels of cattle with subclinical ketosis were higher in comparison to the group that served as the control, and that there was a positive correlation between the serum leptin levels and the insulin levels.

As the time for parturition draws nearer, dairy cows have a decrease in their dry matter intake, which is one of the most significant physiological changes that take place throughout the transition phase (Drackley et al., 2001). As a direct consequence of this, the cow will frequently suffer from a wide variety of metabolic illnesses such as ketosis, fatty liver, hypocalcemia during the period in which it is transitioning (Goff et al., 1997). Intake of food is controlled by a variety of elements, including hormones and metabolites, and is a mechanism that is quite complex (Ingvartsen et al., 2000). Ghrelin is a peptide hormone that is produced in cattle by the abomasal and ruminal tissues (Hayashida et al., 2001; Gentry et al., 2003). According to the findings of a study, the amount of the hormone ghrelin found in the plasma of dairy cows during the time when they were making the transition from pregnancy to lactation increased (Melendez et al,, 2006). Although increased ghrelin secretion is consistent with experimental models of energy deficiency, 36 hours of fasting has been reported to increase plasma ghrelin concentration more than fivefold (Wertz-Lutz et al,, 2006). An rise in serum ghrelin levels was discovered in cattle with subclinical ketosis in the current study ($p<0.05$), which is consistent with the findings of the studies that were discussed before. However, although many studies have reported a negative relationship between serum leptin and ghrelin levels (Nowroozi-Asi et al., 2016; Vargova et al., 2015), a positive relationship was found between serum leptin and ghrelin concentrations in the current study. This relationship is thought to be a part of the acute phase response seen in subclinical ketosis (El-Deeb et al., 2017) rather than hormonal regulation. It has been reported that both serum leptin and serum ghrelin levels increase simultaneously as part of the acute phase response, especially in inflammatory conditions such as sepsis (Das et al., 2011).

Irisin is a recently reported new myokine and adipokine (Boström et al., 2012) produced by cleavage of the precursor protein fibronectin type III domain-containing protein 5 (FNDC5). Various studies in humans and rodents (Boström et al., 2012, Perakakis et al., 2017) show that irisin plays a role in energy homeostasis and irisin is a regulator of glucose metabolism.

Irisin is a hormone that plays a role in the metabolic pathways of the body. This hormone increases the uptake of glucose and fatty acids by the muscles, decreases gluconeogenesis and stimulates glycogenesis in the liver, and converts white adipose tissue (WAT) into brown adipose tissue. Irisin has also been demonstrated to lessen the intensity of inflammation and to have an effect on the function of the kidneys, neurons, bones, endothelial cells, and beta cells of the pancreas (Perakakis et al., 2017; Momenzadeh et al., 2022). A significant number of research organizations have established a connection between irisin level and coronary heart disease, hypertension, and some malignancies (hepatocellular, prostate). It has been suggested that the irisin has a protective effect against these pathologies (Ma and Chen, 2021). Irisin has been linked to having beneficial effects on metabolic disorders, including obesity, type 2 diabetes, dyslipidemia, and nonalcoholic fatty liver disease (NAFLD) (Polyzos et al., 2018). Irisin and NAFLD have a contentious association, and studies that were just recently published have revealed that the concentration of this myokine is elevated in patients who have fatty liver disease (Kosmalski et al., 2022).

Although there has not been a study that measures serum or serum irisin levels in cases of fatty liver or ketosis in ruminants, irisin hormone levels are expected to increase in subclinical ketosis, just as they do in non-alcoholic fatty liver syndrome in humans. In the current study, it was determined that serum irisin hormone levels increase in subclinical ketosis.

Some studies have reported that serum irisin and leptin hormones are positively correlated and these two hormones increase in metabolic diseases such as fatty liver syndrome, obesity and insulin resistance (Sahin-Efe et al., 2018; Li et al., 2019). As a matter of fact, in the current study, both serum leptin levels and serum irisin levels were increased in cows with subclinical ketosis and they had a positive correlation. In addition, other studies examining metabolic disorders (Stengel et al., 2013; De Meneck et al., 2018) reported a positive correlation between insulin hormone and irisin hormone, which is consistent with our findings in the current study.

CONCLUSION

In this study, correlation between serum levels of leptin, irisin, ghrelin and insulin hormones are observed in animals with subclinical ketosis. However, it is thought that leptin, ghrelin, insulin and irisin hormones, which are associated with lipid and carbohydrate metabolism, can be used as important biomarkers in the diagnosis of subclinical ketosis and in the follow-up of its prognosis.

DECLARATIONS

Ethics Approval

This study was approved by Burdur Mehmet Akif Ersoy University Rectorate, Animal Experiments Local Ethics Committee.

Conflict of Interest

There is no conflict of interest.

Consent for Publication

Not applicable

Author contribution

Idea, concept and design: HEE, OM, KB, KV, TA

Data collection and analysis: HEE, OM, KB, KV, TA

Drafting of the manuscript: HEE, OM, KB, KV, TA

Critical review: HEE, OM, KB, KV, TA

Data Availability:

The data of this study are available from the corresponding author upon reasonable request.

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