Turkish Veterinary Journal, 6(2): 56-60, 2024
DOI: 10.51755/turkvetj.1530723

Turkish Veterinary Journal

| tvj.cumhuriyet.edu.tr | Founded: 2019 Available online, ISSN: 2667-8292 Publisher: Sivas Cumhuriyet Üniversitesi

The Effect of Birth Type on Serum Irisin Levels in Awassi Lambs

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Research Article

History

Received: 09/08/2024
Accepted: 03/12/2024

Teşekkür
Bu çalışma Türkiye Bilimsel ve Teknolojik Araştırma Kurumu (TÜBİTAK) tarafından 1919B012333371 (2209-A) numaralı proje kapsamında desteklenmiştir.

ABSTRACT

Irisin is a thermogenic adipomyokine that plays a crucial role in energy metabolism, promoting the conversion of white adipose tissue to brown adipose tissue and contributing to metabolic health. The present study aims to reveal the effects of normal birth and dystocia on serum irisin levels in Awassi lambs. The study material consisted of 20 lambs born from Awassi sheep. Only sheep that had previously given birth normally, had no postnatal problems, and had single births were included to objectively evaluate the parameters under investigation. The newborn lambs were divided into two groups based on the type of birth. Group 1 (n=10) consisted of lambs born from normal births, while Group 2 (n=10) consisted of lambs born from dystocia. Blood samples were collected from the vena jugularis of lambs in both study groups before colostrum intake, after colostrum intake, and on the 15th, 30th, 45th, and 60th days of the postnatal period (a total of six times) and were then centrifuged. Serum irisin levels were assessed using a commercial kit. The obtained data were analyzed using two-way ANOVA. In the intergroup assessment, the serum irisin level in the dystocia group was found to be higher before colostrum intake ($P<0.001$). In the within-group assessment, significant time-dependent differences were observed in the measurements before colostrum intake, after colostrum intake, and during the postnatal period for both groups ($P<0.001$). Additionally, a significant difference was found in the group*time interaction for serum irisin levels ($P<0.001$). In conclusion, significant differences were observed between birth type and serum irisin levels, particularly before colostrum intake. It was concluded that administering colostrum, which plays a crucial role in neonatal nutrition, to lambs without delay, especially in cases of dystocia, ensures protection against diseases, promotes rapid recovery, and provides a healthy start for the newborns.

Keywords: Dystocia, irisin, lamb, newborn, normal birth

İvesi Irkı Kuzularda Doğum Şeklinin Serum İrisin Düzeylerine Etkisi

Süreç

Geliş: 09/08/2024
Kabul: 03/12/2024

Acknowledgement
This study was supported by the Scientific and Technological Research Council of Turkey (TÜBİTAK) under project number 1919B012333371 (2209-A).

ÖZ

Irisin, enerji metabolizmasında önemli bir rol oynayan termojenik bir adipomiyokindir; beyaz yağ dokusunun kahverengi yağ dokusuna dönüşümünü teşvik eder ve metabolik sağlığa katkıda bulunur. Sunulan çalışmada, İvesi ırkı kuzularda normal doğum ve güç doğumun serum irisin düzeyleri üzerindeki etkisini ortaya koymak amaçlanmıştır. Çalışma materyalini, İvesi ırkı koyunlardan doğan 20 adet kuzu oluşturdu. Çalışmada; alınan anamnezde önceki doğumlarını normal olarak yapmış, doğum sonrası herhangi bir problem şekillenmemiş ve bakılması düşünülen parametrelerin objektif değerlendirilebilmesi için sadece tekiz doğum yapan koyunlar çalışmaya dahil edildi. Doğan kuzular, doğum şekline göre 2 gruba ayrıldı. Grup 1 (n=10) normal doğum sonucu meydana gelen kuzulardan, Grup 2 (n=10) ise güç doğum sonucu meydana gelen kuzulardan oluştu. Her iki çalışma grubundaki kuzulardan; kolostrum alımı öncesinde, kolostrum alımı sonrasında ve postnatal dönemin 15., 30., 45. ve 60. günlerinde (toplam altı kez) vena jugularis'ten kan örneği alınarak santrifüj edildi. Serum İrisin düzeyleri ticari kit kullanılarak değerlendirildi. Elde edilen veriler iki yönlü varyans analizi ile analiz edildi. Grup içi değerlendirmede ise, her iki grup için kolostrum alımı öncesi, kolostrum alımı sonrası ve postnatal dönemin ölçümlerinde zamana bağlı anlamlı farklılıklar gözlemlendi ($P<0.001$). Ayrıca, serum irisin düzeyinde grup*zaman etkileşiminde de anlamlı farklılık bulundu ($P<0.001$). Sonuç olarak, doğum şekli ile serum İrisin düzeyi arasında özellikle kolostrum alımı öncesi önemli farklılıklar gözlendi. Yavru beslenmesinde önemli bir yeri olan kolostrumun, özellikle güç doğum olgularında zaman kaybetmeden yavrulara verilmesinin, yenidoğanların hastalıklara karşı korunmasını, hızlı toparlanmasını ve sağlıklı bir başlangıç yapmasını sağladığı sonucuna varıldı.

Anahtar Kelimeler: Güç doğum, irisin, kuzu, normal doğum, yenidoğan

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How to Cite: Akkus T, Yaprakci O, Bayazit R, Ekici M, Coskun A (2024) The Effect of Birth Type on Serum Irisin Levels in Awassi Lambs, Turkish Veterinary Journal, 6(2): 56-60

Introduction

In lambs, the neonatal period, which covers the first 28 days after birth, is a critical time during which the incidence of diseases and deaths is highest (Aydoğdu, 2016). Globally, the mortality rates in lambs (those born alive but dying before weaning) range from 8% to 30% on average (Dwyer et al., 2016). High neonatal mortality rates threaten the economic efficiency and sustainability of the global sheep farming industry (Shiels et al., 2022). This

period requires physiological, morphological, and behavioral changes to adapt to the extrauterine environment. Therefore, newborns are particularly vulnerable to diseases and mortality during the first week of life (Piccione et al., 2007). In the clinical monitoring of newborn lambs, correcting acid-base imbalances and maintaining thermal and metabolic homeostasis can play a crucial role in the early detection of adaptation failure (Camargo et al., 2012). The survival of the newborn is closely related to thermogenesis due to the significant decrease in body temperature that occurs during the transition from the warm uterine environment to the external environment. During birth, the control of body temperature in lambs is primarily dependent on heat production through the oxidation of brown adipose tissue (BAT), mediated by uncoupling protein-1 (UCP1) and endocrine factors (Vannucchi et al., 2012). Brown adipose tissue is the primary tissue responsible for non-shivering thermogenesis, increasing energy expenditure due to its high mitochondrial content and UCP1. In young lambs, the heat produced by BAT provides approximately half of the heat required by newborns, with the remaining heat produced through muscle thermogenesis (Zhang et al., 2019). The heat produced by BAT is essential for the survival of neonatal mammals in cold environments. However, it rapidly decreases in the postnatal period and is difficult to detect in adults (Kajimura et al., 2015).

Irisin is a thermogenic protein discovered in 2012, gaining recognition for its pivotal role in energy metabolism and thermogenesis. As primarily an adipomyokine, irisin is secreted not only by skeletal muscles but also by subcutaneous and visceral adipose tissues, reflecting its widespread physiological significance (Bayraktar & Tekce, 2021). The primary physiological role of irisin is to enhance energy expenditure by promoting the conversion of white adipose tissue into brown adipose tissue through circulation. This process is crucial, as brown adipose tissue is known for its ability to generate heat and facilitate fat breakdown, thereby contributing to weight management and metabolic health (Boström et al., 2012). Moreover, irisin exhibits various additional functions that underline its importance in maintaining overall health. For instance, it has been shown to reduce insulin resistance, which is vital for glucose homeostasis and preventing metabolic disorders such as type 2 diabetes. Furthermore, irisin positively influences musculoskeletal connections, enhancing muscle function and promoting overall physical performance (Yano et al., 2021). Recent studies have also suggested that irisin may play a critical role in thermoregulation mechanisms, particularly during periods of temperature fluctuation. Its presence in colostrum, the first milk produced after childbirth, is of particular interest, as it is believed to contribute to postnatal thermoregulation, assisting newborns in maintaining their body temperature during the critical hours following birth (Lidell & Enerbäck, 2010). Given these multifaceted roles, irisin has emerged as a promising target for further research, especially in understanding its potential therapeutic applications in obesity, metabolic disorders, and overall health maintenance.

This study was conducted to investigate the effects of birth type on serum irisin levels in Awassi lambs. A review of the literature found no studies evaluating the effects of normal and dystocic births on serum irisin levels in Awassi lambs. Therefore, this study is considered the first contribution to the literature on this topic.

Materials and Methods

This study was conducted with the permission of Harran University Animal Experiments Local Ethics Committee (HRU-HADYEK) (dated 16/05/2024 and numbered 2024/003).

Animal Material

The study material consisted of 20 Awassi lambs born on a private farm located in the Harran District of Şanlıurfa Province. The lambs were randomly selected from ewes aged 3-5 years, with an average weight of 56.73±5.16 kg, that had no issues with their reproductive systems and were kept under the same nutritional and management conditions. The ewes used in the study had given birth at least once, experienced normal births, and had no postnatal issues. Only those ewes that had single births were included in the study. When a decrease in rumination and separation from the flock were observed in the ewes a few days before parturition, they were placed in separate pens (2x2 m) to monitor their births.

Formation of Experimental Groups and Sample Collection

The lambs were divided into two groups based on the type of birth. Group 1 (n=10) consisted of lambs born from normal births (average weight 4.18±0.14 kg, 5 males, 5 females), while Group 2 (n=10) consisted of lambs born from dystocia (average weight 4.24±0.16 kg, 6 males, 4 females). When the duration of labor exceeded a total of 90 minutes or if no progress was observed for 30 minutes after the fetal membranes had ruptured, the situation was classified as dystocia. Ewes that gave birth on their own without any assistance were considered to have had a normal birth. Both groups of lambs were given 50 mL/kg (Nowak & Poindron, 2006) of colostrum within 1 hour after birth. For both groups of lambs, blood samples (10 mL each) were collected from the vena jugularis using a 20 G sterile syringe before colostrum intake, 1 hour after colostrum intake, and on the 15th, 30th, 45th, and 60th days of the postnatal period (a total of six times). The blood samples were transferred to tubes and centrifuged at 3000 rpm for 10 minutes to obtain serum. The resulting samples were stored at -20°C until analysis.

Laboratory Analyses

The serum irisin levels of the lambs in the study groups were assessed using the ELISA method (Sheep Irisin (IS) ELISA Kit, MBS9348152 [Measurement Range: 0.625 ng/mL - 20 ng/mL, Sensitivity: 0.1 ng/mL], MyBioSource, San Diego, USA) at the MEGATIP Laboratory in Gaziantep. Measurements were conducted according to the

manufacturer's instructions using the Molecular Devices SpectraMax M5 Plate Reader (Pleasanton, California, USA).

Statistical Analyses

The statistical analysis of the data was conducted using the Statistical Package for the Social Sciences (SPSS for Windows; version 26.0) software. The normality of the variables was assessed using visual methods (histograms and Q-Q plots) and analytical methods (Shapiro-Wilk tests). Descriptive analyses were reported as mean ± standard error of the mean (SEM) for variables that exhibited a normal distribution. To detect time-dependent changes within groups, the data were evaluated using a two-way ANOVA with repeated measures. In cases where the time factor or time*group interaction was significant, post-hoc analysis of time-dependent changes within each group was performed using additional coding in the General Linear Model (GLM) procedure. A p-value of <0.05 was considered statistically significant for all analyses.

Results and Discussion

The mean serum irisin levels for the study groups are provided in Table 1 and Figure 1. In the intergroup assessment, the serum irisin level in the dystocia group was found to be higher before colostrum intake (P<0.001). Although the serum irisin level was higher in the dystocia group after colostrum intake and on the 15th, 30th, 45th, and 60th days of the postnatal period, this difference was not statistically significant (P>0.05). In the within-group assessment, significant time-dependent differences were observed in the measurements before colostrum intake, after colostrum intake, and during the postnatal period for both groups (P<0.001). However, no differences were observed on the 15th and 30th days of the postnatal period (P>0.05). Additionally, a significant difference was found in the group*time interaction for serum irisin levels (P<0.001).

Table 1. Mean serum irisin levels for the study groups

Days	IRISIN (ng/mL)				P_{ANOVA} (Group)
	Normal Birth		Dystocia		
	n	$\bar{X} \pm SEM$	N	$\bar{X} \pm SEM$	
BCI	10	$1.516 \pm 0.372^{A,a}$	10	$2.037 \pm 0.410^{B,a}$	**<0.001**
ACI	10	$1.252 \pm 0.021^{A,b}$	10	$1.292 \pm 0.009^{A,b}$	0.107
15	10	$1.003 \pm 0.032^{A,c}$	10	$1.045 \pm 0.045^{A,c}$	0.463
30	10	$0.955 \pm 0.024^{A,c}$	10	$1.028 \pm 0.029^{A,c}$	0.071
45	10	$0.809 \pm 0.013^{A,d}$	10	$0.856 \pm 0.024^{A,d}$	0.110
60	10	$0.751 \pm 0.012^{A,e}$	10	$0.784 \pm 0.012^{A,e}$	0.082
P_{ANOVA} (Time)		<0.001		<0.001	
P value (Group*Time)		<0.001		<0.001	

a, b, c, d, e: Different letters within the same column represent time-dependent statistical differences within the group. A, B: Different letters within the same row represent time-dependent statistical differences between groups. ANOVA: analysis of variance, SEM: standard error of the mean, BCI: Before colostrum intake, ACI: After colostrum intake

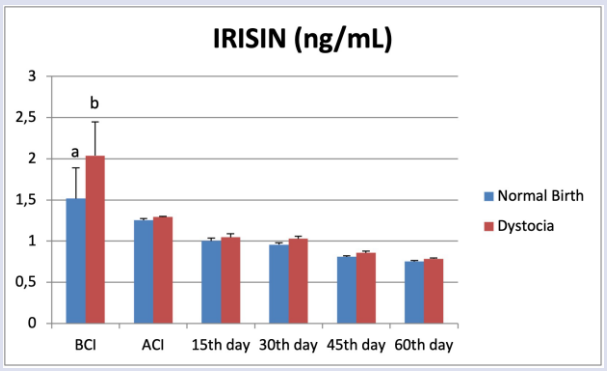

Figure 1. Mean serum irisin levels for the study groups. BCI: Before colostrum intake, ACI: After colostrum intake. a, b: Different letters indicate time-dependent statistical differences within the group

Irisin performs several functions, including converting white adipose tissue into brown adipose tissue, reducing insulin resistance, and improving musculoskeletal connections. Brown adipose tissue is crucial for thermogenesis during the neonatal period, thereby aiding the survival of the newborn (Ojha et al., 2013). It rapidly generates heat by increasing energy expenditure, providing protection against hypothermia that may occur in the extrauterine environment (Asakura, 2004; Cannon & Nedergaard, 2004). Therefore, irisin may play a significant role in the thermoregulation mechanisms of newborns, and its levels during the postnatal period could contribute to thermoregulation. Our study, as the first to evaluate the effect of birth type on serum irisin levels in lambs, provides valuable data that will contribute to elucidating this relationship. Additionally, serum irisin levels were measured and compared at different time points after both normal birth and dystocia.

The activity of irisin and serum concentration vary depending on physiological and/or pathological conditions (Korta et al., 2019). Kızıl et al. (2022), in their study evaluating irisin levels in calves based on birth type, found higher serum irisin levels in the dystocia group compared to normal births. Consistent with the literature, our study also found higher serum irisin levels in the dystocia group. High serum irisin levels in the dystocia group may indicate increased stress and muscle activity during birth. In the normal birth group, lower serum irisin levels may suggest that normal birth induces metabolic stress and energy expenditure to a lesser extent (Blum &

Hammon, 2000). Before colostrum intake in our study, serum irisin levels in the dystocia group were significantly higher compared to the normal birth group. This suggests that dystocia may lead to greater stress and energy expenditure on the fetus, potentially increasing irisin release. Dystocia can result in increased neonatal stress and various biochemical changes (Singh et al., 2009). Irisin, a hormone secreted by muscles that regulates energy metabolism, is known to increase its secretion under stress (Joro et al., 2021). In this context, stress during birth may elevate irisin levels, and this increase is particularly pronounced in cases of dystocia. In our study, serum irisin levels were highest in the first days after birth and decreased over time. This may be related to the postnatal metabolic adaptation process. Differences in serum irisin levels before and after colostrum intake highlight the impact of nutrition and energy intake on irisin levels (Vannucchi et al., 2012). A significant decrease in irisin levels was observed after colostrum intake, suggesting that colostrum's energy density and nutritional content stabilize the metabolic state. After colostrum intake and on the 15th, 30th, 45th, and 60th days of the postnatal period, similar trends in serum irisin levels were observed in both groups. Although irisin levels were higher in the dystocia group, these differences were not statistically significant. This finding indicates that both groups underwent similar recovery processes during the postnatal period, and this process did not lead to substantial differences in irisin levels (Blum & Hammon, 2000).

In the presented study, within-group evaluations revealed significant time-dependent differences between pre-colostrum intake and post-colostrum intake, as well as on different days of the postnatal period (15th, 30th, 45th, and 60th days) for both groups. This finding indicates that the irisin hormone plays a significant physiological role during the postnatal period and exhibits changes over time. However, the absence of differences on the 15th and 30th days of the postnatal period suggests that the metabolic state during these times is stable (Huh et al., 2012).

Additionally, in our study, a significant difference was found in the group*time interaction for serum irisin levels. This finding suggests that both the type of birth and time influence irisin levels, and this interaction may differ for each group. Specifically, the serum irisin levels in both groups change differently over time. This suggests that dystocia may have a longer-lasting impact on irisin levels. The difference in serum irisin levels over time between the normal birth and dystocia groups is important for understanding the long-term metabolic effects of birth type. Specifically, the observation that lambs born from dystocia exhibit high irisin levels in the first days after birth indicates that the difficulty of the birth increases the metabolic burden (Boström et al., 2012).

Considering the effects of irisin on energy homeostasis and thermoregulation, it is thought that the levels of this hormone in the postpartum period may contribute to the adaptation process of newborn lambs. The high levels of irisin following dystocia support the possible role of this hormone in stress and adaptation mechanisms. However, the lack of statistical significance in these differences during the post-colostrum intake periods suggests that postnatal care and nutrition may balance irisin levels (Aladag et al., 2023). Studies evaluating the effects of birth type on irisin levels are limited in the literature. Therefore, our findings are believed to provide significant contributions to the existing literature. Understanding the changes in serum irisin levels based on the birth type of İvesi lambs can help in developing strategies related to the breeding and management of this breed. Additionally, understanding the role of irisin during the postnatal period can contribute to the development of potential intervention methods to enhance the health and welfare of newborn lambs.

Conclusion

In conclusion, this study has shown that birth type has significant effects on serum irisin levels in Awassi breed lambs, particularly indicating that dystocia can increase serum irisin levels. However, it also highlights that these differences can be balanced over time through postnatal care and nutrition. To better understand the effects of birth type on irisin levels and their long-term impacts on metabolic health, comprehensive and long-term studies should be conducted.

Conflict of Interest

The authors declare that there is no conflict of interest.

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