

The relation of fat tissue hormones and some galactopoietic hormones with milk yield in Holstein and Simmental cows

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Abstract: The present study was conducted to investigate the physiological changes in leptin, adiponectin, and ghrelin hormones, which are effective in the regulation of energy metabolism, and the galactopoietic hormones including growth hormone (GH), insulin, insulin-like growth factor-I (IGF-I), cortisol, thyroid stimulating hormone (TSH) and thyroxine (T_4) levels, which are also effective in the regulation of metabolic activities and their correlation with milk yield levels in Holstein and Simmental cows with low, medium, and high milk yield. Holstein (n = 30) and Simmental cows (n = 30) with low, medium, and high milk yield were used. Cows were divided into 6 groups with 10 animals in each group, and body condition scores (BCS) were determined. Serum GH, insulin, IGF-I, cortisol, TSH, T_4 , leptin, adiponectin, and ghrelin levels were measured. GH level increased in both breeds ($p < 0.05$). As a result, in cases when the energy requirement increases during lactation without reaching a critical point, the GH, cortisol, and ghrelin hormone levels increase, while the plasma T_4 level decreases and insulin, IGF-I, TSH, leptin, and adiponectin levels remain unchanged. An interaction between leptin, adiponectin, and ghrelin and galactopoietic hormones was noted.

Key words: Adiponectin, growth hormone, ghrelin, leptin, milk yield

1. Introduction

The main objective of animal husbandry is to obtain the highest possible product from the animal. However, if the yield level exceeds the metabolic reserve capacity of the animal, this causes metabolic disorders. A problem-free metabolism is the main factor affecting the sustainability of the milk production of the cow during lactation. The continuation of milk production is defined as galactopoiesis. Galactopoietic hormones (growth hormone, insulin, insulin-like growth factor-I, thyroid hormones, and cortisol) and regular milking are necessary for continuation of lactation. Due to its importance in milk production, the decrease in galactopoietic hormones suppresses the milk production [1].

The lactation period in dairy cows is a period in which significant changes in energy metabolism occur, and the total energy demand increases four-fold [2]. During this period, the increased energy needs are met by physiological adaptation mechanisms such as increasing feed consumption and use of body stores and increasing the metabolic activity through thyroid hormones. The central nervous system (CNS) plays an important role in this process [3]. Growth hormone (GH), insulin, cortisol, insulin-like growth

factor-I (IGF-I), thyroid-stimulating hormone (TSH), and thyroxine (T_4) are effective in galactopoiesis, and they are metabolic hormones involved in energy/protein homeostasis together with leptin, adiponectin, and ghrelin [4]. The circulating concentrations of these metabolic hormones provide important clues regarding the adequacy of feeding and the metabolic status of the animal.

White fat tissue is the largest energy store in the body and is very important for energy homeostasis. White adipose tissue regulated by numerous hormonal signals, nuclear hormone receptors, and the central nervous system has recently been recognized as an important endocrine organ that secretes hormones called adipokines. Leptin and adiponectin, the main adipokines secreted from white fat tissue, are in protein structure and are associated with energy balance, and they are the hormones that directly or indirectly affect the glucose and lipid metabolism by insulin signaling [5]. There is a small number of studies reflecting the relationship between the milk yield level and physiological changes of hormones that are effective in energy metabolism.

In this study, we aimed to determine the physiological changes in leptin, adiponectin and ghrelin hormones,

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which are effective in the regulation of energy metabolism and the physiological changes in thyroid hormones (TSH and T_4), growth hormone (GH), insulin, cortisol, insulin-like growth factor-I (IGF-I), which are effective in the regulation of metabolic activities and the relationship between these physiological changes and low, medium and high milk yield of Holstein and Simmental cows during lactation periods. The results of this study will contribute to the prevention of problems related to nutrition and metabolic diseases, which cause serious economic losses in milk yield.

2. Materials and methods

2.1. Animal materials

In the study, 4-5 years-old Holstein ($n = 30$) and Simmental ($n = 30$) breed cows were used; their body weights were between 520 and 610 kg. The cows were grown under the same care conditions by free breeding system supplying a total mix of clover, corn silage, and milk feed (Dry Matter: 67%, Crude Protein: 14-19%, NEL: 1.2-1.72 Mcal / kg). The cows were milked twice a day. For this purpose, a special milk production facility was used in the province of Aksaray. The study was carried out in accordance with the code of ethics (2017/10), with the approval of the Local Ethics Committee of the Animal Experiments of Van Yüzüncü Yıl University.

2.2. The study groups

Holstein and Simmental cows with low, medium, and high milk yields compatible with the milk yields of their lactation periods (low milk yield: the 305-280th days of lactation; medium milk yield: the 280-140th days of lactation; and high milk yield: the 140-70th days of lactation) were divided into 6 independent groups detailed below.

Each group included 10 of dairy cows.

Group 1 : The Holstein cows with low milk yield (average daily milk, 18.00 ± 0.67 kg) and on the 305-280th days of lactation ($n = 10$)

Group 2 : The Holstein cows with medium milk yield (average daily milk, 24.00 ± 1.09 kg), and on the 280-140th days of lactation ($n = 10$).

Group 3 : The Holstein cows with high milk yield (average daily milk, 32.00 ± 1.15 kg) and on the 140-70th days of lactation ($n = 10$).

Group 4 : The Simmental cows with low milk yield (average daily milk, 17.30 ± 0.79 kg) and on the 305-280th days of lactation ($n = 10$).

Group 5: The Simmental cows with medium milk yield (average daily milk, 22.60 ± 0.95 kg), and on the 280-140th days of lactation ($n = 10$)

Group 6: The Simmental cows with high milk yield (average daily milk, 29.9 ± 0.96 kg), and on the 140-70th days of lactation ($n = 10$)

2.3. Determination of body condition scores

Body condition scores of the animals in the dry period (time of delivery) were determined as 1-5 points according to Rodenburg [6]. Accordingly, score 1 was considered as 'very thin' and score 5 was considered as 'too fat'.

2.4. Blood sampling and hormone analysis

Blood samples (10 mL) were drawn from V. Jugularis of all animals into anticoagulant-free vacutainer tubes. The blood samples were incubated for 30 min at room temperature for clotting and then centrifuged at 3000 RPM for 15 min. The sera obtained were stored at -80 °C temperature until the analysis. Growth hormone (YL Biont, catalog no: YLA0335BO), insulin (YL Biont, catalog no: YLA0041BO), IGF-I (YL Biont, catalog no: YLA0052BO), cortisol (YL Biont, catalog no: YLA0060BO), TSH (YL Biont, catalog no: YLA0132BO), T_4 (YL Biont, catalog no: YLA0079BO), leptin (YL Biont, catalog no: YLA0083BO), adiponectin (SunRed catalog no:201-04-0211) and ghrelin (YL Biont, catalog no: YLA0105BO) levels were determined using ELISA kits.

2.5. Statistical analysis

Body condition scores, average milk yields, serum levels of galactopoietic hormones, and hormones that were effective in the regulation of energy metabolism (leptin, adiponectin, and ghrelin) were compared between the different study groups using the one-way ANOVA test. The Duncan multiple test was used to determine the statistically significant differences. The Pearson's correlation analysis was used to test the correlation between BCS and hormone levels in low, medium, and high milk yield cows. Statistical analyses were performed using the SAS 9.4 statistical software program.

3. Results

3.1. Milk yield levels and body condition score

The body condition scores (BCS) in dry period and milk yields of Holstein and Simmental cows according to lactation periods have been presented in Table 1.

The mean milk yields of Holstein cows according to the lactation periods were 18.00 ± 0.67 kg/day (low milk yield), 24.00 ± 1.09 kg/day (medium milk yield), and 32.00 ± 1.15 kg/day (high milk yield), while it was 17.30 ± 0.79 kg/day (low milk yield), 22.60 ± 0.95 kg/day (medium milk yield), and 29.90 ± 0.96 kg/day (high milk yield) in Simmental cows. The difference between the milk yield levels in the same breeds was statistically significant ($p < 0.05$), whereas there was no significant difference in the milk yield levels between the two breeds.

In Holstein cows with low, medium, and high milk yield, the BCSs were 3.95 ± 0.050 , 3.85 ± 0.076 , and 3.65 ± 0.107 points, respectively, while they were 4.4 ± 0.067 , 4.35 ± 0.076 , and 4.30 ± 0.082 points, respectively in Simmental

cows with low, medium, and high milk yield. BCS was significantly lower in Holstein cows with high (32.00 ± 1.15 kg/day) compared to those with low milk yield (18.00 ± 0.67 kg/day) ($p < 0.05$). There was no statistically significant difference in BCS between in Simmental cows with different milk yield levels. When compared the Holstein and Simmental cows in terms of BCS values, it was found that the BCS values of the Holstein were lower than those of the Simmental ($p < 0.05$).

3.2. Galactopoietic hormones and hormones effective in regulation of energy metabolism

The physiological levels of galactopoietic hormones and hormones which are effective in regulating energy

metabolism in Holstein and Simmental cows with low, medium, and high milk yield have been presented in Table 2.

The plasma GH levels in Holstein cows with low, medium, and high milk yield were 3.129 ± 0.128, 2.522 ± 0.088, and 2.780 ± 0.223, respectively, while those were 3.014 ± 0.223, 2.450 ± 0.091, and 3.079 ± 0.168, respectively, in the Simmental cows. The growth hormone levels were high in Holstein cows with low milk yield and Simmental cows with low and high milk yield ($p < 0.05$). There was no significant difference between the Holstein and Simmental cows in terms of plasma GH levels. The plasma cortisol levels in Holstein cows with low, medium, and high milk yield were 0.958 ± 0.027, 0.887 ± 0.017, and 0.905 ± 0.012,

Table 1. Milk yield levels (low, medium and high) and dry body condition scores of Holstein and Simmental cows according to lactation periods

	Holstein			Simmental		
	Low (305–280th days)	Medium (280–140th days)	High (140–70th days)	Low (305–280th days)	Medium (280–140th days)	High (140–70th days)
ADMY kg/day	18.00 ± 0.671 ^c	24.00 ± 1.09 ^b	32.00 ± 1.15 ^a	17.30 ± 0.792 ^c	22.60 ± 0.954 ^b	29.90 ± 0.959 ^a
BCS	3.950 ± 0.050 ^{Ba}	3.850 ± 0.076 ^{Bab}	3.650 ± 0.107 ^{Bb}	4.400 ± 0.067 ^A	4.350 ± 0.076 ^A	4.300 ± 0.082 ^A

ADMY: Average daily milk yield, kg/day; BCS: Body condition score; ^{ab}: The differences between the averages shown with different letters in the same row and in the same breed groups are significant ($p < 0.05$). ^{A,B}: The differences between the averages shown with different letters in the same row and in the different breed groups are significant ($p < 0.05$).

Table 2. Physiological values of galactopoietic hormones and the hormones (leptin, adiponectin and ghrelin) effective in the regulation of energy metabolism with in Holstein and Simmental cows at different milk yield levels according to lactation periods.

Hormones	Holstein			Simmental		
	Low (305–280th days)	Medium (280–140th days)	High (140–70th days)	Low (305–280th days)	Medium (280–140th days)	High (140–70th days)
GH (ng/mL)	3.129 ± 0.128 ^a	2.522 ± 0.088 ^b	2.780 ± 0.223 ^{ab}	3.014 ± 0.223 ^x	2.450 ± 0.091 ^y	3.079 ± 0.168 ^x
Insulin (mIU / L)	3.185 ± 0.051	3.102 ± 0.060	3.378 ± 0.198	3.357 ± 0.126	3.280 ± 0.110	3.375 ± 0.099
IGF-I (ng/mL)	6.157 ± 0.251	6.051 ± 0.331	6.057 ± 0.310	6.200 ± 0.316	5.945 ± 0.242	6.136 ± 0.185
Cortisol (µg/mL)	0.958 ± 0.027 ^a	0.887 ± 0.017 ^b	0.905 ± 0.012 ^{ab}	1.024 ± 0.078	0.907 ± 0.027	0.897 ± 0.018
TSH (mIU / L)	1.670 ± 0.176	1.854 ± 0.113	1.700 ± 0.148	1.812 ± 0.126	1.799 ± 0.107	1.789 ± 0.130
T4 (ng/mL)	28.954 ± 1.277 ^{Aa}	26.254 ± 1.679 ^{ab}	23.948 ± 1.020 ^b	23.307 ± 1.240 ^B	28.250 ± 2.672	27.992 ± 2.132
Leptin (ng/mL)	0.378 ± 0.021	0.332 ± 0.019	0.361 ± 0.027	0.411 ± 0.039	0.358 ± 0.040	0.375 ± 0.020
Adiponectin (µg/mL)	9.195 ± 0.763	8.281 ± 0.513	8.915 ± 0.692	10.492 ± 0.981	9.030 ± 0.539	8.994 ± 0.715
Ghrelin (ng/mL)	167.172 ± 15.583	144.669 ± 7.576	162.451 ± 7.474	180.404 ± 15.949 ^x	140.733 ± 8.973 ^y	154.124 ± 7.363 ^{xy}

GH: Growth hormone; IGF-I: Insulin-like growth factor I; TSH: Thyroid stimulating hormone; T4:Thyroxine. ^{ab;xy}: The differences between the averages shown with different letters in the same row and in the same breed groups are significant ($p < 0.05$). ^{A,B}: The differences between the averages shown with different letters in the same row and in the different breed groups are significant ($p < 0.05$). ^{A,B}: In the same row, the differences between the averages shown with different letters at the same yield levels in different breed groups are significant ($p < 0.05$).

respectively, while they were 1.024 ± 0.078 , 0.907 ± 0.027 , and 0.897 ± 0.018 , respectively, in Simmental cows.

The plasma cortisol level was significantly higher in Holstein cows with low and high milk yield ($p < 0.05$); it tended to be high numerically in the low milk yielding Simmental cows. There was no significant difference between the Holstein and Simmental cows in terms of plasma cortisol levels.

The plasma T_4 hormone levels were 28.954 ± 1.277 , 26.254 ± 1.679 , and 23.984 ± 1.020 in Holstein cows with low, medium, and high milk yield levels, respectively, while they were 23.307 ± 1.240 , 28.250 ± 2.672 , and 27.992 ± 2.132 , respectively, in Simmental cows. It was observed that the plasma T_4 levels decreased in Holstein cows with high milk yield ($p < 0.05$), and this level increased while the milk yield decreased ($p < 0.05$). In Simmental cows, there was no significant difference in the plasma T_4 levels between the cows with different milk yield categories. The plasma T_4 levels were higher in Holstein cows with low milk yield compared to Simmental cows with low milk yield in the same period ($p < 0.05$).

The plasma ghrelin levels in Holstein cows with low, medium and high milk yield levels were 167.172 ± 15.583 , 144.669 ± 7.576 , and 162.451 ± 7.474 , respectively, while they were 180.404 ± 15.949 , 140.733 ± 8.973 , and 154.124 ± 7.363 , respectively in Simmental cows. The ghrelin level was significantly higher in the Simmental cows with low and high milk yield ($p < 0.05$), whereas it tended to be high numerically in the Holstein. There was no significant difference between the Holstein and the Simmental cows in terms of plasma ghrelin levels. The plasma insulin, IGF-I, TSH, leptin, and adiponectin levels were not significantly different between the two breeds, and they were not statistically significant between the different milk yield categories of the same breed either.

The correlations between hormones in the low, medium, and high milk yield categories regardless of the cow breeds have been presented in Tables 3, 4, and 5.

The plasma leptin levels were positively correlated with insulin ($r = 0.95$, $r = 0.85$, $r = 0.84$; $p < 0.01$, for all) and the GH level ($r = 0.57$, $r = 0.53$, $r = 0.89$; $p < 0.01$, for all) in low, medium, and high milk yield cows regardless of the breed. The plasma adiponectin levels were positively correlated with the leptin level ($r = 0.71$, $r = 0.58$, $r = 0.67$; $p < 0.01$, for all) and the insulin level ($r = 0.63$, $r = 0.62$, $r = 0.67$; $p < 0.01$, for all) in each milk yield category.

The body condition scores of the cows did not show a statistically significant correlation with either the plasma leptin level or the adiponectin level in the low, medium, and high milk yield categories regardless of the breed. There was a negative correlation between the plasma ghrelin levels and BCS in cows with high milk yield ($r = -0.44$, $p < 0.05$) (Table 5).

4. Discussion

The onset of milk secretion with delivery is defined as 'lactation', and the continuation of lactation is defined as 'galactopoiesis'. The energy need of the organism increases, and significant changes occur in the energy metabolism during the lactation period. In this period, the increased energy demand is met by increased feed consumption and physiological adaptation mechanisms such as the use of body energy stores. Recently discovered leptin and adiponectin are hormones secreted from adipose tissue and ghrelin secreted from the stomach, and they all play an important role in the regulation of energy balance. The galactopoietic hormones are the necessary hormones for continuation of milk production, which include GH, insulin, IGF-I, cortisol, TSH, and T_4 . For galactopoiesis, it is also necessary to maintain the milk secretion reflex, to maintain the number of alveolar cells and their synthesis capabilities. The milk yield in animals of the same and different breeds is affected by many genetic and environmental factors during lactation, which increases after delivery, persists at a high level for 2–8 weeks and then gradually decreases. In this study, after categorization of Holstein and Simmental cows as low, medium, and high milk yield groups according to the lactation periods, the dry period BCS (parturition) and the relationship between BCS and the milk yield levels were investigated. In addition, both of the physiological values of galactopoietic hormones and the hormones (leptin, adiponectin and ghrelin) effective in regulating energy metabolism and their correlation with milk yield levels were also investigated.

The body condition scores was lower in the Holstein compared to the Simmental. Cows of the same breed were compared according to milk yield levels in terms of BCS; BCS was higher in low milk yield Holstein cows, but lower in high milk yield cows ($p < 0.05$) (Table 1). On the other hand, there was no significant difference in BCSs between different milk yield categories of Simmental cows. The body condition of the cow at the time of delivery significantly affects the milk yield. A good body condition gained during this period ensures the healthy onset of milk secretion after delivery and the safe continuation of lactation. Rodenburg [6] reported that the ideal BCS was 3.0–4.0 in cows during the dry period and calving. Mishra et al. [7] reported that the optimal BCS in the dry period was 3.0 to 3.75, while some researchers have suggested that the ideal BCS should be 3.0–3.25 during this period and the milk yield is negatively affected in cows with BCS of 3.5 and above at parturition [8]. It is known that Holstein cows genetically have better milk yield than Simmental cows [9]. BCS of cows of the same and different breeds with higher milk yield was lower than that of genetically low milk yield cows. Cows with genetically high milk yield capacity are

Table 3. Correlations between hormone levels and body condition score (BCS) studied in low milk yielding cows.

	Cortisol	Leptin	Insulin	IGF-I	Adiponectin	Ghrelin	T4	TSH	GH	BCS
Cortisol		0.95148**	0.94452**	0.88205**	0.65997**	0.71979**	0.19920	0.01775	0.63612**	0.35987
Leptin			0.95024**	0.93280**	0.71072**	0.81043**	0.24909	0.03056	0.57975**	0.32971
Insulin				0.82492**	0.63048**	0.81451**	0.14495	-0.02981	0.66435**	0.41337*
IGF-I					0.75342**	0.75528**	0.26078	-0.03898	0.46541*	0.17126
Adiponectin						0.57709**	-0.02199	0.35615	0.26420	0.15426
Ghrelin							0.30040	-0.13495	0.54225*	0.29624
T4								-0.27732	0.11756	-0.31632
TSH									-0.25650	0.00620
GH										0.03171
BCS										1

IGF-I: Insulin-like growth factor I; T4:Thyroxine; TSH: Thyroid stimulating hormone; GH: Growth hormone; BCS: Body condition score; * : Correlation is significant at the $p < 0.05$ level; ** : Correlation is significant at the $p < 0.01$ level.

Table 4. Correlations between hormone levels and body condition score (BCS) studied in medium milk yielding cows.

	Cortisol	Leptin	Insulin	IGF-I	Adiponectin	Ghrelin	T4	TSH	GH	BCS
Cortisol		0.79855**	0.60947**	0.57148**	0.45536*	0.51181*	-0.07210	-0.13422	0.60081**	0.35927
Leptin			0.84866**	0.71126**	0.58129**	0.54228**	-0.08763	-0.14589	0.53240**	0.38344
Insulin				0.74403**	0.62255**	0.68445**	0.02813	-0.12633	0.36877	0.45415*
IGF-I					0.73613**	0.65863**	-0.10703	-0.07839	0.76840**	0.18405
Adiponectin						0.39798*	-0.42891	-0.26697	0.63578**	0.25544
Ghrelin							0.06743	-0.01397	0.40985*	0.06696
T4								0.21706	-0.25765	0.13530
TSH									-0.17787	0.08778
GH										0.19230
BCS										1

IGF-I: Insulin-like growth factor I; T4:Thyroxine; TSH: Thyroid stimulating hormone; GH: Growth hormone; BCS: Body condition score; * : Correlation is significant at the $p < 0.05$ level; ** : Correlation is significant at the $p < 0.01$ level.

more prone to use body fat stores to maintain high milk production. This explains the difference between the milk yield levels of different breeds in the same lactation period.

The growth hormone level was high in the low milk yield category in Holstein cows and in the low and high milk yield categories in the Simmental ($p < 0.05$). There was no significant difference between the Holstein cows and the Simmental cows in terms of plasma GH levels. The growth hormone, which is accepted as the main galactopoietic hormone in ruminants, is responsible for the initiation and continuation of lactation and shows its effect by increasing the nutrient flow to the mammary gland and stimulating the synthesis of IGF-I in the liver [10]. Increased plasma GH levels were reported in high-

yielding dairy cows and malnourished ruminants, while the GH level did not change significantly in balanced fed animals [11]. In present study, Holstein and Simmental cows demonstrated a low milk yield during the middle and last lactation period and Simmental cows displayed a high milk yield during the peak dry matter period, which may be associated with high plasma GH levels and increased energy and nutrient requirements.

The last 3 weeks of gestation is the period in which fetal development is the fastest and when colostrum production begins. The energy demand that starts to increase in this period continues in the postpartum period together with the increase in milk yield [12]. While the rumen volume decreases due to the pregnant uterus, the consumption

Table 5. Correlations between hormone levels and body condition score (BCS) studied in high milk yielding cows.

	Cortisol	Leptin	Insulin	IGF-I	Adiponectin	Ghrelin	T4	TSH	GH	BCS
Cortisol		0.30442	0.23175	0.15844	0.41127*	0.20531	-0.04596	-0.37915	0.25095	-0.08648
Leptin			0.83839**	0.90720**	0.67420**	0.55084**	0.09821	-0.03801	0.89245**	0.06169
Insulin				0.86593**	0.75944**	0.38025	0.20098	-0.02204	0.77314**	0.04635
IGF-I					0.65970**	0.49631*	0.05854	-0.05187	0.83166**	0.03218
Adiponectin						0.52966**	0.11286	-0.03895	0.64110**	-0.08261
Ghrelin							-0.19641	0.03013	0.45370*	-0.44865*
T4								-0.12890	0.28121	0.37268
TSH									-0.11027	0.24048
GH										0.23623
BCS										1

IGF-I: Insulin-like growth factor I; T4:Thyroxine; TSH: Thyroid stimulating hormone; GH: Growth hormone; BCS: Body condition score; * : Correlation is significant at the $p < 0.05$ level; ** : Correlation is significant at the $p < 0.01$ level.

of dry matter and the decrease in the absorption capacity of rumen papillae cannot be met by ration, resulting in an energy deficiency. In the case of insufficient energy consumption, the body reserves begin to be used as an energy source [13]. On the other hand, the energy requirement that starts to increase in the middle and last lactation period continues in the postpartum period by the increase in milk yield [12]. During the high milk yield period, which is the postpartum 5th to 14th week, being an average 8 weeks, 30% of the body reserves are used for milk synthesis and negative energy balance occurs [14]. In present study, increased energy and nutrient needs were attempted to be fulfilled by increased GH levels in cows with low and high milk yield categories. An elevated GH level after delivery increases the lipolysis in adipose tissue and gluconeogenesis in the liver by showing opposite effects to insulin [15]. The growth hormone stimulates the mobilization of nutrients in the peripheral tissues and, thus, directs them to the mammary gland. Block et al. [16] reported that the plasma GH level increases and the insulin level decreases when the lactating cows are fed to fulfil 33% of their nutritional needs. Insulin exhibits the opposite effects of GH. It induces glycogenolysis and gluconeogenesis in the liver, suppresses the lipolysis in fat tissue, and promotes lipogenesis [15]. As a result of this insulin effect, the glucose production is suppressed, and the glucose consumption in peripheral tissues is increased [17]. The plasma level of insulin, which is effective in the regulation of energy metabolism, decreases with the onset of lactation and remains low throughout the lactation. This is an adaptation of the organism to cope with the increased need for energy starting with lactation [18]. Since insulin promotes the entrance of glucose into cells, a decrease in the plasma level of insulin results in a

limitation of glucose entry into tissues and an increase in plasma glucose levels. On the other hand, glucose inflow in the lactating mammary gland is independent of insulin. During lactation, high GH levels and low plasma insulin levels support gluconeogenesis and lipolysis, enabling the mobilization of nutrients from body reserves [19]. The growth hormone provides the coordination of tissue metabolism to promote the flow of nutrients and energy from other tissues to the mammary gland and its use in milk synthesis [20]. Genetically high milk yield Holstein cows have been reported to have low plasma insulin levels [21]. In present study, no significant difference between the Holstein and Simmental cows in terms of plasma insulin levels was observed. There was no difference between the milk yield categories in terms of plasma insulin levels either. This result supported that the diminished plasma insulin level, which decreases with the onset of lactation and remains low during lactation, is an adaptation mechanism to cope with increased energy requirements, reported by Wathes et al. [18] and Djkovic et al. [19].

Cortisol is one of the hormones that are effective in glucose metabolism in ruminants. High cortisol levels are required for gluconeogenesis. Plasma cortisol levels are elevated in malnourished ruminants [17]. Glucocorticoids have a two-way effect on the regulation of energy balance. Despite its catabolic effect in the peripheral tissues, it increases the intake of nutrients in the central nervous system. In the current study, a statistically significant increase in plasma cortisol levels was observed in Holstein cows with low and high milk yield, whereas the numerical plasma cortisol levels in Simmental cows tended to increase, suggesting that the food intake was inadequate. Indeed, elevated plasma cortisol levels have been reported to regulate the milk yield via gluconeogenesis [22].

Negative energy balance develops in malnourished cows during lactation. In the presence of NEB, the plasma GH, IGF-I, and the ghrelin levels increase, while the plasma leptin, adiponectin, and insulin levels decrease [23]. Kurose et al. [24] reported that energy restriction increases the plasma ghrelin levels. The plasma ghrelin level, regulated by caloric intake, increases in the fasting state and decreases in satiety [25]. Increased secretion of ghrelin in the case of fasting is thought to be a fasting signal that enforces food intake. The plasma ghrelin levels have been reported to be at low levels as long as the balance between energy intake and expenditure is maintained in ruminants, and it increases in the presence of NEB [26]. In the present study, a high plasma GH levels in Holstein and Simmental cows with low milk yield was noted. The plasma ghrelin level's tendency to increase numerically in Holstein cows and having statistically increased in Simmental cows supports this report. The increased plasma ghrelin levels detected in cows with low milk yield compared to those with high milk yield support our opinion that energy deficiency has developed due to inadequate nutrient intake.

Studies with rats and humans show that ghrelin is the most potent secretor of GH [27]. Roche et al. [23] also reported that ghrelin produced in auxinric cells in the abomasum of ruminants binds to growth hormone-releasing receptors and acts as a growth hormone-releasing hormone to increase GH secretion. The appetite-enhancing effect of ghrelin is independent of its effects on GH and occurs via the effect of leptin [28]. No significant differences were found in plasma leptin and ghrelin levels between the two cow breeds with different milk yield levels. On the other hand, positive correlations between plasma leptin and ghrelin levels in cows with low, medium, and high milk yield support the previous reports regarding ghrelin hormone, which is well explained in humans and rhodates, but there is scarce information about its release and effects in ruminants.

One of the hormones that are reported to be essential for galactopoiesis is IGF-I, which is synthesized in the liver under the influence of GH and mediates the functions of GH through autocrine and paracrine routes. Inadequate protein or energy intake leads to a decrease in IGF-I levels by decreasing the number of GH-receptors in liver cell membranes [11]. In this study, it was determined that high plasma GH, ghrelin, and cortisol levels detected in Holstein and Simmental cows with low milk yield are associated with low energy intake. In this case, the plasma IGF-I levels are expected to be low, but we did not detect a significant difference in the plasma IGF-I levels in the current study. This can be explained by the fact that the energy deficiency, which is thought to occur in cows with low milk yield, was not so serious. Breier [11] and Fenwick

et al. [29] reported that in the presence of a significant negative energy balance, there were significant changes occurring in IGF levels as a result of the down-regulation of hepatic GH-receptors together with GH levels and GH-resistance in the liver tissue.

In the present study, the plasma T_4 hormone concentrations were lower in Holstein cows with high milk yield compared to those with medium and low milk yield ($p < 0.05$). No significant difference was found between the different milk yield categories in Simmental cows. This result was consistent with previous studies reporting lower T_4 levels in early lactation compared to those of late gestation and mid-lactation periods (19, 30). In our study, the Holstein cows that have high milk yield during the lactation period have mobilized their body reserves for milk yield. Kasagic et al. [30] suggested that hypothyroidism developed due to mobilization of the body stores in cows during early lactation and the high milk yield period. Peripheral tissues try to regulate their energy metabolism to adapt to increased catabolic activities after delivery, resulting in decreased circulating thyroid hormone levels [31]. We found low plasma T_4 levels in Holstein cows with high milk yield during the lactation period. This result was consistent with the opinion of Pezzi et al. [32] that at the beginning of galactopoiesis, the number of T_3 receptors with type-3 deiodinase enzyme activity, which converts T_4 hormone into T_3 hormone in mammary gland secretory cells, increases and therefore, the plasma T_4 level decreases. An increase in T_4 levels was found in accordance with the decrease in the milk yield in Holstein cows with low milk yield according to the lactation period ($p < 0.05$). This supports the report of Klimiene et al. [33] saying that there is a negative correlation between the plasma T_4 concentration and the milk yield during the lactation period. We found that the mean plasma T_4 level of Holstein cows with low milk yield was higher than the Simmental in the same lactation period. This finding supports that thyroid hormones are related to and change with age, breed, milk yield level, and many environmental factors as well [34].

Leptin, secreted by white adipose tissue, is a secretion that controls the nutritional behavior, metabolism, and endocrine system to maintain the energy homeostasis and acts as an energy reserve signal for hypothalamic regions. As in other species, the leptin concentration in ruminants varies according to changes in body weight and body fat percentage. It has been reported that dairy cows lose more than 60% of their body fat during the early lactation period, and consequently the leptin concentration decreases shortly before the time of delivery [35]. Conflicting results have been reported about the plasma leptin levels during the lactation period. Kadokawa et al. [35] reported an increase, whereas Huszeniczka et al. [36] reported no change;

Holtenius et al. [37] reported a decrease, and Liefers et al. [38] reported a temporary increase in the plasma leptin level after delivery. The plasma leptin levels did not change in Holstein and Simmental cows with different milk yields. The opposite interaction between insulin and GH was reported to affect the regulation of the plasma leptin level, and the insulin level was supposed to be the main factor in regulating the plasma leptin level [16]. The high plasma GH levels despite the lack of a decrease in plasma insulin levels in Holstein and Simmental cows with low milk yield may have led to the maintenance of plasma leptin levels. A positive correlation between the plasma leptin and the insulin levels in cows with low, medium, and high milk yield categories supports that insulin regulates the leptin levels in lactating cows.

The production of adiponectin, which is secreted from adipose tissue and is effective in the regulation of energy metabolism, is related to glucose and fatty acid metabolism [39]. There are scarce data regarding the effect of nutrient and energy intake on circulating adiponectin levels in dairy cows. Ohtani et al. [40] reported that the plasma adiponectin levels reached the lowest level in the first two weeks after delivery in cows and increased steadily for 6–8 weeks and remained stable thereafter. Singh et al. [41] reported that changes in plasma adiponectin levels occurred during the peri-labor period in cows and that the plasma adiponectin levels decreased during the first 3 weeks after delivery, gradually increased during the following 4 weeks and remained stable thereafter. In the current study, a significant difference in the plasma adiponectin levels between low, medium, and high milk yield cows according to lactation periods in either of the two breeds were not found. This finding supports the finding of Ohtani et al. [40] and Singh et al. [41] that decreased plasma adiponectin levels at the time of delivery remains stable until an average of 8 weeks after delivery.

It is known that ruminants fulfil the vast majority of their glucose needs with gluconeogenesis. Gluconeogenesis is important for the maintenance of glucose flow into the mammary glands. Adiponectin increases the glucose uptake of skeletal muscles, reduces the expression of molecules related to gluconeogenesis in the liver, suppresses gluconeogenesis and increases insulin sensitivity [39]. Therefore, Singh et al. [41] suggested that the plasma adiponectin levels should be low in cows during the lactation regarding the energy intake. However, Gross et al. [42] demonstrated that, despite a more negative energy balance than the negative energy balance in cows at the beginning of the lactation period, limiting feed consumption and subsequent re-feeding did not change the plasma adiponectin level, while Singh et al. [41] reported that energy restriction did not affect the plasma adiponectin levels in humans and dairy cows. A

high GH and cortisol levels in Holstein and Simmental cows with low and high milk yield, high ghrelin levels in the Simmental and tendency of high ghrelin levels numerically in Holsteins, and the low T_4 levels in high milk yield Holstein cows suggest that the energy intake was inadequate in these groups. However, there was no decrease in the plasma insulin and leptin levels, suggesting that the lack of energy intake was not so severe. In this context, our findings related to plasma adiponectin levels support the opinions of Singh et al. [41], Gross et al. [42] and Mielenz et al. [43] that the adiponectin level is effective in adapting to rapid metabolic changes during the NED period at early lactation and is not affected by energy restriction and balance after early lactation in cows. Krumm et al. [44] reported that the plasma leptin and insulin levels may be effective in regulating the plasma adiponectin levels, while Koebnick et al. [45] reported no correlation between the plasma adiponectin and leptin levels; Blümer et al. [46] showed a positive correlation between the plasma adiponectin and insulin concentrations. The plasma adiponectin levels were positively correlated with leptin and insulin levels in the low, medium and high milk yield categories regardless of the cow breeds. This finding was consistent with the view that leptin and insulin levels may be effective in the regulation of plasma adiponectin levels and there is a positive correlation between the plasma concentrations of adiponectin and insulin. Adiponectin secretion from adipose tissue is stimulated by insulin. It is thought that adiponectin and leptin, whose secretions are regulated by insulin, regulate the biological functions of the body independent of each other.

One-third of the total milk solids produced at the beginning of lactation is produced from body fat tissue reserves [47]. For this purpose, cows use their fat deposits as the energy source during lactation. Mishra et al. [7] reported that energy sources were directly related to BCS, indicating the situation and deprivation of the energy store, and, therefore, the differences in BCS affected the milk yield. However, no significant correlation was determined between BCSs, which represent the body fat reservoirs that can be used as energy sources for animals, and plasma leptin and adiponectin levels, which are accepted as indicators of energy stores in the low, medium, and high milk yield categories regardless of breeds (Tables 3, 4 and 5).

Energy stores are the key component of milk production, since hormone-sensitive lipase breaks down triglycerides in adipose tissue into fatty acids to be used as a source of energy, thus accelerating lipolysis. As the lactation period progresses, lipolysis gradually decreases, and lipogenesis increases [48]. Low intake of dry matter and high milk yield cause a negative energy balance, while the body depots consumed during this period are

replaced in the middle and late lactation period, and the animal returns to the positive energy balance again. This fluctuation in energy balance causes changes in BCS, milk yield, and reproductive performance. If fluctuations in energy balance are within physiological limits, the milk yield and reproductive performance improve [23, 49].

The plasma ghrelin levels were negatively correlated with BCS in the high milk yield group (Table 5). In the case of hunger, the secretion of the ghrelin hormone increases, which stimulates food intake. Itoh et al. [50] reported that the plasma ghrelin level increased during the high milk yield period. Since 1/3 of the total milk solid produced at the beginning of lactation are produced from body fat tissue reserves, fat tissue loss occurs during first lactation period, and BCS decreases accordingly. This explains the negative correlation between BCS and the plasma ghrelin hormone levels in cows with high milk yield.

The main findings of the present study were as follows: 1) The dry period body condition score affected the milk yield; 2) The growth hormone, cortisol, and ghrelin hormone levels physiologically increased, the plasma T_4 level decreased, and the insulin, IGF-I, TSH, leptin and adiponectin levels did not change in the periods with high energy need according to the lactation periods and milk yield levels; 3) There was a positive correlation between adiponectin, leptin and insulin at different levels of milk yield (low, medium and high), independent of

breed, and also between leptin and adiponectin, which is effective in the regulation of energy metabolism; 4) Secretion of leptin and adiponectin from adipose tissue was controlled by insulin and regulated the biological functions in galactopoiesis in both breeds; 5) The body condition score, which expresses the body fat reserves that can be used as an energy source of the animal, was not significantly correlated with the plasma levels of leptin and adiponectin, which are accepted as indicators of energy stores, and regardless of breeds at low, medium and high milk yield categories according to the lactation periods. As a result, it was concluded that individual feeding programs should be carried out in line with the energy requirements to achieve the highest milk yield without disturbing the animal's health together with keeping the continuity of the milk yield. Moreover, in accordance with the physiological needs of animals, more comprehensive and molecular studies are needed to increase the milk yield while preserving their health.

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Conflict of interest

The authors declare that there is no conflict of interest.

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