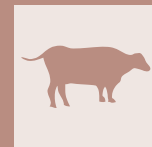


Relationships between milk insulin-like growth factor-I (IGF-I) concentration and body condition score with reproductive performance and milk yield in Jersey cows



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SUMMARY

Introduction - One of the most important hormones affecting reproductive and milk yield is insulin-like growth factor I (IGF-I). IGF-I is closely related to nutritional status and the post-partum ovarian activity in dairy cows. It also stimulates milk synthesis and its secretion. Body condition score (BCS) is a useful management tool routinely used to predict body fat storage and energy status in dairy cows and has a strong influence on milk production and reproductive efficiency for the upcoming lactation after calving. Many publications have been available on BCS and serum or plasma IGF-I in Holstein cows. However, the information regarding the milk of Jersey cows has been limited. Therefore, further studies have been required to reveal the effect of IGF-I and BCS on reproduction and milk yield traits of this breed.

Aim - This study was conducted to investigate the relationships between milk IGF-I concentration and body condition score (BCS) with reproduction performance and milk yield of Jersey cows raised at Karakoy State Farm in Samsun.

Materials and methods - The mean milk IGF-I concentration and BCS were calculated by taking the mean of the three lactation periods (70 ± 14 , 140 ± 14 , and 210 ± 14 days) using the repeated measures analysis procedure. The enzyme-linked immunosorbent assay (ELISA) method was applied for milk IGF-I analyses. BCS was assessed using a scale of 1 to 5 points with 0.25 unit increments.

Results and discussion - The effects of stage of lactation on IGF-I and BCS classes were significant ($P < 0.001$). The effects of mean IGF-I concentration on interval calving to first service (ICFS) (0.041), calving interval (CI) (0.042), and dry period (DP) (0.030) were found statistically important. Significant correlations were also determined between mean IGF-I and ICFS (-0.184), CI (-0.183), or lactation length (LL) (-0.155), and ICFS, CI, and LL were found to be shorter in cows with higher IGF-I. Both reproduction and milk yield traits were not affected by BCS.

Conclusions - The results of the study revealed that milk IGF-I concentration may be used as an indicator to detect reproduction characteristics of dairy cows.

KEY WORDS

Jersey cow, insulin-like growth factor-I, body condition score, milk production, reproduction.

INTRODUCTION

The fertility of lactating dairy cows over the last 20 years has declined in association with increased genetic capability for milk production, coupled with changes in nutritional management and larger herd sizes¹. Today's dairy cows tend to have lower greater days open (DO) and conception rates² and more reproduction diseases³. This decline in fertility can be explained by the negative correlation between milk production and reproduction. The solution for improving fertility in high-producing dairy cows will include both short-term and long-term components. Immediate short-term solutions involve changes in the diet so that dietary ingredients invoke hormonal responses that benefit the reproduction of the dairy cow⁴. One of growth promoting hormones affecting reproductive and milk yield is insulin-like growth factor I (IGF-I)⁵.

IGF-I is closely related to nutritional status and the post-partum ovarian activity in dairy cows⁶. IGF-I stimulates growth, cell development, differentiation into a variety of cell types and organizes the DNA synthesis into follicles via IGF-I receptors⁷. Furthermore, IGF-I plays an important role in the survival of the embryo and it can act directly to regulate the growth of embryo⁸. Milk IGF-I concentration, which reflects the concentration of the hormones in blood⁹, is an indication of the cows physiological state and it plays several important roles in controlling reproduction⁸. Magistrelli et al.¹⁰ determined that milk IGF-I is 13% of blood level. Researches were also reported that IGF-I in milk was correlated to plasma IGF-I levels ($r = 0.88$; $P < 0.01$). IGF-I is also a mammary apoptosis inhibitor⁷. IGF-I synthesized from the mammary gland stimulates cellular proliferation, cell survival¹¹, mammary gland development and alveolar differentiation¹². It also stimulates the synthesis and secretion of milk¹³.

Like IGF-I, BCS is a useful management tool routinely used to appraise the body fat reserve and energy status in dairy cows¹⁴, and has a strong influence on milk production and reproductive efficiency for the upcoming lactation after calving¹⁵. Cows with low BCS due to a lack of adequate feed in-

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take during early lactation have increased incidence anestrus and anovulation cycles, reduced conception rates and fertility². Excessive BCS causes difficult calving and increases the incidence of certain metabolic diseases¹⁵. Ruegg et al.¹⁶ reported increased reproductive problems in very fat or very thin high-yielding cows. Thus, the goal was to obtain cows in good condition meaning that they were not too thin or not too fatty¹⁵. Unlike these findings, Gillund et al.¹⁷ reported no effect of BCS on reproduction in dairy cows. A great amount of literature has been available about BCS and serum or plasma IGF-I in Holstein cows. However, the information regarding the milk of Jersey cows has been limited. Therefore, further studies are required to reveal the effect of IGF-I and BCS on reproduction and milk yield traits of this breed. The objective of this study was to determine the relationships between milk IGF-I concentration and BCS with reproduction and milk yield traits in Jersey cows.

MATERIALS AND METHODS

Sample collection and management

A total of 166 Jersey cows with 1-3 lactation number were used raised at Karakoy State Farm in Samsun province, which is located in the Black Sea region of Turkey. The farm was visited monthly at 28 day intervals. Milk samples for IGF-I analyses from the evening milking were collected three times from the cows within 70±14, 140±14, and 210±14 days of lactation, and these cows were scored for body condition score (BCS) on these days. After cleaning the teats with tepid water, the first stream of foremilk was discarded, and a 15 mL milk sample was obtained from each teat into sterile tubes. Milk samples were stored at 4°C in an ice-cooled box and analyzed within 12 h.

On this farm, the cows were kept in free-stall barns during the whole year. They were milked twice a day. The cows were fed a total mixed ration (TMR) ad libitum twice a day using a mixer wagon, and grazed on pasture during the dry season. TMR consisted of concentrate feed, silage (corn and vetch), and hay (grass and wheat straw). Diets were fed twice daily in equal proportions before milking. The daily milk yield of each cow was automatically recorded on a computer via transponders.

Milk IGF-I Determination

The IGF-I concentration in milk was measured by bovine enzyme-linked immunosorbent assay (ELISA) by using the IGF-I EIASIA KAP1581 kit (DIAsource ImmunoAssays S.A., Rue de l'Industrie, 8, B-1400 Nivelles, Belgium)^{18,19}.

This study was carried out as a pre-treatment and experiment according to the kit procedure. During the pre-treatment, each of the milk samples were centrifuged for 10 minutes at 5.000 rpm. After the supernatants were obtained, 400 µl of pre-treatment solution was added into this tube. The tubes were closed, vortexed, and incubated for 30 minutes at room temperature. Then, these tubes were centrifuged for 2 minutes at 10.000 rpm. Next, 100 µl of supernatant was obtained and transferred it into the polypropylene tube, and 600 µl of the neutralization were added to this tube; each tube was vortexed.

During the experimental stage, low and high controls, calibrators, and samples were studied two parallel according to

the kit procedure. First, 100 µl of IGF-I-HRP conjugate solution was pipetted into all the wells, respectively. It was incubated for 1 hour at room temperature, then the liquid from each well was aspirated. The plate was washed three times by dispensing 0.4 ml of wash and aspirating the content of each well. Afterwards, 200 µl of the chromogenic solution was pipetted into each well within 15 minutes following the washing step. Then the microtiterplate was incubated for 15 minutes at room temperature, and kept from direct sunlight. One hundred µl of stop solution was pipetted into each well. Finally, the plates were read at 450 nm (reference filter 630 nm or 650 nm) by means of an Epoch Microplate Spectrophotometer (Model No: SN242136, BioTek, USA). The absorbance was inversely proportional to the IGF-I concentration. A four-parameter logistic function curve fitting was used to calculate the IGF-I concentration.

Body Condition Score

BCS was assessed using a scale of 1 to 5 (1=very thin; 5=very fat) with 0.25 unit increments described by Ferguson et al.²⁰. Scoring was made by the same person from the cows within 70±14, 140±14, and 210±14 days of lactation.

Reproduction and Milk Yield Traits

In the current study, the reproduction and milk yield traits of each Jersey cow were calculated from the official herd book and computer records.

The reproduction traits included interval calving to first service (ICFS), days open (DO), gestation length (GL), calving interval (CI), and number of services per conception (NSC). The milk yield traits included daily milk yield (dMY), lactation length (LL), lactation milk yield (LMY), 305-day milk yield (305-dMY), and dry period (DP). Milk yield and lactation length were calculated by the Holland method²¹.

Statistical Analysis

To determine the sample size, power and sample size analyses were performed in the MINITAB statistical package program (Minitab - Version 12)²² using the study data of Falkenberg et al.²³. The necessary sample size was established as at least 35 at a 90% confidence interval.

The mean milk IGF-I concentration and BCS were calculated by the mean of three lactation periods (70±14, 140±14, and 210±14 days) and statistically analyzed by using the repeated measures analysis procedure.

The following model was used to examine the influence of stage of lactation on IGF-I and BCS;

$$\gamma_{ij} = \mu + \alpha_i + \varepsilon_{ij}$$

γ_{ij} = The j^{th} observation in the i^{th} stage of lactation (IGF-I and BCS)

μ = overall mean

α_i = effect of i^{th} stage of lactation (i : 70±14, 140±14 and 210±14)

ε_{ij} = random error term

Mean milk IGF-I concentration was divided into three groups: low (<20 ng/ml), moderate (20-25 ng/ml), and high (>25 ng/ml). To determine the effect of the change in IGF-I on the reproduction and milk yield traits above, the following model was used:

$$\gamma_{ij} = \mu + \alpha_i + \varepsilon_{ij}$$

γ_{ij} = The j^{th} observation in the i^{th} IGF-I group (ICFS, DO, GL, CI, NSC, dMY, LL, LMY, 305-dMY and DP)

μ = overall mean

α_i = effect of i^{th} IGF-I group (i : <20, 20-25 and >25)

ε_{ij} = random error term

Mean BCS was divided into three groups: low (<3.00), moderate (3.00), and high (>3.00). To determine the effect of the change in BCS on the reproduction and milk yield traits above, the following model was used:

$$\gamma_{ij} = \mu + \alpha_i + \varepsilon_{ij}$$

γ_{ij} = The j^{th} observation in the i^{th} BCS group (ICFS, DO, GL, CI, NSC, dMY, LL, LMY, 305-dMY and DP)

μ = overall mean

α_i = effect of i^{th} BCS group (i : <3.00, 3.00 and >3.00)

ε_{ij} = random error term

The statistical analysis were performed using SPSS 13.0 for Windows²⁴. The values were presented as least squares means \pm SE. The differences between means were determined by Tukey's multiple range test. In addition, phenotypic correlations between IGF-I and BCS with reproduction and milk yield traits were calculated.

RESULTS

The effect of stage of lactation on IGF-I concentration was given in Figure 1. As seen that, the lowest IGF-I concentration was detected in the early lactation period (70 ± 14), and the highest in the 140 ± 14 and 210 ± 14 days of lactation ($P < 0.001$).

As seen that Figure 2, BCS was affected by stage of lactation ($P < 0.001$). The lowest BCS was found in first lactation period (70 ± 14), and highest in third lactation period (210 ± 14). The effects of the overall means of milk IGF-I concentration and BCS grouping on reproduction and milk yield traits were presented in Table 1.

The results had shown that ICFS values were significantly ($P = 0.041$) different among IGF-I groups. DP values according to IGF-I group were statistically different at $P = 0.030$. The

present study found that dMY, LL, LMY and 305-dMY were not affected by IGF-I groups.

The effects of BCS groups on ICFS, DO, GL and NSC were not significant (Table 2). As seen in Table 2, dMY, LL, LMY, 305-dMY and DP were not affected by BCS groups.

In the present study, the negative phenotypic correlations between mean IGF-I concentration with ICFS (-0.184) and CI (-0.183) of reproductive traits were determined ($P < 0.05$) (Table 3), but no significant with DO and GL. There was a negative and statistically significant relationship ($r = -0.155$) between IGF-I concentration and only LL from milk yield traits; however, there were no relationships with other milk yield traits.

DISCUSSIONS

In the present study, the determined change in IGF-I concentration by lactation stage is shown in Figure 1. As seen that, IGF-I concentration was the lowest in early stage of lactation (70 ± 14) ($P < 0.001$) compared to the later stage 140 ± 14 and 210 ± 14 days of lactation. This result was in agreement with the result of Spicer et al.²⁵, who obtained the lowest serum IGF-I concentration in early lactation period. Sejrnsen et al.²⁶ reported that the content of lowest IGF-I in milk was determined in middle lactation and high content in late lactation. These results were different from those reported by Kang et al.⁷, who IGF-I content in the early, middle, and late stages of lactation did not change significantly throughout the entirety of the lactation period.

Decreasing milk IGF-I concentration in the early lactation period can be explained as a result of the negative energy balance (NEB). As is known, during the early postpartum period, the energy demand for maintenance and production exceed and dairy cows enter a period of NEB during which they due to mobilize body reserves from milk production⁵. Thus, dairy cows have to consume enough feed to meet energy demand during early²⁵. Thus, IGF-I concentrations are decreased in early lactation when cows are in peak milk yield. Early lactation in dairy cattle is characterized by low concentrations of IGF-I in serum¹. During NEB, serum growth hormone (GH) concentration in dairy cows increase, but decreases serum IGF-I and GH receptor expression in the liv-

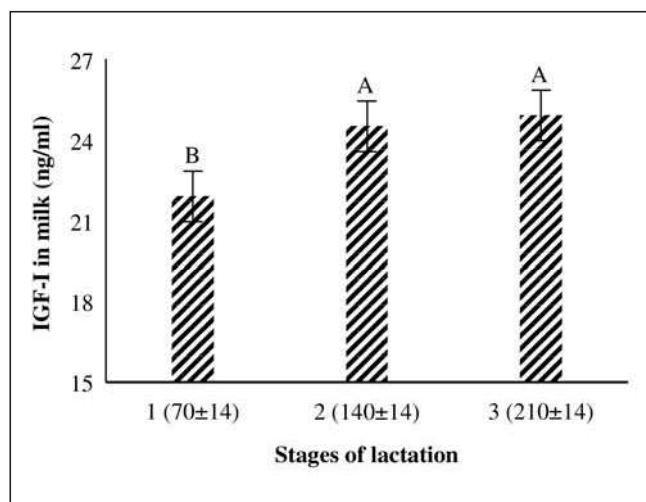


Figure 1 - Effect of stage of lactation on IGF-I.

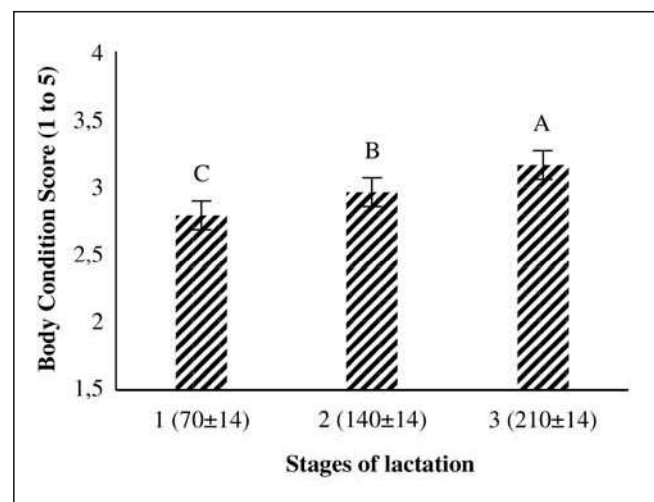


Figure 2 - Effect of stage of lactation on BCS.

Table 1 - The effects of milk IGF-I concentration on reproduction and milk yield traits (mean \pm SE).

	Traits	IGF-I group (ng/ml)							
		< 20 (n=39)		20 - 25 (n=63)		> 25 (n=64)		Overall	
		n	$\bar{X} \pm S\bar{x}$	n	$\bar{X} \pm S\bar{x}$	n	$\bar{X} \pm S\bar{x}$	n	$\bar{X} \pm S\bar{x}$
Reproduction	ICFS, d	34	69.5 \pm 3.69 ^A	66	63.3 \pm 2.10 ^{AB}	65	59.5 \pm 2.07 ^B	165	63.1 \pm 1.42
	DO, d	32	99.3 \pm 7.67	61	85.8 \pm 4.98	59	96.9 \pm 6.11	152	92.9 \pm 3.51
	GL, d	26	283.0 \pm 0.84	41	281.4 \pm 1.02	38	281.8 \pm 0.91	105	282.0 \pm 0.55
	CI, d	26	386.2 \pm 8.41 ^A	40	362.1 \pm 5.70 ^B	38	367.8 \pm 5.91 ^B	104	370.2 \pm 3.80
	NSC, n	34	1.9 \pm 0.18	61	1.7 \pm 0.10	63	1.9 \pm 0.11	161	1.8 \pm 0.07
Milk yield	dMY, kg	35	15.5 \pm 0.41	67	15.6 \pm 0.32	64	15.7 \pm 0.27	166	15.6 \pm 0.19
	LL, d	64	342.5 \pm 9.54	67	312.5 \pm 8.23	63	321.1 \pm 9.11	164	322.0 \pm 5.29
	LMY, kg	64	5358.4 \pm 204.17	67	4835.9 \pm 150.54	63	5057.7 \pm 171.50	164	5029.4 \pm 100.40
	305-dMY, kg	34	4939.8 \pm 148.53	65	4722.1 \pm 103.16	64	4872.0 \pm 114.30	163	4826.4 \pm 68.24
	DP, d	26	64.0 \pm 2.17 ^A	41	56.8 \pm 1.54 ^B	38	60.6 \pm 1.89 ^{AB}	105	59.9 \pm 1.08

AB: Means in the same line with no common superscripts differ (P<0.05).
 Non-significant (P>0.05).
 IGF-I: Insulin-Like Growth Factor-I, BCS: Body Condition Score, ICFS: Interval Calving to First Service, DO: Days Open, GL: Gestation Length, CI: Calving Interval, NSC: Number of Services per Conception, dMY: Daily Milk Yield, LL: Lactation Length, LMY: Lactation Milk Yield, 305-dMY: 305-day Milk Yield, DP: Dry Period.

Table 2 - The effects of BCS on reproduction and milk yield traits (mean \pm SE).

	Traits	BCS group					
		< 3.00 (n=64)		3.00 (n=43)		> 3.00 (n=59)	
		n	$\bar{X} \pm S\bar{x}$	n	$\bar{X} \pm S\bar{x}$	n	$\bar{X} \pm S\bar{x}$
Reproduction	ICFS, d	40	65.4 \pm 3.13	71	63.1 \pm 2.26	54	61.4 \pm 2.14
	DO, d	36	96.4 \pm 6.90	64	91.7 \pm 5.12	52	92.1 \pm 6.64
	GL, d	16	282.1 \pm 0.97	44	282.8 \pm 0.59	45	281.1 \pm 1.10
	CI, d	16	381.3 \pm 5.89	44	371.3 \pm 5.64	44	365.1 \pm 5.78
	NSC, n	39	1.8 \pm 0.12	68	1.8 \pm 0.11	54	1.8 \pm 0.13
Milk yield	dMY, kg	41	15.6 \pm 0.35	71	15.8 \pm 0.29	54	15.3 \pm 0.33
	LL, d	39	312.8 \pm 13.61	71	320.7 \pm 8.14	54	330.3 \pm 6.93
	LMY, kg	39	4960.0 \pm 242.33	71	5055.4 \pm 152.86	54	5045.4 \pm 151.93
	305-dMY, kg	39	4890.9 \pm 134.24	71	4865.3 \pm 105.00	53	4726.7 \pm 121.49
	DP, d	16	57.4 \pm 3.57	44	60.1 \pm 1.26	45	60.7 \pm 1.82

Non-significant (P>0.05).
 IGF-I: Insulin-Like Growth Factor-I, BCS: Body Condition Score, ICFS: Interval Calving to First Service, DO: Days Open, GL: Gestation Length, CI: Calving Interval, NSC: Number of Services per Conception, dMY: daily Milk Yield, LL: Lactation Length, LMY: Lactation Milk Yield, 305-dMY: 305-day Milk Yield, DP: Dry Period.

Table 3 - Correlations between IGF-I and BCS with reproduction and milk yield traits.

	Reproduction traits					Milk yield traits				
	ICFS	DO	GL	CI	NSC	dMY	LL	LMY	305-dMY	DP
IGF-I	-0.184*	-0.057	-0.020	-0.183*	-0.030	0.125	-0.155*	-0.058	0.033	-0.063
BCS	-0.165	-0.052	-0.081	-0.103	-0.012	-0.077	0.090	0.010	-0.087	0.018

*P<0.05
 IGF-I: Insulin-Like Growth Factor-I, BCS: Body Condition Score, ICFS: Interval Calving to First Service, DO: Days Open, GL: Gestation Length, CI: Calving Interval, NSC: Number of Services per Conception, dMY: daily Milk Yield, LL: Lactation Length, LMY: Lactation Milk Yield, 305-dMY: 305-day Milk Yield, DP: Dry Period

er²⁷. Because blood IGF-I is the primary negative feedback hormone for GH. Furthermore, mammary tissue may have more receptors for IGF-I in early than in late lactation. GH initiates the mobilization of fatty acids from adipose tissue and IGF-I level in blood and mammary gland decreases²⁸. Stage of lactation markedly affected BCS of Jersey cows

(P<0.001). BCS showed an increase from the first to the third lactation period. The lowest BCS was determined in first lactation period and highest in third lactation period (Figure 2). Similar results have previously been reported^{14,29}. Rossoni et al.³⁰ determined that BCS in primiparous Italian Brown Swiss cattle decreases slightly during first 90 milking days,

but increases during the end of lactation. Peak lactation is a critical time in terms of metabolic stress in the dairy cow³¹. They mobilize their lipid reserves and lose BCS for meeting the energy requirements for growth and milk production when the cows go into the NEB³².

ICFS values were the lowest in cows with IGF-I > 25 ng/ml, and the highest in cows with IGF-I < 20 ng/ml. (Table 1). Similar to ICFS, the highest CI was recorded in cows with IGF-I < 20 ng/ml (P=0.042). However, the CI values that were the lowest in IGF-I 20-25 ng/ml were not statically different than in milk with IGF-I > 25 ng/ml. For this reason, it can be said that ICFS and CI tended to decrease with increased milk IGF-I concentration. According to the mean IGF-I group, no statistical differences were found between DO, GL and NSC values.

The current results are agree with that of Beam and Butler³³, in which increased serum IGF-I concentration was associated with early first ovulation in dairy cows. Meikle et al.³⁴ reported that the better reproductive performance in dairy cows had higher plasma IGF-I concentrations. Previous studies have reported that dairy cows with high serum IGF-I concentrations at the beginning period of the early luteal activity had better reproductive performance³⁵. In this regard, IGF-I plays an important role in higher success rate of first inseminations, shorter CI³⁶ and higher pregnancy rates³. Jorritsma et al.³⁷ emphasized that IGF-I during the early postpartum period may be important for oocyte quality. However, Falkenberg et al.²³ emphasized that the routine measurement of IGF-I in serum after calving was neither practical nor economically suitable for reproductive management in dairy cows. The maximum DP was recorded in IGF-I < 20 ng/ml, but the lowest in IGF-I 20-25 ng/ml (P=0.030). DMY, LL, LMY and 305-dMY were not affected by IGF-I groups. ICFS, DO, GL, and NSC were not affected by BCS groups (Table 2). These results are different from the results of some studies those reported that cows with lower BCS had longer CI^{31,38}. Yaylak³⁹ reported that cows with BCS ≥ 3.50 had lower ICFS and DO. While Heuer et al.⁴⁰ indicated that conception rates at first insemination in fatty cows were lower than in cows with normal body condition, Buckley et al.⁴¹ reported that cows with a very low nadir BCS (≤ 2.5) had lowest pregnancy to first service conception rate. Amer² emphasized that the cows with moderate BCS showed shorter DO and lower NSC than thin or fatty cows. Parallel to present findings, Ruegg and Milton²⁹ determined that ICFS was not affected by BCS. Gillund et al.¹⁷ reported that effect of BCS on reproduction was not significant, and it was not related to conception at first service, interval from calving to the second insemination, DO and NSC. In contrast, the research results of Yaylak³⁹ concluded that BCS in dairy cows could be used as a management and selection tool to improve reproductive performance, which were also supported by Pryce et al.³⁸.

The effects of BCS on dMY, LL, LMY, 305-dMY and DP were not significant (Table 2). This differed from the study of Ruegg and Milton²⁹, who emphasized that over conditioned cows would have extended LL. Distinct from our research results, Yaylak and Kumlu⁴² reported that there was a linear relationship between BCS and 305-dMY. In other words, the milk yield steadily increases towards in cows with good condition compared to cows with a thin condition. Markusfeld et al.⁴³ reported that cows with a higher condition score at calving produced more milk in the first 90 days of lactation. This is due to the fact that an additional energy reserve may

be required to support the milk yield. These results indicated the importance of having adequate body reserves available to support high milk yield.

Phenotypic correlations between mean IGF-I concentration with ICFS and CI of reproductive traits were found negative with statistically important (P < 0.05) (Table 3), but no significant with DO, GL and NSC. According to these relationships, ICFS and CI were shorter in cows having high mean IGF-I concentration. These data were consistent with the hypothesis that IGF-I level in plasma in the postpartum period may be a useful indicator to improve reproductive performance in a herd³⁴. Thus, the farm benefits, both economically and in terms of herd management.

In this study, a negative relationship (r = -0.155) was also estimated between IGF-I concentration and LL. However, the relations of IGF with other milk IGF-I and other milk yield traits were not statistically important. At this point, shorter LL in cows with high IGF-I concentration can be explained by shorter CI. Parallel to the current findings, Spicer et al.²⁵ indicated that IGF-I may stimulate mammary growth, but have no effect on milk synthesis. In contrast, the previous studies emphasized that cow milk contains high levels of IGF-I, which plays an important role in regulating cell proliferation, differentiation and apoptosis⁴⁴, and may contribute to mammary development⁴⁵ and milk production⁴⁶. However, there is no evidence for a genetic association between IGF-I and milk yield. Thus, further studies are required to identify the interrelationships between IGF-I concentration and milk yield traits. As similar to mean BCS groups, no significant relationships were found between BCS and both reproduction traits or milk yield traits (Table 3).

CONCLUSIONS

The present results demonstrated that ICFS and CI were shorter in cows with higher IGF-I concentration. Thus, it can be said that milk IGF-I concentration may be used as a parameter to detect the reproduction characteristics of dairy cows. Additionally, further studies are required to reveal the relationships between IGF-I and BCS with reproduction and milk yield traits in different dairy breeds.

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