Exploring the Relationship between Changes in Postpartum BCS (Body Condition Score) and Reproductive Performance in Dairy Herds

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ABSTRACT: The current research aims to explore the effects of body condition score (BCS) reduction on some reproductive factors such as pregnancy rate, number of artificial inseminations (AI) per pregnancy, clinical endometritis, and ovarian activity in 1613 lactating Holstein cows, and also the effects of the number of calving and its difficulty, on changes in the BCS. Cows were divided into 6 groups from 1 (no changes in the BCS) to 6 (1.25 units’ changes and more). The reproductive system examination and diagnosis of the pregnancy were conducted using an ultrasound machine. To evaluate the endometritis, the vaginal evaluation was performed after external examining. Grading of vaginal internal mucus was done from 0 to 3 based on the color and amount of pus in secretions. Presence or absence of cyst in ovaries and also ovaries status including static, follicles >1cm, or an active corpus luteum were evaluated. All cows were monitored until 33±3 and 65±5 days of a new pregnancy. Data analysis shows that with a further decrease in BCS the first month after parturition, the percentage of non-pregnant cases and the number of cows with inactive ovaries increases significantly. By decreasing 0.5 units or more in BCS, the rate of endometritis significantly increases. The number of inseminations necessary for creating pregnancy in cows with a reduction of 0.5 units or more in BCS was significantly higher. The odds ratios of developing clinical endometritis, inactive ovaries, and Pregnancy with more than three inseminations in cows with BCS changes> 0.5 were significantly greater. BCS changes of cows in calving 3 and 4 were significantly higher than cows in the first calving. Regarding the type of delivery only dystocia has been associated with significant BCS changes (P<0.05). According to these results, it can be concluded that a further reduction in BCS was associated with a reduction in reproductive performance.

Keywords: Body condition score (BCS) ; Reproductive performance; Dairy cows; Endometritis.
INTRODUCTION

A favorable reproductive period and faster conception of cows are important goals in the dairy herd industry. One calf is expected to be born from each cow every year to have such a profitable system. In this matter, energy metabolism is a factor affecting the reproductive performance of dairy cows, which is critical in the transition period (Carvalho et al., 2014). The transition period refers to 3 weeks before and 3 weeks after calving, which is a challenging period for dairy cows because it increases milk production and food intake. The lack of optimal coordination between these two processes can lead to the creation of negative energy balance (NEB), mobilization of adipose tissues, and reduction of the body condition score (BCS), and the body weight (Carvalho et al., 2014). The energy metabolism status can be controlled by assessing the BCS of herds since the evaluation of BCS is a simple and available evaluation method for managing cow feeding based on their physiological conditions (Delgado et al., 2004). Therefore, the current research aims to explore the main effects of reducing the BCS on some reproductive factors such as pregnancy rate, number of inseminations per pregnancy, clinical endometritis, and ovarian activity. This research also investigates the effects of factors, such as the number of calving and dystocia, on changes in the BCS.

MATERIALS AND METHODS

This study was performed for about 24 months on 1613 lactating Holstein cows, in a large commercial dairy herd in Fars province, located in the south of Iran. We recorded the data of each cow, including the number of calving, type of calving (normal, with a little help, dystocia), ovarian status (static, presence of follicle, presence of corpus luteum, existence of cyst), pregnancy status, number of inseminations per pregnancy, presence or absence of endometritis, as well as the BCS. Cows had a TMR diet and were fed two times a day with a diet formula adjusted by a nutritionist using NRC 2001 software. The cows were housed in a free-stall barn with sand bedding, and milking was done 3 times a day at intervals of 8 hours. The average milk production in the cows was 45 kg on 30±3 days after calving. Estrus synchronization was established in all cows after clean test on 30±3 days in milk (DIM) by using the Presynch-heatsynch method. Cows that did not pregnant after the first insemination synchronized by using the heatsynch program in the absence of uterine infection. Estrus was detected three times a day by a technician and all animals presenting signs of standing estrus were artificially inseminated by an AI technician. Pregnancy diagnosis was performed 33±3 days after AI via trans rectal ultrasonography (Easyscan, BCF, UK) and it was reconfirmed 65±5 days postAI.

The evaluation of their BCS was performed at two different times (the first time around the calving and the second time on 30±3 days after calving) and each time by two expert technicians, and finally the mean scores were recorded. To assess the BCS, the amounts of subcutaneous fat in the relevant areas of the cows’ body were estimated and it was rated 1 to 5 (at intervals of 0.25 units) based on the severity of obesity and thinness of the cows (Radostits et al, 2010) and finally, after calculating the amounts of changes in the BCS, cows were divided into 6 groups, The groups included 1 (unchanged cases), 2 (decrease of 0.25 units), 3 (decrease of 0.5 units), 4 (decrease of 0.75 units), 5 (decrease of 1 unit), and 6 (decrease of 1.25 units and more).

Clean test for examination of the reproductive system of the cows after parturition was done by using an ultrasound machine (Easyscan, BCF, UK) and rectal palpation at 30±3DIM. The vaginal examination was also used to diagnose the postpartum endometritis in the clean test. After inspection for the presence of fresh discharge around the vulva perineum or tail, if the discharge was not visible externally, a vaginal examination was performed. After cleaning the cow’s vulva with a dry paper towel, a clean, lubricated, and gloved hand was inserted through the vulva. By palpation of all directions in the vagina, mucous contents of the vagina were withdrawn manually for examination. According to the color and proportion of pus in mucus, the vaginal discharge was scored on a 0inatione (Sheldon et al., 2006; Williams et al., 2005), 0 = normal uterine discharge, 1 = flakes of purulent exudates in the uterine discharge, 2 = >50% of the uterine discharge is made up of purulent exudates and 3 = hemorrhagic uterine discharge mixed with purulent exudates (Sheldon et al., 2006, Williams et al., 2005).

Ultrasonographic scanning was performed using a 5 MHz rectal linear probe to assess ovarian
structures, the diameter of the uterus, echotexture, the thickness of the uterine wall, and intraluminal fluid accumulation in all cows. The ovaries and uterus were clinically examined via rectal palpation and ultrasonographic scanning based on our previous study (Makki, Ahmadi et al. 2017). Cows were divided into 2 groups in terms of ovarian status, including 1: static (where there was no follicle more than 10 mm and active corpus luteum on the ovary, and the animal was in the anestrus status) and 2: cases with follicles over 10 mm, or cases with an active corpus luteum. In terms of the presence or absence of cysts, cows were divided into two general groups.

Statistical analysis
We finally examined the results using SPSS 21. The Chi-square test was also used to evaluate the rate of re-pregnancy and different degrees of endometritis and its relationship with different degrees of changes in BCSs. Furthermore, we used the Chi-square test and then the odds ratio to examine the relationship between factors such as endometritis, ovarian activity, ovarian cysts, and the number of inseminations with changes in the BCS. To this end, the cows were grouped into the groups with endometritis and healthy cases, cows with ovaries without significant structures, and cases with ovaries with follicles larger than one centimeter or active corpus luteum, cows without ovarian cysts and ovarian cysts, and finally cows pregnant with maximum three inseminations, and the cows with a need for more than three inseminations for pregnancy, also all the cows were divided into two groups of changes≤ 0.5 and >0.5 in terms of BCS from delivery to 30±3 days of lactation. Furthermore, we utilized the double logistic regression test to examine the relationship of changes in BCS with the number of calving as well as the calving difficulty. We used the one-way ANOVA and the LSD post hoc test to compare the mean number of DO (days open) and the number of inseminations leading to pregnancy in different groups with reduction of the body scores. Finally, we took the advantage of Cox regression and Kaplan-Meier survival plot to examine the chances of re-pregnancy in different groups of changes in the BCS.

RESULTS
We obtained the following results after evaluating the BCS of the cows at the target times and also examining the cases in them as follows separately. The results of chi-square test in Table 1 show that all cows had either reduced scores or were unchanged, and no cows had increased scores Based on these results with a further decrease in BCS between delivery and clean test, the percentage of non-pregnant cases in the herd (regardless of the number of inseminations) increases significantly (P <0.05) (Table 1). Table 2 shows that the more the amount of reduction in the BCSs of the livestock at a certain time interval, the more number of inseminations are needed to pregnancy (P <0.05).

Table 1. Relationship between BCS lost and induction of pregnancy regardless of the number of inseminations in the Holstein cows

| BCS Changes | Non pregnant | Pregnant | Total |
|-------------|--------------|----------|-------|
| 0           | 1 (1.6%)     | 61 (98.4%) | 62 (100%) |
| 0.25        | 7 (2.9%)     | 234 (97.1%) | 241 (100%) |
| 0.5         | 9 (1%)       | 900 (99%)   | 909 (100%) |
| 0.75        | 8 (2.5%)     | 308 (97.5%) | 316 (100%) |
| 1           | 4 (5.7%)     | 66 (94.3%)  | 70 (100%)  |
| 1.25        | 1 (6.7%)     | 14 (93.3%)  | 15 (100%)  |
| Total       | 30 (1.9%)    | 1583 (98.1%)| 1613 (100%)|

Table 2. Relationship between BCS lost and number of AI

| BCS change | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|------------|---|---|---|---|---|---|---|---|---|----|
| 0          | 17 (27.4%) | 20 (32.3%) | 10 (11.2%) | 7 (11.3%) | 5 (8.1%) | 2 (3.2%) | 0 (0%) | 1 (1.6%) | 0 (0%) | 0 (0%) |
| 0.25       | 94 (39%)   | 59 (24.5%) | 27 (11.8%) | 30 (12.4%) | 14 (5.8%) | 9 (3.7%) | 2 (0.8%) | 6 (2.5%) | 0 (0%) | 0 (0%) |
| 0.5        | 259 (28.5%)| 241 (26.5%)| 198 (20.9%)| 101 (11.1%)| 52 (5.7%) | 31 (3.4%)| 17 (1.9%)| 5 (0.6%)| 4 (0.4%)| 1 (0.1%)|
| 0.75       | 76 (24.1%) | 67 (21.2%) | 66 (20.9%) | 50 (15.8%) | 30 (9.5%) | 15 (4.7%)| 8 (2.5%) | 2 (1.4%)| 1 (0.3%)| 1 (0.3%)|
| 1          | 13 (18.6%) | 18 (25.7%) | 11 (15.7%) | 11 (15.7%) | 8 (11.4%) | 3 (4.3%) | 3 (4.3%) | 1 (6.7%) | 2 (2.9%)| 0 (0%) |
| 1.25       | 4 (26.7%)  | 1 (6.7%)  | 2 (13.3)  | 3 (20%)   | 1 (6.7%) | 0 (0%)  | 2 (13.3) | 1 (6.7%) | 1 (6.7%)| 0 (0%) |
| Total      | 463 (28.7%)| 406 (25.2%)| 314 (19.5%)| 202 (12.5%)| 110 (6.8%)| 60 (3.7%)| 32 (2%)  | 16 (1%)  | 8 (0.5%)| 2 (0.1%)|
Table 3 presents different degrees of endometritis in different groups of changes in the BCS. The rate of endometritis significantly increases by increasing negative changes in BCS by 0.5 units or more (P<0.05). The table also indicates that the number of cows with inactive ovaries significantly increases by increasing negative changes in the BCS (P<0.05). The number of AI for pregnancy in two groups by a decrease in BCS less than and equal to 0.5 units and the group with a decrease of more than 0.5 units of BCS was also associated with a statistically significant difference (P<0.05) so that a further decrease in BCS has been associated with a higher number of AI.

The relationship between the number of calving and the rate of decrease in BCS at an interval between calving and clean test

Comparing multiparous cows with the primiparous indicated that there was no significant difference between parity 1 and parity 2, while the BCS lost in parity 3 and 4 were significantly higher than parity 1 (P<0.05) (Table 5).

Table 4 presents the odds ratios of cows for pregnancy with three and less than 3 inseminations, clinical endometritis, and inactive ovaries, and ovarian cyst status, up to about 30 days after calving in both groups of BCS change≤ 0.5 and BCS change>0.5 changes. As shown, the odds ratios of developing clinical endometritis and inactive ovaries in cows with BCS changes <0.5 were significantly greater than the other group (P<0.05). In addition, odds ratios of pregnancy with finally 3 AI in cows with changes in BCS≤ 0.5 were significantly different from cows with higher BCS changes (P<0.05). Table 5 compares two factors of the number of calving and dystocia as predisposing factors to reduce BCS and the odds ratio of reducing BCS by more than 0.5 units per cow according to the factors.
The relationship between parturition type and the rate of BCS lost at 30±3 DIM

Comparing the changes in BCS in different calving models in the present study, as mentioned in the method section, indicated that changes in BCS in cows that gave birth with some help compared to those that gave birth easily and without dystocia were not significant, and their odds ratios in declining the BCS were similar. However, this change in cows with calving difficulty was significantly different from cows with easy calving in a way that cases with dystocia had greater odds ratios for reducing the BCS. (P<0.05) (Table 5). Table 6 compares the average number of days open in different groups of BCS reduction in the first 30 days after calving, and it is found that the BCS reduction more than 0.75, increased the average days open. Besides, the average number of AI in a group with a decrease in BCS of 1.25 was significantly higher than other groups ($P$<0.05).

Survival plot

As shown in the Fig. 1, the blue and green lines, which represent the cases without change in BCS and the least decrease in BCS, respectively, are at the lowest level of the plot compared to other cases, indicating that they were associated with fewer days open and got pregnant earlier. Medians for survival time of Days Open of different BCS change group were 123.41 (108.23-138.60) days ($n=62$, 1.6% censored), 125.38 (116.00-134.77) days ($n=241$, 2.9% censored), 132.35 (127.81-136.88) days ($n=909$, 1.0% censored), 143.72 (135.51-151.94) days ($n=316$, 2.5% censored), 146.88 (128.39-165.38) days ($n=70$, 5.7% censored) and 178.06 (124.79-231.34) days ($n=15$, 6.7% censored) in 0, 0.25, 0.5, 0.75, 1.0 and 1.25 BCS change groups, respectively.

Table 6. Relationship between days open and different degrees of BCS reduction and comparison with unchanged cases in the Holstein cows

| BCS Change | N     | Days open Mean | AI Mean |
|------------|-------|----------------|---------|
| 0.00       | 62    | 122.61 ± 58.75 | 2.58±1.55 |
| 0.25       | 241   | 123.76 ± 70.53 | 2.48±1.73 |
| 0.50       | 909   | 132.05 ± 69.06 | 2.62±1.60 |
| 0.75       | 316   | 142.56 ± 72.05 | 2.97±1.72 |
| 1.00       | 70    | 144.41 ± 75.06 | 3.30±2.00 |
| 1.25       | 15    | 174.53 ± 100.58| 4.00±2.69 |
| Total      | 1613  | 133.44 ± 70.41 | 2.71±1.69 |

a, b, c Values with different superscript letters within a column differ significantly at $P<0.05$

Fig. 1. Kaplan-Meier survival curve for days open of different BCS change group
DISCUSSION

A profitable system for maintaining dairy cows depends mainly on their reproductive performance (Delgado et al., 2004). Numerous factors affect the fertility of cows, including BCS. The effect of BCS has been mentioned in various articles, but the evaluation of BCS has been done at different times, and in general, no specific coherence and effect have been reported in these studies. It is noteworthy that, unlike other studies which focused more on the BCS of each cow, the present study examined the effects of changing BCS at an interval of a month after calving on the reproductive performance.

BCS changes and fertility

For instance, Delgado et al., 2004 found that cow fertility could be affected by the BCS during calving and its changes. Some researchers have found that BCS at calving time does not affect the reproductive performance (Gillund et al., 2001; Buckley et al., 2003; Waltner et al., 1993), while other researchers have reported specific effects of this issue (Roche et al., 2007). According to some researchers, the differences in the above studies may be due to the difference in the number of the samples or the number of times of the BCS evaluation (Delgado et al., 2004). Also, the difference in results of different previous articles may be due to their greater focus on the cow's BCS at a particular time rather than its changes.

Mulliniks et al. (2012) reported that the BCS did not affect the overall pregnancy rate. Ciccioli et al. (2003) also reported that the BCS at calving time did not affect the estrus, ovarian function, or reproductive performance. As we know, the negative energy balance of the early lactation will disappear with the progress in lactation days, and the cows will be in the energy balance status, but the consequences of this imbalance can affect the reproductive performance (McArt et al., 2013;Vanholder et al., 2006), which is difficult to be assessed because other variables can be involved in this issue in addition to nutritional factors. NEB and loss of the BCS are associated with a reduction of progesterone concentrations during the pre-ovulatory period and lower pregnancy rates. In general, a NEB leads to lower fertility in dairy cows. NEB also leads to defects in the secretion of LH and causes negative effects on ovulation because the frequency of LH pulse is significantly lower during the first postpartum follicle, leading to no ovulation in the dominant follicle (Butler, 2003). On the other hand, the ovary responses to LH stimuli also decrease in such conditions. It should also be emphasized that the NEB is associated with lower insulin and glucose (Butler, 2001). The existence of insulin is important to increase the ovarian follicular response to gonadotropins through the reset of LH receptors. Furthermore, the plasma concentration of IGF1, which is directly related to energy status, is vital for the growth of ovarian follicles (Butler, 2003). Blood insulin levels are lower than normal in cows with a reduction of BCS.

In general, the ability of the follicles to produce enough estradiol for ovulation during NEB generally depends on the availability of insulin and IGF1 in serum and changes in energy balance profiles. Under normal conditions in herds, it is impossible to directly assess the NEB in each cow, but a change in the BCS makes an indirect assessment possible. A further reduction in the amount of BCS will further decrease the pregnancy rate (Butler, 2001). Buckley et al. (2003) reported significant effects of body weight changes from breeding time up to 90 days later on the pregnancy rates at the first insemination. About changes in the BCS, Butler and Smith (1989) found that cows lost their BCS from 0.5 to 1 unit at the time interval from calving and the first service, gaining 35% pregnancy rate in the first service, while the rate was 17% in cows that lost more than one unit of BCS. Results of a study by Buckley et al. (2003) emphasized the importance of the BCS in achieving appropriate reproductive performance. Their results were consistent with the present study because it seems that changes in BCS and its reduction indicate the intensity of the NEB that occurs in the early lactation. Most studies also indicate a negative relationship between changes in the BCS and reproductive performance (Stockdale, 2001). Various studies have found that there is a 17-38% higher risk of lower fertility in cows at a rate of 1 unit or more BCS in a 5-point system in the early lactation. It seems that the obvious reduction of the BCS by more than 1.25 units is associated with a reduction of more than 50% in successful conception in the first insemination compared to cows with a lower reduction in the BCS (Gillund et al., 2001).

Gillund et al. (2001) studied the Norwegian dairy cows and claimed that cows with a significant reduction in the BCS in the early lactation got pregnant with a probability of 50% in the first service in comparison with those with a moderate BCS reduction. They also found that the reduction of BCS in the postpartum period was associated with an increase in the
interval between calving and pregnancy. Pryce et al. (2001) found that a one unit increase in BCS at week 10 of lactation was associated with a 6.2-day reduction in the interval between calving to the first service and a 9% greater probability of pregnancy in the first service; and a one unit increase in the BCS during the week 1 to 10 of lactation was associated with a decrease in the interval between calving and the first service by 10.6 days, and a 9% increase in the probability of pregnancy in the first estrus.

Lopez-Gatius et al. (2003) found that the reduction of BCS by 0.5-1 units in the early lactation was not associated with specific effects on the number of days open, but the number of days open increased by 10.6 days in cows with a severe reduction of BCS more than one unit in comparison with cows with lower reduction of the BCS. Drackley (1999) found that the moderate changes in BCS (both decrease and increase) had no specific effects on the number of days open, but a sharp decrease in the postpartum period led to a sharp increase in the number of days open (Drackley, 1999). In a study by Garnsworthy (2006), it was also reported that the loss of 0.5-1 units of BCS was equal to an increase of days open by 3.5 in cows compared to those that lost 0.5 units of BCS. In the present study, as the decrease in body score became higher than 0.75, the number of days open also changed significantly compared to cows without any changes in the BCS, and cows showed a lower probability of getting pregnant than the group without changes.

**BCS changes and clinical endometritis**

For the impact of the BCS on the occurrence of clinical endometritis, it should be noted that the prevalence of clinical endometritis in cows with lower and equal to 2.5 BCS was higher than cows with higher body scores (Carneiro et al., 2014). Of course, the role of BCS on the occurrence of subclinical endometritis has also been mentioned in several studies (Bacha & Regassa, 2010). Priest et al. (2013) reported that the rates of BCS were lower in cows with endometritis. Dubuc et al. (2010) stated that subclinical endometritis was more seen in cows with BCS of less than 2.75. Giuliodori et al. (2013) also reported that cows with a NEB had a higher risk of developing clinical endometritis, and the NEB had detrimental effects on neutrophil function, and thus the uterine health. The above studies have only paid attention to the BCS of cows at the endometritis examination stage, but the present study emphasized the amount of changes in BCS so that the probability of developing endometritis was significantly higher in cows with a reduction in BCS higher than 0.5.

Wathes et al. (2009) reported that the numbers of WBC and lymphocytes were lower in cows with severe NEB in the postpartum period than cows with mild NEB, and the inflammatory responses in the uterus of cows with severe NEB were still active at 2 weeks after pregnancy while the endometrium of cows with mild NEB largely returned to normal. Roche et al. (2013) found that the number of multinucleated cells in uterine secretions was higher in cows with low or medium BCS than those with higher BCS at the first 3 weeks after calving, indicating a higher risk of developing uterine diseases. Çolakoğlu et al. (2019) also mentioned a change in BCS before calving and stated that the change of more than 0.25 units in BCS was accompanied by an increase in BHBA on the 30th day after calving, and as we know, the higher BHBA was associated with a weaker immune response and lower reproductive performance. Furthermore, Galvão et al. (2010) reported that NEFA was higher in cows with subclinical endometritis and metritis than healthy cows. As mentioned earlier, the poor body condition was associated with a higher probability of uterine problems, including clinical endometritis, but it should be noted that none of the above studies investigated the changes in the BCS during the transitional period after delivery like the present study. Results of the present study indicated that postpartum changes could also affect the occurrence of clinical endometritis.

**Parturition type and BCS reduction**

There is little information about the impact of calving difficulty on the postpartum BCS in dairy herds (Berry et al., 2007). Drennan and Berry (2006) studied the broiler cows and reported a greater reduction of BCS in cows with calving difficulty than those without any pregnancy help. It was also reported that the higher BCS during calving and NEB along with a sharp reduction of BCS after calving was associated with an increase in cases of calving difficulty, retained placenta, metritis, etc. (Roche et al., 2009; Garnsworthy, 2006). According to studies, the rate of decrease in BCS after calving was very clear and significant in the group with dystocia. According to Ingvartsen et al., the stress caused by dystocia as well as possible subsequent problems such as retained placenta, metritis, and lameness could affect the food intake of livestock and predisposed it to a sharp decline in BCS.
Parity and BCS changes

The present study also considered the relationship between the parity number and the amount of loss in the BCS on the first 30 days of lactation, as addressed in various studies, and the results of the present study were consistent with the results of most of them. However, it should be noted that in most of the studies cows are often divided into two groups (the first and multiple calving), while in the present study cows were divided into 4 groups (first, second, third, and fourth calving). In general, in cows of third calving onwards with complete growth period, the results were similar to previous studies. In a study by Lee and Kim (2006) and according to the divisions, it was found that the rate of reduction in the BCSs in calving 3, 4, and 5 was significantly higher than the first calving. According to a study by Ettema and Santos (2004), the depletion and recovery of body reserves in the first calving cows were less severe than those with multiple calving. Sakaguchi (2009) found that the parity number could affect BCS changes after calving so that the reduction of BCS at an interval between calving and about 3 weeks later was higher in multiparous cows than primiparous. Piñeyrúa et al. (2018) found that the energy balance was more affected in the multiparous cows, and the amounts of postpartum blood NEFA and BHBA were lower in the primiparous cows than the multiparous. Primiparous cows tended to produce less milk than multiparous cows probably due to the fulfillment of their growth needs. According to a report by Piñeyrúa et al. (2018), the curves of NEFA and BHBA were different in multiparous cows with the primiparous cows as the multiparous cows had 2 peaks in the curve (weeks 1 and 4), while there was only one peak in the primiparous cows probably due to the continuity of fat transfer in multiparous cows in the early lactation. On the contrary to the above content which uses the TMR system, some studies with the grazing system reported that the primiparous cows had a more metabolic problem, less BCS, and lost higher BCS than multiparous cows. (Meikle et al. 2004; Cavestany et al., 2009). In the grazing system, receiving energy and protein were lower than feeding by TMR (Piñeyrúa et al., 2018).

CONCLUSION

According to the present study results, a further reduction in BCS was associated with a reduction in reproductive performance, but there is a need for more studies to investigate the possible reasons for this effect.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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