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ABDULKADİR KESKİN

HIDIR GENÇOĞLU

GÜLNAZ MECİTOĞLU

İSMAİL ÇETİN

ÇAĞDAŞ KARA

See next page for additional authors

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The effects of varying levels of dietary starch on reproductive traits in lactating dairy cows

Abdulkadir KESKİN[1,2,*], Hıdır GENÇOĞLU[3], Gülnaz MECİTOĞLU[1], İsmail ÇETİN[3], Çağdaş KARA[3], Duygu UDUM KÜÇÜKŞEN[4], Ebru BİLEN[1], Barış GÜNER[1], Abdulkadir ORMAN[5], Ahmet GÜMEN[1]
[1]Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, Uludağ University, Görükle, Bursa, Turkey
[2]Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, Kyrgyzstan-Turkey Manas University, Bishkek, Kyrgyzstan
[3]Department of Animal Nutrition and Nutritional Disease, Faculty of Veterinary Medicine, Uludağ University, Görükle, Bursa, Turkey
[4]Department of Biochemistry, Faculty of Veterinary Medicine, Uludağ University, Görükle, Bursa, Turkey
[5]Department of Zootechnics, Faculty of Veterinary Medicine, Uludağ University, Görükle, Bursa, Turkey

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Abstract: The aim of this study was to determine the effects of three different dietary starch levels on postpartum milk yield, blood metabolites, and reproductive traits in cows. A total of 23 primiparous Holstein–Friesian cows were fed three different diets including different starch levels on a dry matter basis from parturition until day 80. Cows were randomly assigned into the three groups according to the starch levels: low (LS, 16%, n = 7), medium (MS, 20%, n = 8), and high (HS, 24%, n = 8). Milk yields were recorded daily, and body condition score (BCS) was determined every week. Transrectal ultrasonography was performed, and blood samples were taken three times each week to determine reproductive traits and blood metabolites. Although the milk yield was greater in the LS group compared to the other groups, BCS and blood metabolites did not differ among the groups. The postpartum follicular patterns, times of first postpartum ovulation, and involution processes did not differ among the groups. In addition, the ovulatory follicle and corpus luteum sizes and the progesterone levels after induced estrus were numerically greater in HS than other groups. Thus, there were no effects of starch levels on postpartum reproductive traits.

Key words: Nutrition, starch, postpartum, reproductive traits, cow

1. Introduction

Milk production and reproduction are the main economic sources in the dairy industry. Specifically, the aim of the dairy industry is to improve profit by maximizing milk yield. Genetic selections and better management options have been used to increase milk production for the last 50–60 years (1). These techniques resulted in increased milk yield; however, the increased milk yield has caused decreased fertility and increased nutritional problems (2,3). Decreasing dry matter intake combined with high milk yield after calving causes a negative energy balance (NEB). Depending on its severity and duration, NEB can be associated with depressed immune systems and some metabolic and infectious diseases, thereby causing infertility in dairy cows (1,4).

Some studies have been performed to determine the effects of NEB on fertility (1–5), indicating that more severe and longer durations of NEB were associated with greater delays to the first postpartum ovulation, extended calving intervals, and decreased pregnancy per artificial insemination (P/AI) rates. Specifically, NEB causes ovarian dysfunction through negative effects on dominant follicle growth (decreases in size). Smaller dominant follicles produce less estradiol, which causes suppression of pulsatile luteinizing hormone (LH) and decreases ovarian responsiveness to LH (6,7). In addition, NEB has negative effects on hormones that regulate the proliferation and steroidogenesis of follicular cells, such as insulin and insulin-like growth factor 1 (IGF-1). These hormones are positively associated with follicular growth and ovulation (7). Thus, the severity and duration of NEB play critical roles in reproductive traits during the postpartum period.

Lactating dairy cows are usually fed high-energy diets, primarily based on high starch contents, to reduce the severity and duration of NEB (3,4,8). Some research (4,9,10) has indicated that feeding cows high dietary starch levels causes increases in plasma insulin and suppresses fat mobilization through the antilipolytic effects of insulin.

* Correspondence: kadirk@uludag.edu.tr

Therefore, reducing the negative effects of NEB may result in improved fertility. However, some other studies (2,3) showed that high starch levels in diets induced high plasma insulin levels, but did not affect follicular growth and had negative effects on oocyte quality (11). Thus, there is disagreement regarding the levels of dietary starch that should be used to improve reproductive parameters in lactating dairy cows. The aim of this study was to determine the effects of different dietary starch levels on postpartum ovarian follicular development, first postpartum ovulation day, involution of the genital tract, ovulation time and ovulatory follicle size after induced estrus, luteal size after ovulation, and the increase in progesterone levels 7 days after ovulation.

2. Materials and methods

2.1. Animals, housing, and nutrition

This study was performed at the Research and Application Farm of the Faculty of Veterinary Medicine at Uludağ University from September 2012 to May 2013. The Animal Experimentation Ethics Committee of Uludağ University approved all the experimental procedures. A total of 23 primiparous Holstein–Friesian cows were included in the study following parturition, and the cows were randomly assigned to one of three groups according to their feeding strategy. The cows in the low (LS, n = 7), medium (MS, n = 8), and high (HS, n = 8) starch groups were fed diets containing 16.3%, 20.1%, and 24.5% starch on a dry matter basis, respectively. Cows were assigned to the experimental diets immediately after calving and remained on their respective diets until 80 days in milk (DIM). Ingredients and chemical compositions of the diets were determined according to NRC recommendations (12) and are presented in Table 1. The nutrient requirements of cows were calculated on body weight basis that was recorded weekly throughout the trial. Cows were individually fed the total mixed ration once daily (0900 hours) to allow for ad libitum consumption and animals were allowed access to feed at all times, except during milking times. The orts samples were collected to determine dry matter intake

Table 1. Ingredients and chemical compositions of the diets.

	Low starch	Medium starch	High starch
Ingredients (% of DM bases)			
Corn silage	30.0	30.0	30.0
Alfalfa hay	20.0	20.0	20.0
Ground corn	4.0	5.9	10.9
Ground barley	4.2	6.2	10.2
Soybean meal	9.0	9.3	10.6
Corn dry distillers grains plus soluble	12.5	10.2	9.4
Wheat bran	10.2	14.1	5.9
Soybean hulls	4.9	0.0	0.0
Bypass fat	2.2	1.3	0.0
Molasses	1.7	1.7	1.7
Limestone	1.07	1.07	1.07
Vitamin and mineral supplement	0.05	0.05	0.05
Salt	0.18	0.17	0.18
Chemical composition			
Crude protein, DM %	17.4	17.2	17.0
Ether extract, DM %	6.1	5.1	3.70
NDF, DM %	39.0	37.5	35.4
ADF, DM %	22.3	20.7	20.1
Starch, DM %	16.3	20.1	24.5
NFC[1], DM %	33.6	36.1	39.7
NE_L[2], Mcal/kg DM	1.63	1.61	1.59

[1] NFC (nonfiber carbohydrate) = 100 – (% NDF + % CP + % ether extract + % ash).
[2] Net energy for lactation, estimated using NRC (2001).

(DMI) that was measured daily for individual cows during the whole experimental period. The cows had unlimited access to water and they were milked three times daily (0600, 1400, and 2200 hours), and milk yield was recorded daily. Body condition score (BCS) was measured every other week thereafter until 80 DIM by the same researcher, who was blinded to the groups. BCS was assessed using a scale from 1 to 5, with 1 = very thin and 5 = obese (13).

2.2. Ultrasonographic examinations

Examinations were performed three times a week during the study from day 6 to 80 days postpartum.

2.2.1. Monitoring postpartum follicular dynamics

To monitor the postpartum follicular dynamics, the ovaries of all of the cows were examined using ultrasonography (IBEX Pro with a 5–7.5 MHz transrectal probe, Promed, Turkey) three times per week during the first 50 days postpartum. The ovaries and/or ovarian structures were measured and recorded to create individual ovarian maps. Follicular waves were monitored by determining the point when the cohort follicles began to increase in size between ultrasonographic examinations, after which they differentiated into the subordinate stage and, subsequently, into a single dominant follicle. The end of a follicular wave was considered to occur when the diameter of the dominant follicle began to decrease due to the regression phase in an anovulatory follicular wave or when ovulation of the dominant follicle occurred (7). The lengths of follicular waves with or without ovulation and the durations of luteal phases in cows that ovulated were determined and recorded. The first postpartum ovulation was characterized by the disappearance of the dominant follicle and the appearance of a new corpus luteum in the following days. Ovulation was also confirmed through serum progesterone levels above 1 ng/mL in serum samples that were taken on the days of examinations.

2.2.2. Monitoring of involution process of genital tract

Monitoring of the involution process was performed once a week using transrectal ultrasonography (IBEX Pro with a 5–7.5 MHz transrectal probe). To monitor the involution process, the cervical diameters were measured and recorded over the first 7 weeks postpartum. Measurements of the uterine horns began in the third postpartum week. The cervical diameters and uterine horns were measured by placing the transducer longitudinally over the middle of the cervix (14) and transversally ~2 cm from the internal uterine body bifurcation (15).

2.2.3. Estrus synchronization and determining of ovulation time, ovulatory follicle size, and luteal size

To determine the effects of dietary starch levels on ovulation times and ovulatory follicle sizes during estrus and luteal sizes after ovulation, estrus was synchronized at 60 days postpartum. GnRH (Buserelin acetate, 10 µg, im, Receptal, Intervet, İstanbul, Turkey) and CIDR (Eazi-Breed, 1.38 g progesterone, Zoetis, İstanbul, Turkey) were administered at 60 days and PGF2α (Cloprostenol, 500 µg, im, Estrumate, CevaDIF, İstanbul, Turkey) was administered and the CIDR was removed at 67 days. Scans of the cows began 1 day after the PGF2α injections; ultrasonography was performed every 6 h to determine ovulation. Ovulatory follicle sizes were recorded; ovulation was characterized by the disappearance of the ovulatory follicle. Luteal sizes were measured using ultrasonography every day until 7 days after ovulation.

2.3. Blood sampling

Blood samples were obtained from the coccygeal vein three times a week during the study from day 6 to day 80 postpartum. Blood samples were collected using evacuated tubes with and without EDTA for plasma and serum, respectively. All of the tubes for both serum and plasma were kept on ice or in a refrigerator before being centrifuged at 3000 rpm for 15 min at 4 °C; serum and plasma samples were stored at –20 °C until the analyses were performed.

2.3.1. Measurements of progesterone, IGF-1, and insulin levels

The first postpartum ovulation and ovulation times of ovulatory follicles after induced estrus were confirmed through progesterone levels of greater than 1 ng/mL in serum samples. During the study, plasma IGF-1 and insulin levels were determined at the same sampling times as progesterone. Blood samples were taken daily until 7 days after ovulation to determine the postovulatory levels of progesterone after induced estrus. Serum progesterone (Bovine Progesterone Kit, ELISA, Demeditec Diagnostics GmbH, Kiel, Germany), plasma IGF-1 (Bovine IGF-1 Kit, ELISA, Demeditec Diagnostics GmbH), and plasma insulin (Bovine Insulin Kit, ELISA, Cusabio, China) levels were determined by ELISA (ELX808IU Ultra Microplate Reader, Bio-Tek Instruments, Inc.) according to the manufacturer's instructions.

2.3.2 Determination of nonesterified fatty acid (NEFA) and beta-hydroxybutyric acid (BHBA) levels

NEFA and BHBA levels were measured every week during the study period before and 4 h after each feeding, respectively. Plasma NEFA (Bovine Non-Esterified Fatty Acid ELISA Kit, Cusabio) and BHBA (β-OHB ELISA Kit, Cusabio) levels were determined by ELISA according to the manufacturer's instructions.

2.4. Statistical analyses

A general linear model (GLM) using SAS software (Version 9.2, SAS Institute Inc., 2010) was employed to statistically analyze the experimental data. The differences between the treatment means were separated by Duncan's multiple comparison test. Moreover, a logistic regression test was used to determine the interactions between blood metabolites and time, milk yields and BCS, and milk yields and blood metabolites. The chi-square test was also used to compare percentages of ovulatory and anovulatory cows between the groups.

3. Results

In all of the cows, milk yield increased gradually within the first 2 weeks of calving and reached a constant value at 5 weeks after calving, as shown in Figure 1. The average daily milk production (kg/day) during the study was 27.73 ± 0.20 for the LS group, 25.81 ± 0.19 for the MS group, and 24.45 ± 0.19 for the HS group. Milk production was found to be different between the groups, with cows in the LS group producing more milk ($P < 0.01$) than other groups. The DMI was different in groups ($P < 0.05$) and it averaged 21.0, 19.8, and 19.0 kg/day for cows fed the LS, MS, and HS diets respectively.

However, there were no detectable interactions between milk yield and BCS or blood metabolites. During the study, there were no significant effects ($P > 0.05$) of different starch levels on BCS (2.79 ± 0.05 in LS, 2.77 ± 0.05 in MS, 2.75 ± 0.04 in HS). No treatment or times × treatment interactions were detected from the measured blood metabolites (insulin, IGF-1, NEFA, and BHBA).

The mean size of the largest follicle during the first week postpartum (6 ± 1 days postpartum) was 9.6 ± 0.5 mm; this did not differ significantly ($P > 0.05$) among the groups. The times to the emergence of the first follicular wave after parturition (8.83 ± 1.0 days, 9.37 ± 0.95 days, and 10.12 ± 0.95 days in the LS, MS, and HS groups, respectively) did not differ significantly ($P > 0.05$) between the groups. In addition, the length of the first follicular wave tended to be shorter ($P < 0.08$) in the LS group (7.33 ± 1.29 days) than in the other groups (11.25 ± 1.11 days in the MS group and 10.25 ± 1.12 days in the HS group, respectively). The maximum sizes of the dominant follicles in the first follicular wave (13.00 ± 0.83 mm, 14.43 ± 0.72 mm, and 13.06 ± 0.72 mm in the LS, MS, and HS groups, respectively) were found to be similar between the groups. Similarly, when subsequent follicular waves were evaluated with respect to their lengths and maximum dominant follicle sizes, there were no significant differences ($P > 0.05$) between the groups (Table 2).

The calving to first ovulation intervals (18.20 ± 4.75 days, 27.60 ± 4.75 days, and 25.25 ± 5.31 days in the LS, MS, and HS groups, respectively) and maximum ovulatory follicular sizes (13.00 ± 1.85 mm, 14.60 ± 1.85 mm, and 16.87 ± 2.07 mm in the LS, MS, and HS groups, respectively) were found to be similar. Although a higher percentage of anovulatory cows were detected in the HS group before the 50th day postpartum (50.0%, 4/8), there were no significant differences ($P > 0.05$) between the groups (28.6%, 2/7 in the LS group and 37.5%, 3/8 in

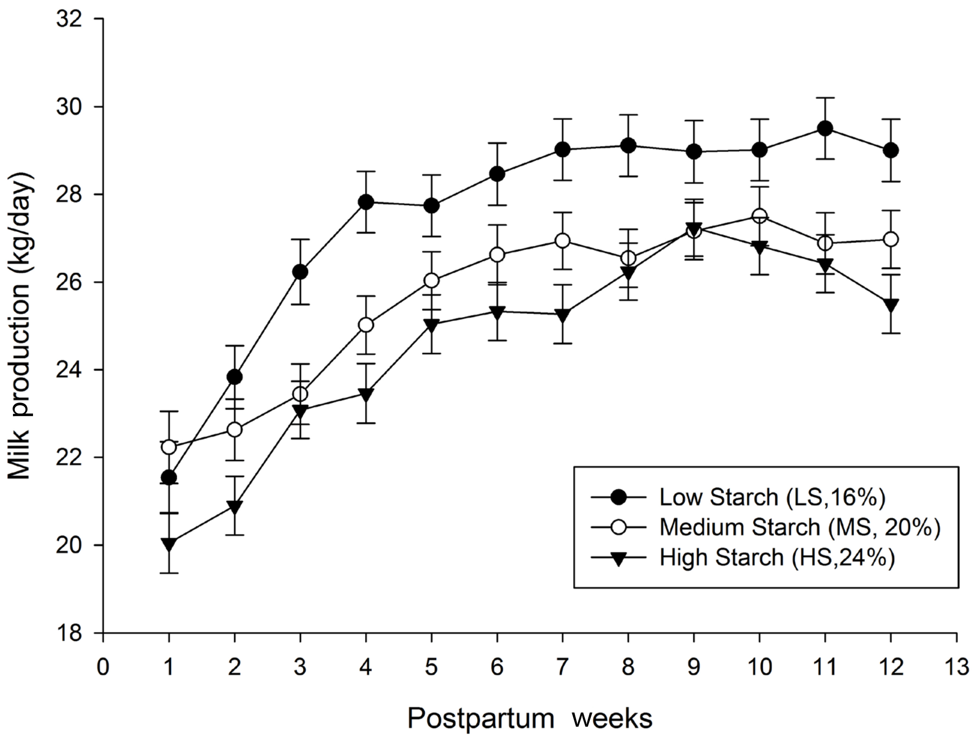

Figure 1. Milk production over 12 weeks by group.

Table 2. Follicular patterns following the first postpartum follicular wave.

Groups*	LS group	MS group	HS group	P-value
Length of follicular waves (days)				
2nd wave	10.83 ± 1.63	11.57 ± 1.51	11.87 ± 1.51	0.89
3rd wave	10.33 ± 1.51	10.00 ± 1.39	10.28 ± 1.39	0.98
4th wave	10.77 ± 1.27	9.66 ± 2.20	8.75 ± 1.90	0.99
5th wave	10.00 ± 0.70	9.50 ± 0.50	10.72 ± 0.52	0.81
Maximum dominant follicle size of waves (mm)				
2nd wave	14.83 ± 1.54	15.57 ± 1.42	14.92 ± 1.42	0.92
3rd wave	15.50 ± 0.97	15.00 ± 0.90	13.78 ± 0.90	0.42
4th wave	13.61 ± 0.74	14.16 ± 1.29	13.50 ± 1.11	0.90
5th wave	14.00 ± 1.07	15.25 ± 0.75	16.00 ± 1.06	0.59

*LS: Low starch (16%), MS: medium starch (20%), HS: high starch (24%).

the MS group). In addition, the numbers of anovulatory follicular waves during the postpartum period did not differ significantly (P > 0.05) between the groups (2.66 ± 0.68, 1.57 ± 0.44, and 2.00 ± 048 in the LS, MS, and HS groups, respectively). However, two luteal phases were detected in the groups prior to 50 days postpartum. When the luteal phase durations in the groups were evaluated, it was found that the average durations did not differ significantly (P > 0.05) between the groups (durations of first and second luteal phases: 15.28 ± 1.86 and 18.66 ± 1.43 days, 16.66 ± 2.01 and 21.50 ± 2.48 days, and 16.75 ± 1.74 and 18.33 ± 2.02 days in the LS, MS, and HS groups, respectively).

Cervical diameters in the first week postpartum were measured as 47.25 ± 4.3 mm, 40.33 ± 4.9 mm, and 47.20 ± 3.8 mm in the LS, MS, and HS groups, respectively. The cervical diameters following the first week postpartum did not differ significantly (P > 0.05) among the groups. In addition, cervical measurements during the involution period were found to be similar between the groups in the following 7 weeks (Figure 2). The diameters of the right and left uterine horns at the third week postpartum were 11.67 ± 1.03 and 10.50 ± 1.02 mm in the LS group, 14.28 ± 0.96 and 13.85 ± 0.94 mm in the MS group, and 12.28 ± 0.96 and 11.85 ± 0.94 mm in the HS group, respectively. There were no significant differences (P > 0.05) between the sides of the uterine horns or between the groups in the involution process.

When ovulation times and ovulatory follicle sizes were evaluated after estrus was induced at 60 days postpartum, the ovulation times of the dominant follicles were similar between the groups (Table 3). The sizes of the ovulatory follicles and the corpus luteum were found to be greater in the HS group than in the LS and MS groups, but the differences were insignificant (P > 0.05, Table 3). The percentage of anovulatory cows also did not differ significantly (P > 0.05) among the groups (Table 3). In addition, the increases in progesterone levels were evaluated from ovulation to 7 days after ovulation; although increasing serum progesterone levels were greater in the HS group than in the other groups, the differences were insignificant (P > 0.05, Figure 3).

4. Discussion

The aim of this study was to determine the effects of 3 different dietary starch levels on milk production, blood metabolites, and reproductive traits. The main results of this study were that blood metabolites and reproductive traits were found to be similar in all of the groups even though milk production was higher in the LS group than in the others. The high milk yield in the LS group can be explained by its greater dry matter intake in comparison to the other groups. The DMI for cows fed the LS diet was 1.2 and 2.0 kg/day greater than for cows fed the MS (P < 0.05) and HS (P < 0.01) diets, respectively.

Although gonadotropic hormones (follicle-stimulating hormone (FSH) and LH) are released by the hypothalamus and pituitary gland, the ovaries do not respond to them during the early postpartum period (16) because the high progesterone levels present during late gestation cause a decrease in ovarian responsiveness to gonadotropic hormones (17). The average initiation time of ovarian responsiveness to gonadotropins and the initiation time of the first follicular wave is 7–10 days after parturition

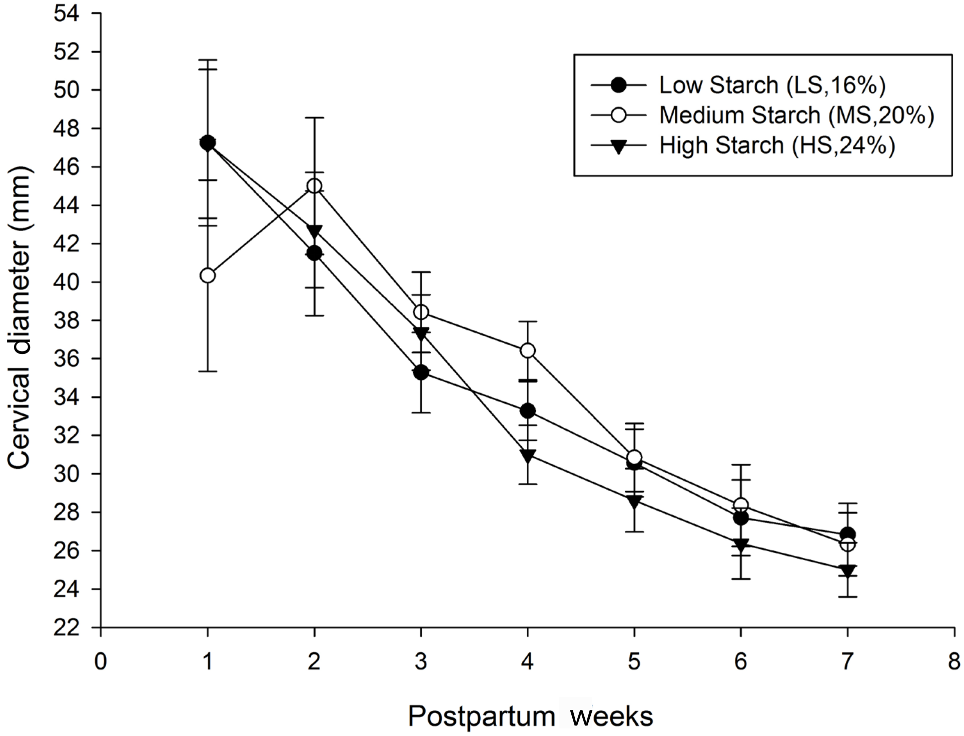

Figure 2. Involution of cervix uteri during the postpartum period.

Table 3. Ovulation times, ovulatory follicle, and CL sizes and progesterone levels after ovulation.

Groups*	Ovulation time (h)	Maximum follicle size (mm)	Maximum corpus luteum size (mm)	Anovulatory cows (%)
LS (n = 7)	95.00 ± 13.5	14.58 ± 0.67	21.00 ± 1.37	42.8% (3/7)
MS (n = 8)	91.67 ± 11.1	15.57 ± 0.62	24.60 ± 1.25	25.0% (2/8)
HS (n = 8)	92.80 ± 12.1	16.60 ± 0.74	23.70 ± 1.37	37.5% (3/8)
P-value	0.98	0.16	0.17	0.86

*LS: Low starch (16%), MS: medium starch (20%), HS: high starch (24%).

(4,7,16–18). Problems that occur during the transition period can delay the initiation of follicular activity (16,17). However, some authors have indicated that the initiation of the first follicular wave is not affected by problems during the transition period (17). As was observed in previous studies (4,16,18), the average initiation time of the follicular wave in the present study was 9 days after parturition (8.8 days, 9.4 days, and 10.1 days in the LS, MS, and HS groups, respectively), and it did not differ between the groups. These results demonstrated that different dietary starch levels did not affect the timing of the onset of the first follicular wave.

Although many factors affect the resumption of postpartum cyclicity, the maximum follicular size in the first follicular wave is important to the onset of cyclicity in dairy cows. The size and ability of the first dominant follicle to ovulate postpartum are the decisive factors (7,19). It is known that the ovulatory follicle size must be greater than 10 mm (7,16). In the present study, the preovulatory follicle size at the first postpartum ovulation did not differ among the groups; the preovulatory follicle size was 13.00 mm in the LS group, 14.60 mm in the MS group, and 16.87 mm in the HS group. This indicates that, similar to the findings of Dyck et al., (19), starch/energy levels did not affect the ovulatory follicle size at the first postpartum ovulation. In addition, the maximum follicle size in the first follicular wave and the following waves is important to cyclicity in lactating dairy cows. In one study, the maximum follicle

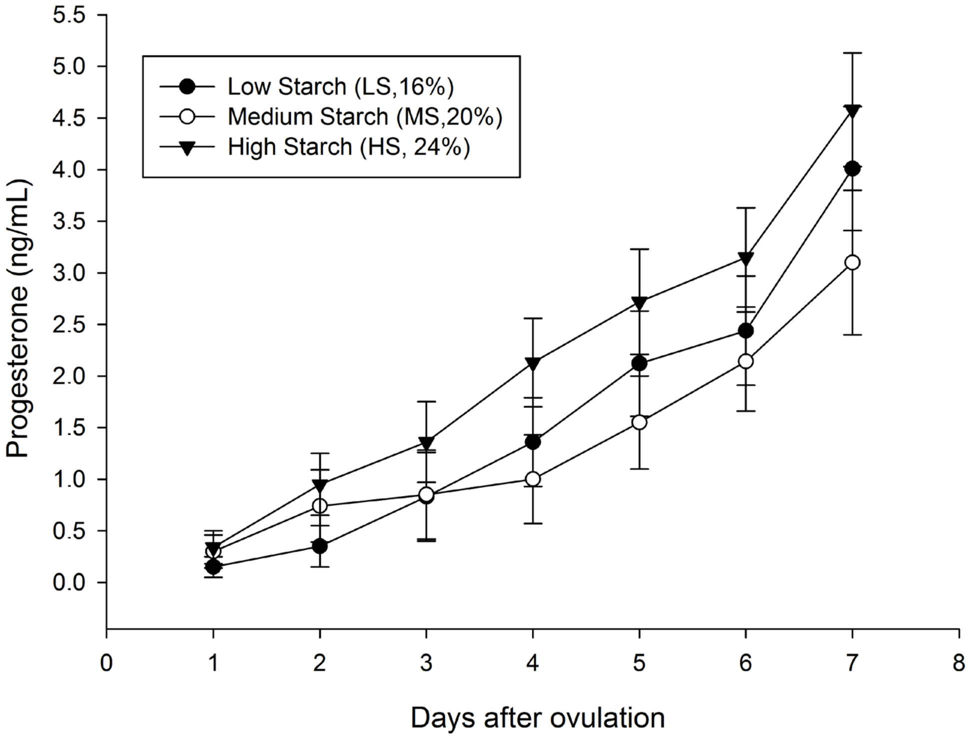

Figure 3. Progesterone levels 7 days after ovulation by group.

size was greater in cows that were fed high-energy diets (11.0 mm) than in heifers that were fed low-energy diets (8.1 mm) (11). Rizos et al. (18) showed that the maximum follicle sizes in the first follicular waves after parturition were, on average, 16.8 mm and 18.3 mm in high-energy and control groups, respectively, and differences were found to be insignificant. In one study, Dyck et al. (19) found that the maximum follicle size in the first follicular wave was approximately 17.5 mm, and different dietary starch levels (these were 23.3%, 25.2%, and 26.7% in the groups in the study) did not affect ovarian dynamics. We found a smaller maximum follicle size in the first follicular wave than these studies, with no differences among the groups. With these results, it can be stated that different dietary starch levels have no effect on the first postpartum follicular wave.

Many researchers have indicated that NEB causes a decrease in critical hormones such as plasma insulin, glucose, and IGF-1, which diminishes both GnRH and gonadotropic (FSH and LH) hormones (1,2,17,20). These negative effects impede the initiation of follicular waves (16,17). The initiation of antral follicle growth, the subsequent selection of a dominant follicle, and ovulation or atresia of the dominant follicle depends on gonadotropic hormones (7,16). The length of the first follicular wave following parturition is determined by FSH and LH (11,17). Many studies have indicated that LH causes the dominant follicle to grow after selection and promotes its growth until it reaches ovulatory size. It is known that NEB decreases the amount of LH released and negatively affects positive feedback (17). Therefore, NEB has an important effect on the stage of the growing follicle. Wiltbank et al. (7) classified follicles according to their stages of growth as follows: a) growth from selection to deviation (5–10 mm); b) growth from deviation to ovulatory size (10–18 mm); and c) greater than the ovulatory size (>20 mm). As shown in Table 2, our study demonstrated that the sizes of dominant follicles of follicular patterns (between 13 and 16 mm) until 50 days postpartum reached ovulatory size (10–18 mm) as classified by Wiltbank et al. (7), and there were no differences among the groups. Our results could indicate that NEB has the same effect on cows in all three groups or that dietary starch levels did not affect follicular growth. The IGF-1, insulin, NEFA, and BHBA levels also supported these results, as blood metabolite levels did not differ among the study groups. One study (18) that focused on the negative effects of NEB on the follicular wave tested the effects of administering propylene glycol against NEB on follicular wave during the postpartum period. The results of that study showed that high-energy diets did not have positive effects on the follicular wave.

In addition, it was previously reported that the length of the follicular wave is approximately 7–10 days in lactating dairy cows (7,18,20,21). As in previous studies, the present study found that the duration of follicular waves was detected between 8.5 and 11.8 days in follicular patterns and lengths of follicular wave in both groups were found to be similar.

The early onset of cyclicity during the postpartum period is critical to the reproductive performance of lactating dairy cows (5,17,22). On average, 80% of cows are cyclic by 50–60 days following parturition (22–24). It was previously reported that the first postpartum ovulation occurs approximately 10–26 days after parturition (7,22,23). This study observed first postpartum ovulation times of 18.2 days in the LS group, 27.6 days in the MS group, and 25.2 days in the HS groups, with the differences found to be insignificant. These results were found to be similar to those of previous studies (7,22,23). In addition, one study that evaluated the effect of high-energy diets on the onset of cyclicity (4) showed that high-energy diets induced cyclicity earlier in cows that had both high (41 days) and low (28 days) milk production compared to a control group (54 vs. 43 days, respectively). The authors emphasized that high-energy diets increased plasma insulin levels, causing cyclicity to begin earlier than in the control group. In contrast to our study, one study showed that increasing starch levels in diets reduced the time from calving to first ovulation (19), with the first ovulation detected at 30.6 days postpartum. However, the first ovulation times in this study were found to be 18.2 days in the LS group, 27.6 days in the MS group, and 25.2 days in the HS group, and the first ovulation occurred earlier than in that study regardless of starch levels. Differences between studies might be due to levels of milk production.

Noncyclic conditions in dairy cows are one of the main causes of infertility in cows and economic losses in the dairy industry (22). Previous studies have indicated that the incidence of noncyclic cows was between 13% and 44% (22,24,25). In this study, the percentages of noncyclic cows prior to 50 days postpartum were found to be 28.6%, 37.5%, and 50% in the LS, MS, and HS groups, respectively. These results were similar to those of previous studies (22,24,25). However, Gilmore et al. (5) found that the percentage of noncyclic cows was approximately 10% between 40 and 50 days postpartum in their high-energy group (high-starch/high-fat group). However, the authors did not find differences between the groups, and they indicated that high-energy diets did not affect the same reproductive traits.

Uterine and cervical involution is typically completed within 5–7 weeks postpartum (26). This period can be confirmed using transrectal ultrasonography (26,27). In this study, the involution periods of the cervixes and uteri were monitored using ultrasonography during the first 7 weeks postpartum, and the involution periods were found to be similar between the groups. Previous studies indicated that many factors affect involution, including uterine infections, parity, and seasons (27). One study indicated that suppression of the early resumption of cyclicity improved uterine involution in cows with and without uterine diseases (28). In this study, the time of the first postpartum ovulation was determined to have occurred on approximately the expected day (7,16,22). Although the number of cyclic cows was greater than the number of noncyclic cows, though not statistically so, there were no differences in involution periods of the cervixes and uteri between the groups, which is similar to the findings of Heppelmann et al. (28). Thus, the involution periods of the cervixes and uterine horns were normal, as expected, in all of the groups. However, another study indicated that, despite marked differences in metabolic statuses caused by lactation, uterine involution periods were found to be similar among lactating and nonlactating cows (27). This means that NEB cannot affect uterine involution periods. Similarly, our results showed that different dietary starch levels in groups did not affect involution periods.

The other results in this study were ovulation times and ovulatory follicle sizes after induced estrus, maximum corpus luteum sizes, and progesterone and insulin levels 7 days after ovulation. Our results indicated that even though follicle sizes did not differ among the groups, larger follicle sizes produced larger corpus luteum sizes and progesterone levels, as shown in Figure 3. Armstrong et al. (11) indicated that high-energy diets increased ovulatory follicle sizes; consequently, progesterone levels were greater. As in this study, even though there were no differences between groups, cows in the HS group had larger follicle and CL sizes and higher progesterone levels than other groups. This result may be associated with fertility in cows. Some studies have demonstrated that larger ovulatory follicle sizes increased pregnancy rates (29,30). Larger follicle sizes were found to be associated with higher estradiol and progesterone levels and larger corpus luteum sizes in these studies. Higher estradiol levels improve gamete transportation in the reproductive tract and the uterine environment needed for early embryonic development (29,30). In addition, larger corpus lutea, which form following ovulation of larger follicles, are capable of producing more progesterone. High progesterone levels are a decisive factor for embryonic survival in lactating dairy cows.

In conclusion, in this study, different dietary starch levels did not affect blood metabolites or early or late postpartum reproductive traits. However, milk yield was found to be higher in the LS group than in other groups.

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