Turkish Journal of Veterinary & Animal Sciences

Volume 32 | Number 2 | Article 6

1-1-2008

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Turk. J. Vet. Anim. Sci.
2008; 32(2): 99-106

Research Article

The Effects of a Nutritionally Unbalanced Diet on Some Blood and Postpartum Fertility Parameters in Dairy Cows

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Received: 20.12.2006

Abstract: This study was carried out to determine the effects of mineral imbalances and negative energy balance on reproductive functions during the early and later stages of the postpartum period in dairy cows. The study included 100 randomly selected multiparous Holstein cows aged between 2 and 8 years (mean weight: 650 kg). The animals' diets were not manipulated and they were fed the rations used by the study farm. Blood samples were taken on postpartum days 0, 20, 24, 30, 31, 38, 45, 60, and 90. Cows with calving and/or puerperium problems were recorded. Analyses were made for non-esterified fatty acids, calcium, phosphorus, magnesium, urea nitrogen, SGOT (AST), and progesterone levels in the blood. Non-esterified fatty acid levels in blood serum were 670, 660, 660, 620, and 640 µmol/l on postpartum day 0, 20, 30, 60, and 90, respectively. It was assumed that those levels might be indicators of significant negative energy levels in the rations. The Ca:P ratio in blood samples was 1:1.25. In all, 31% of the animals' serum progesterone levels increased until the 45th postpartum day, whereas progesterone levels were below 1 ng/ml in 69% of the animals. The fertility parameters of the cows with or without postpartum problems were, respectively, as follows: calving to first service interval: 102.44 ± 36.41 and 108 ± 28.61 days; calving to conception interval: 132.69 ± 34.00 and 128.63 ± 35.50 days; services per conception: 2.00 ± 0.70 and 2.12 ± 0.71; repeat breeder rate: 33.3% and 37.93%. These parameters indicate infertility and economic lose for the study farm.

Key Words: Dairy cow, unbalanced ration, negative energy balance, postpartum fertility

Süt İneklerinde Dengesiz Beslemenin Bazı Kan Parametreleri ile Postpartum Fertiliteye Etkisi

Özet: Çalışmada, yüksek verimli süt ineklerinde beslenmeye bağlı olarak şekillenen ve subklinik seyreden mineral madde dengesizlikleri ile negatif enerji dengesinin erken ve ileri postpartum dönemde bulunan ineklerdeki üreme fonksiyonlarına etkileri araştırıldı. Bu amaçla, özel bir sütçü inek işletmesinden, random yöntemiyle seçilen 2-8 yaşlı, ortalama 650 kg ağırlığında, 100 baş Holstein materyal olarak kullanıldı. İşletmenin uyguladığı rasyonlarda herhangi bir değişiklik yapılmadı. Deneme hayvanlarından kan örnekleri, postpartum 0. , 20., 24., 30., 31., 38., 45., 60. ve 90. günlerde alındı. Doğum ve/veya puerperum sorunları gösteren inekler kayıt edildi. Kan örneklerinde esterleşmemiş yağ asitleri, kalsiyum, fosfor, magnezyum, üre azotu, SGOT (AST) ve progesteron hormonu analizleri yapıldı. Bu çerçevede kan serumunda esterleşmemiş yağ asitleri (EYA) düzeyleri post partum 0., 20., 30., 60. ve 90. günlerde sırasıyla, 670, 660, 660, 620 ve 640 µmol/l olarak saptandı. Söz konusu değerlerin önemli derecede negatif enerji dengesinin bir göstergesi olabileceği kanısına varıldı. Kan serumu örneklerinde Ca/P oranının 1/1.25 arasında olduğu saptandı. Deneme hayvanlarından % 31'inde postpartum 45. güne kadar serum progesteron değerlerinde yükselme izlenirken % 69'unda progesteron değerleri 1 ng/ml'nin altında seyretti. Fertilite parametreleri, postpartum sorunlu ve sorunsuz ineklerde sırasıyla, doğum-ilk tohumlama süresi 102,44 ± 36,41 ve 108 ± 28,61 gün, doğum-gebe kalma aralığı 132,69 ± 34,00 ve 128,63 ± 35,50 gün, gebelik başına tohumlama sayısı 2,00 ± 0,70 ve 2,12 ± 0,71 adet, repeat breeder inek oranı % 33,33 ve % 37,93 olarak hesaplandı. Anılan parametreler sürü fertilitesine ilişkin olarak infertilite ve ekonomik kayıplara işaret etmektedir.

Anahtar Sözcükler: Süt ineği, dengesiz rasyon, negatif enerji dengesi, postpartum fertilite

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Introduction

In dairy cows milk yield usually peaks between postpartum weeks 4 and 8, but maximum dry matter intake usually peaks between postpartum weeks 10 and 14 (1). Nonetheless, dry matter intake needs to be maximized as soon as possible after calving because cows must ingest nutrients sufficient for high milk yield and for resumption of follicular activity so that they can conceive within 12-16 weeks after calving. Negative energy balance (NEB) usually occurs during postpartum weeks 1-8, when milk yield is the highest. NEB has a variable duration depending on the severity of the imbalance (2).

At least 80% of high-yielding dairy cows have NEB during early lactation; therefore, the postpartum anestrous period is extended and survival of embryos during implantation is negatively affected. Cow nutrition plays an important role in follicular growth and maturation, and ovulation capacity. NEB extends the time between calving and first ovulation time. It is known that NEB decreases the pulsatile secretion of LH (3). Similarly, it increases the number of medium-size follicles and decreases the number of large follicles; it also decreases corpus luteum functioning. There is an insufficient quantity of data concerning the effect of energy balance on the fertilization capacity of oocytes (4).

In cows with NEB the risk of metritis, cystic ovaries, and anestrous is high. Due to a reduction in the LH level and response against this hormone, fewer follicles ovulate. This also reduces the likelihood of embryo survival (5).

It was stated that there was a high correlation between the amount of non-esterified fatty acids (NEFA) and energy balance; therefore a high NEFA value is a significant indicator of NEB. As a matter of fact, fatty acid mobilization begins just before the lactation period because of NEB and hormonal changes. Before calving, the level of fatty acid mobilization is determined by the concentration of NEFA in blood. After calving, there is a positive correlation between NEFA concentration and metabolic diseases (6). When calving approaches, an increase in the plasma NEFA value is considered to be dependent on hormonal changes, pregnancy-based stress, and a decrease in dry matter (DM) intake (7).

Among the factors that cause negative changes to fertility parameters are the feeding program, high protein content of rations, fatty liver syndrome, low body condition score, high milk yield, dystocia, placental retention, metritis, cystic ovaries, clinical mastitis, hypocalcemia, hypoglycemia, and abomasal displacement (8). Following insemination the main problem with cows that don't become pregnant is that either fertilization did not occur or repeat breeder syndrome caused by early embryonic death occurs. This syndrome has a varied etiopathogenesis, including the lack of nutrition and nutritional imbalance (9).

The purpose of this study was to determine the effects of mineral imbalances and NEB, in relation to the feeding program, on some blood parameters and reproductive functions of high-yielding cows in the early and later postpartum periods.

Materials and Methods

Animals: The study included 100 multiparous Holstein cows aged between 2 and 8 years (mean weight: 650 kg) that were selected with the random parcels method from among the cattle of a private dairy farm located in Karacabey, Bursa, Turkey. Contents of the rations fed to the experimental animals remained unchanged throughout the experiment. The animals were housed in a free stall system and were placed in different paddocks depending on their milk yield and lactation days.

Feed Material: Animals were fed a total mix ration (TMR) calculated by a computer program according to their milk yield. During the experimental period the following feeding programs were administered to the animals according to their yield periods. Accordingly, during the first 70 days of lactation (early lactation period) ration 1 was supplied to cows with a 30-kg milk yield. On the 70th-140th days of lactation the cows were divided into 2 groups according to their yield and ration 2 (milk yield over 25 kg) was administered to the first group and ration 3 (average 22-kg milk yield) was administered to the second group. The cows in the late lactation period (between 140 days and dry period) were fed ration 4. According to the feeding program, cows with > 28-kg milk yield between lactation days 0 and 70 and those with > 26-kg milk yield in the high yield group between the days 70 and 140 were given 1 kg of concentrate feed for each 2 kg of milk. The composition of the rations given to the animals is presented in Table 1.

Table 1. The constituents of the rations calculated for each animal (kg/head/day).

	Ration 1 (0-70 days, early lactation)	Ration 2 (70-140 days, high milk yield group)	Ration 3 (70-140 days, moderate milk yield group)	Ration 4 (between 140 days and dry period)
Alfalfa hay	5.06	4.47	-	-
Corn silage	16.31	17.79	16.46	12.94
Straw	-	1.51	4.27	5.02
Corn	2.27	2.37	-	-
Barley	3.95	3.10	2.21	1.15
Sunflower seed meal	-	1.51	2.36	1.12
Cotton seed meal	3.16	2.41	0.79	1.41
Wheat middlings	-	2.39	3.37	3.71
Bypass oil	0.35	-	-	-
Limestone	0.11	0.10	0.11	0.10
$NaHCO_3$	0.18	0.22	0.22	-
Salt	0.05	0.05	0.05	
Vitamin premix	0.10	0.10	0.10	0.10

Feed Analysis: The quantity of nutrients in the roughage and concentrates was determined according to the analysis methods of AOAC (10), and metabolizable energy content was calculated with the method suggested by the Turkish Standard Institute (11).

Blood Analysis: Blood samples were taken from the jugular vein of each cow on postpartum days 0, 20, 24, 30, 31, 38, 45, 60, and 90. The samples were centrifuged at 3000 rpm and stored at –20 °C for subsequent analysis. Analysis of progesterone was performed on postpartum days 24, 31, 38, and 45 (12). To determine progesterone concentration, the radioimmunoassay (RIA) method was used. Analysis of calcium, phosphorus, magnesium, urea nitrogen, and SGOT (AST) in blood serum obtained on postpartum days 0, 20, 30, 60, and 90 was made spectrophotometrically using commercial kits (Teco Diagnostics, USA). NEFA analysis was made spectrophotometrically according to the method of Brenner and Reinhard (13).

Parameters Evaluated for Fertility Purposes: Calving and/or puerperium problems in cows with dystocia (with external help for calving to take place), stillbirths, placental retention, abortion, clinical mastitis, and metabolic diseases were monitored and recorded. Ovarian functioning during the postpartum period until insemination time was determined by evaluating the quantitative values of progesterone in the blood samples taken at 1-week intervals between postpartum days 24 and 45. Confirmation of fertility after insemination was made by detecting standing heats after the 45th postpartum day. In order to define fertility after insemination, the following parameters were considered: Calving to first service interval, calving to conception interval, services per conception, and repeat breeder rate. The parameters measured in cows with problems during the early postpartum period and in those with a normal puerperium were compared.

Statistical Analysis*:* Data collected during the experiment were treated as completely randomized blocks and were subjected to two-way analysis of variance. When the animal groups were accepted as units, except the blood parameters, and when significant differences between means were observed, the data were further subjected to Duncan's multiple range test (14). Blood parameters were regressed against the time that blood samples were collected. All statistical analyses were performed using SPSS v.10.0 statistical software (SPSS, Inc., Chicago, Il, USA).

Unless otherwise noted, $P < 0.05$ was accepted as significant.

Results

Nutrient contents and metabolizable energy levels of the silage and concentrate feed given to the animals are shown in Table 2.

Calcium, phosphorus, magnesium, urea nitrogen, urea, SGOT (AST), and NEFA concentrations in the sera of the blood samples taken on postpartum days 0, 20, 30, 60, and 90 are shown in Table 3.

In all, 40 cows had postpartum problems, with the following incidence rates: Dystocia (50%), stillbirths (25%), placental retention (10%), abortion (5%), milk fever (5%), and clinical mastitis (5%).

Among all the blood samples, 31% were determined to have had increasing levels of serum progesterone until the 45th postpartum day, while in the remaining 69% progesterone levels remained below 1 ng/ml. The first days on which increases in progesterone were detected and the incidence rates are given in Table 4.

As a functional problem from postpartum days 24 to 45, ovarian inactivity was observed in 69% of the experimental animals, and in 2 cows progesterone levels remained elevated for a period of 3 weeks (persistent luteal period). Calving to postpartum insemination times and post-insemination fertility parameters are given in Table 5.

Table 2. Nutrient contents (%) and calculated metabolizable energy levels (kcal/kg) of corn silage and concentrate feed.

	Dry Matter	Crude Protein	Ether Extract	Crude Fiber	Crude Ash	ADF	ME
Corn Silage	48.25	6.35	-	-	-	15.95	1395
Concentrate Feed	91.20	18.55	7.00	7.30	6.50	-	2800

Table 3. Mean serum calcium, phosphorus, magnesium, urea nitrogen, urea, SGOT (AST), and NEFA values.

Parameters	Postpartum Days										P
	0		20		30		60		90		
	$\bar{x}$ ± $S\bar{x}$		$\bar{x}$ ± $S\bar{x}$		$\bar{x}$ ± $S\bar{x}$		$\bar{x}$ ± $S\bar{x}$		$\bar{x}$ ± $S\bar{x}$		
Ca (mg/dl)	6.86	1.43^{b}	6.99	1.45^{b}	7.68	0.36^{a}	7.75	1.48^{a}	7.91	2.06^{a}	0.001***
P (mg/dl)	3.42	0.12^{c}	3.68	0.14^{bc}	4.05	0.18^{ab}	4.01	0.15^{ab}	4.43	0.18^{a}	0.001***
Mg (mg/dl)	1.51	0.10	1.46	0.11	1.64	0.12	1.50	0.12	1.66	0.16	0.746
Urea-N (mg/dl)	17.59	2.23	16.98	1.65	15.79	1.87	15.57	2.03	14.25	1.95	0.822
Urea (mg/dl)	37.73	4.78	36.42	3.54	33.87	4.02	33.40	4.36	30.57	4.17	0.822
SGOT (IU/l)	18.70	1.42	13.42	0.75	14.92	1.45	16.80	2.26	16.48	1.19	0.150
NEFA (μmol/l)	670	0.06	660	0.07	660	0.07	620	0.06	640	0.09	0.985

[a,b,c] Mean followed by the same superscript are not statistically different from one another ($P > 0.05$).

***$P < 0.001$.

Table 4. The first increases in progesterone detected on postpartum days and the incidence rates (%).

Experimental Groups	day 24	day 31	day 38	day 45	Total
Cows with calving-postpartum problem (n = 40)	-	12.50	6.25	12.50	31.25
Cows without calving-postpartum problem (n = 60)	8.33	25.00	4.16	16.66	54.16
Total (n = 100)	5.00	20.00	5.00	15.00	45.00

Table 5. Calving to postpartum insemination intervals and fertility parameters after insemination.

Fertility parameters (day)	General (n = 100)		Cows with calving-postpartum problem (n = 40)		Cows without calving-postpartum problem (n = 60)		P
	$\bar{x} \pm S\bar{x}$		$\bar{x} \pm S\bar{x}$		$\bar{x} \pm S\bar{x}$		
Calving to first service interval	105.87	31.55	102.44	36.41	108.00	28.61	0.563
Calving to conception interval	130.31	34.42	132.69	34.00	128.63	35.50	0.749
Services per conception	2.06	0.70	2.00	0.70	2.12	0.71	0.452
Repeat breeders numbers	18 (36.00%)		7 (33.33%)		11 (37.93%)		

Discussion

In the present study 100 dairy cows were metabolically and endocrinologically examined from calving to conception. This process was separately evaluated for the animals with problems that occurred during calving and/or during the postpartum period, and for the animals without problems. To benefit from optimum reproductive performance, the Ca:P ratio should be 1.5-2.5:1 in dairy cows. Altıntaş and Fidancı (15) reported that normal serum mineral concentrations for dairy cows are as follows: Ca: 9.7-12.4 mg/dl; P: 6 mg/dl; Mg: 1.8-2.3 mg/dl. In the present study blood serum obtained from the cows on postpartum days 0, 20, 30, 60, and 90 had the following respective mineral concentrations: Ca: 6.86, 6.99, 7.68, 7.75, 7.91 mg/dl; P: 3.42, 3.68, 4.05, 4.01, 4.43 mg/dl; Mg: 1.51, 1.46, 1.64, 1.50, 1.66 mg/dl. When the adequacy of the energy and nutrients levels of the rations were examined, it was determined that the experimental cows fed ration 1 received more than the minimum requirements (16) of dry matter (20.32% more), metabolizable energy (11.02% more), crude protein (11.05% more), calcium (7.89% more), and phosphorus (45.85% more). It was observed that in the group fed ration 1 the Ca:P ratio was 1.1:1, which was improperly balanced for the Ca requirements of dairy cows. It was determined that the experimental cows fed ration 2 received more than the minimum requirements of dry matter (36.75% more), metabolizable energy (25.62% more), crude protein (20.54% more), calcium (15.22% more), and phosphorus (71.18% more). Similarly, it was observed that in the experimental cows fed ration 2, the Ca:P ratio was 1.05:1, which was improperly balanced for the Ca requirements of dairy cows. Dry matter, metabolizable energy, and P content of ration 3 were higher than those of the minimum requirements for cows (18.71%, 3.71%, and 59.82% higher, respectively). On the other hand, the crude protein and Ca contents of ration 3 were insufficient (5.66% and 22.81%, respectively, less than the minimum requirements). Similarly, the Ca:P ratio was 1:1.25, indicative of a more dramatic Ca imbalance in ration 3. It was determined that ration 4 contained much more dry matter, metabolizable energy, crude protein, and phosphorus than minimally required by cows (42.11%, 12.80%, 7.24%, and 82.61% more, respectively), but that it was lacking in calcium (6.01% less than the minimum requirement). Accordingly, the Ca:P ratio was 1:1.25, indicating a drastically low level of Ca.

The fact that the majority of problems during the calving-early postpartum period are connected to uterine inertia, suggests that they may be a consequence of this period. It was observed that the feeding program administered to the experimental animals generally resulted in the consumption of too much energy, crude protein, and phosphorus. It can be undoubtedly said that an imbalance in the crude protein:energy ratio can cause NEB in dairy cattle. On the other hand, it can be suggested that the high dry matter, energy, and nutrient content of rations 1, 2, and 4 may have caused reproductive dysfunction, irregularity in the sexual cycle, liver malfunction, hypomagnesemia, and fat cow syndrome. The phosphorus content of ration 3 was much higher than required by cattle (59.82%) and the Ca content was lower than required (22.81%), and the Ca:P ratio of the rations 1,2 and 4 was improperly balanced in terms of Ca. This can cause bone or foot problems in cows, decreased milk yield, hypocalcemia, and a predisposition of the genital organs to infection and irregularities in the sexual cycle. Dystocia, uterine prolapse, placental retention, uterine infections, and metabolic diseases that occur during and after calving may negatively affect fertility parameters (17). It was reported that after dystocia the interval from calving to first estrus was extended by 2 days, the interval from calving to first insemination was extended by 2.44 days, and the interval from calving to conception was extended by 8.1 days (8). The same researchers stated that the pregnancy rate in the first insemination decreased by 4.6% and services per conception increased by 0.17%. In field studies it was reported that the service period after calving required assistance, was achieved with excessive force, and was extended from 9.3 to 16.4 days (18). Although hypocalcemia did not have a direct effect on reproduction, it should be considered that it causes a predisposition to calcium deficiency, dystocia, and placental retention. It was reported that the interval between calving and insemination in cows with experimentally established hypocalcemia was 76 days, and the interval from calving to conception was 112 days (17). As a result of placental retention and metritis, the fertility parameters of cows were negatively affected (19). Eicker et al. (20) stated that after placental retention, the interval from calving to first insemination was 75 days, the interval from calving to conception was 108 days, and the pregnancy rate decreased by 14%. Borsberry and Dobson (21) stated that in cattle with this problem the interval between calving and first insemination was 64.8 ± 6.7 days, the interval between calving and conception was 95.9 ± 10.7 days, and services per conception was 2.22 days. In the present study, observed postpartum problems were dystocia (50%), stillbirths (25%), placental retention (10%), abortion (5%), milk fever (5%), and clinical mastitis (5%). The above-mentioned percentages are higher than the normal incidence rates for cattle under an optimum and sufficient feeding program. As a matter of fact, the imbalanced levels of calcium, phosphorus, and magnesium observed during parturition are an important cause of these cases. Similarly, among the cattle in this group, the number of those whose ovarian functions didn't begin until the 45th postpartum day was lower than that of animals without postpartum problems. When we consider the other fertility parameters, data obtained here were generally higher than in the literature; however, no statistically significant difference was found between the study groups.

In the present study, NEFA levels in the blood serum determined on postpartum days 0, 20, 30, 60, and 90 were 670, 660, 660, 620, and 640 µmol/l, respectively. These values support the argument that NEFA levels in dairy cows with NEB can be higher than 600 µmol/l. The findings of this study are similar to the NEFA level of 544.25 ± 104.8 µmol/l reported by Comin et al. (22) for cattle with NEB; however, the NEFA values of this study do not match the findings of McNamara et al. (7). It can be considered that the differences were caused by the rations and propylene glycol, which is given to cattle with NEB. The urea nitrogen values determined on postpartum days 0, 20, 30, 60, and 90 were 17.59, 16.98, 15.79, 15.57, and 14.25 mg/dl, respectively. The urea levels determined by urea nitrogen values during the same period were 37.73, 36.42, 33.87, 33.40, and 30.57 mg/dl, respectively. These values are similar to the results of Miyoshi et al. (23), while the values reported by Bostedt (24) were higher than those of the present study. Serum glutamic oxalacetic transaminase (SGOT = AST) values obtained on the investigated postpartum days were 18.70, 13.42, 14.92, 16.80, and 16.48 IU/l. These values are lower than the values obtained by Parmar and Mehta (25) from cyclic cows.

Alaçam et al. (12) evaluated ovarian functions by measuring progesterone levels in the milk samples collected from cattle on postpartum days 24, 31, 38, and 45; 62.00% of the cows had normal ovarian functions and 38.00% had abnormal functions, including delayed ovulation, ovarian inactivity, persistent luteal phase, and cystic ovaries. They reported that 19.20% of the cows required treatment. In the present study 31.00% of the cows' progesterone levels increased until the 45th postpartum day. This finding indicates lower levels of fertility than reported by Alaçam et al. (12).

McDougall et al. (26) explained that this condition, which was observed in cows whose first postpartum ovulations were detected on postpartum day 45 ± 5.3 and after 4.2 follicular waves, was evidence of severely energy-deficient cow rations. In the present study the cows that had low progesterone levels until the 45th postpartum day were included in the group with significant fertility problems, as reported by O'Farrel (27). As a matter of fact, this condition caused subfertility-infertility cases by negatively affecting several fertility parameters. In the cows whose ovarian functions had started, average serum progesterone level on the 31st postpartum day was 3.02 ± 2.03 ng/ml. Kendrick et al. (28) determined that the serum progesterone level on the 30th postpartum day in cows fed high energy feed was 4.19 ± 0.57 ng/ml, whereas it was 3.15 ± 0.73 ng/ml in cows fed low energy feed. The levels of progesterone observed in the present study were lower than the above-mentioned minimum levels. This condition can be interpreted as more evidence of NEB.

On a modern dairy cattle farm some important parameters affecting reproduction were evaluated in the present study. Similarly, in studies regarding fertility/infertility these kinds of parameters were targeted (27). The most suitable and economic period for insemination of dairy cows is postpartum days 45-60 (29). In the present study the average time from calving to first insemination was 105.87 ± 31.55 days. It has been concluded that in addition to the delayed start of ovarian functions due to NEB and metabolic problems, insufficient herd management regarding the determination of estrus plays an important role. O'Farrel (27), when evaluating services per conception makes the following classification: 1.54-1.66 (normal), 1.67-1.99 (slightly problematic), 2.00-2.22 (moderately problematic), and > 2.22 (significantly problematic). In the present study services per conception was 2.12 ± 0.71 (moderately problematic); however, we think that the extended time of the delay and calving to conception time should be evaluated together with services per conception. In the dairy business, the calving to conception interval is an important parameter within the framework of the goal to produce 1 calf every year. O'Farrel (27), when evaluating this interval in terms of fertility, made the following classification: 80.0-82.5 days (normal), 82.6-85.0 days (slightly problematic), 85.1-100.0 days (moderately problematic), and >100.0 days (significantly problematic). The interval from calving to first estrus and from calving to conception is affected by such factors as the energy and protein level in rations, ovarian dysfunctions, and uterine infections (30). In the present study, the average interval from calving to conception was 130.31 ± 34.42 days. This average is also included among the important problems in terms of fertility. The increase in this parameter was a result of the slight increase in progesterone levels during the first 45 postpartum days, indicating the delay of first postpartum ovulation and the extension of calving to first insemination period.

Considering the 60% pregnancy rate for each insemination in cows without problems, after 3 inseminations 94% pregnancy and 6% repeat breeders was observed. These values are non-negative in terms of herd economy. The repeat breeder rate can have harmless increases, depending on various factors; however, if the incidence of repeat breeders is greater than 16.6%, herd fertility is negatively affected (9,30). In the present study this rate was 36%. It is concluded that this condition appeared as a result of metabolic problems and NEB.

Acknowledgement

This experiment was supported by the Prime Ministry State Planning Organization (Project No: 96K1203500), Ankara, Turkey.

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