Kocatepe Veterinary Journal

Kocatepe Vet J. (2024) 17(2):68-75

DOI: 10.30607/kvj. 1417561

RESEARCH ARTICLE

Effect of Postpartum Drenching of Fluid-Electrolyte and Energy Supplement on Fertility Parameters in Holstein Dairy Cows

Cangir UYARLAR[1*], Oktay YILMAZ[2]

[1] Department of Animal Nutrition and Nutritional Diseases, [2] Department of Obstetrics and Gynaecology, Faculty of Veterinary Medicine, Afyon Kocatepe University, Afyonkarahisar, Türkiye

ABSTRACT

In this study, the effect of orally given fluid-electrolyte and energy supplementation immediately after the delivery on some metabolic and fertility parameters was investigated. Holstein dairy cows which were housed in a private dairy company under identical management and feeding programs were divided into two groups as experiment (n=70) and control (n=75). Cows in the experiment group were treated by orally given 5 kg fluid-electrolyte and energy supplementation dissolved in 20 litres lukewarm water for three consecutive days, whereas the control group received only the 20 litres of lukewarm water. The ear tag numbers of all cows used in the study were recorded and monitored until next delivery. Blood samples were collected from the coccygeal vein of the cows on the postpartum 4th day. It was determined that the concentrations of βHBA in the experiment group were lower than in the control group ($p<0.01$). However, the concentrations of NEFA and glucose did not differ between the experiment and the control groups ($p>0.05$). It was seen that the synchronization rate was lower in the experiment group than that detected in the control group ($p<0.05$). The pregnancy rate did not differ between groups ($p>0.05$). Nevertheless, the service number per pregnancy displayed a higher incidence in the control group as compared to the experiment group ($p<0.001$). Similarly, calving to pregnancy interval and the calving interval was higher in the control group ($p<0.05$). Total lactation milk yield in the experiment group was higher as compared to the control group ($p<0.05$). In conclusion, it was observed that orally given fluid-electrolyte and energy supplementation immediately after delivery improved the fertility parameters in Holstein dairy cows.

Keywords: Fluid-Electrolyte, βHBA, Dairy cow, Fertility

***

Holştayn Süt İneklerinde Doğum Sonrası Sıvı-Elektrolit Ve Enerji Katkısının Fertilite Parametreleri Üzerine Etkisi

ÖZ

Bu çalışmada süt ineklerinin doğumlarından hemen sonra üç gün boyunca içirilen sıvı-elektrolit ve enerji desteğinin bazı metabolik ve fertilite parametreleri üzerine etkisi araştırıldı. Ticari bir süt işletmesinde aynı çiftlik yönetimi ve besleme programı altında barındırılan Holştayn süt inekleri deneme (n=70) ve kontrol (n=75) olmak üzere iki gruba ayrıldı. Deneme grubundaki ineklere üç gün boyunca ağızdan 5 kg/gün sıvı-elektrolit ve 20 litre ılık suda eritilmiş enerji takviyesi verilirken, kontrol grubundaki ineklere sadece 20 litre ılık su verildi. Çalışmada kullanılan tüm ineklerin kulak küpesi numaraları kayıt altına alındı ve bir sonraki doğuma kadar takip edildi. Bütün hayvanların kuyruk venalarından postpartum 4. günde kan örnekleri alındı. Deneme grubundaki kan βHBA konsantrasyonunun kontrol grubuna göre daha düşük olduğu belirlendi ($p<0.01$). Ancak kan NEFA ve glikoz konsantrasyonları deneme ve kontrol grupları arasında farklılık göstermedi ($p>0.05$). Senkronizasyon oranının deneme grubunda kontrol grubuna göre daha düşük olduğu görüldü ($p<0.05$). Gebelik oranı gruplar arasında farklılık göstermedi ($p>0.05$). Kontrol grubunda ise gebelik başına tohumlama sayısı deneme grubuna göre daha yüksek görüldü ($p<0.001$). Benzer şekilde doğum-gebe kalma aralığı ve buzağılama aralığı da kontrol grubunda daha yüksek olduğu izlendi ($p<0.05$). Deneme grubunda laktasyon süt veriminin kontrol grubuna göre daha yüksek olduğu belirlendi ($p<0.05$). Sonuç olarak Holştayn süt ineklerinde doğumdan hemen sonra oral olarak verilen sıvı-elektrolit ve enerji takviyesinin fertilite parametrelerini iyileştirdiği kanısına varıldı.

Anahtar Sözcükler: Sıvı-Elektrolit, βHBA, Süt ineği, Fertilite.

To cite this article: Uyarlar C. Yılmaz O. Effect of Postpartum Drenching of Fluid-Electrolyte and Energy Supplement on Fertility Parameters in Holstein Dairy Cows. ***Kocatepe*** *Vet J. (2024) 17(2):68-75*

Submission: 10.01.2024 **Accepted**: 06.03.2024 **Published Online**: 11.03.2022

ORCID ID; *CU 0000-0002-7803-2454 OY: 0000-0002-9722-5155*

*Corresponding author e-mail: cangiruyarlar@hotmail.com

INTRODUCTION

Dairy cows face a metabolically stressful period after parturition due to the enormous loss of nutrients such as water, energy and electrolytes (Grummer 1995; Enemark et al. 2009). Meanwhile, due to suppressed dry matter intake in the late pregnancy period, animals are not able to meet their nutrient requirements amelioration from the diet. Especially, energy deficiency is quite remarkable and it leads to a severe negative energy balance shortly after parturition (Drackley et al. 1991). Insufficient energy intake causes dramatic metabolic changes such as a decrease in blood glucose whereas there is an increase in non-esterified fatty acids (NEFA) and beta hydroxybutyric acid (βHBA) (Oetzel 2007). Moreover, subclinical ketosis occurs in dairy cows while serum βHBA levels are higher than 1.2 mmol/l. Subclinical ketosis is one of the most dangerous metabolic disorders induced by reduced milk yield, impaired reproductive performance (Collard et al. 2000) and increased incidence of culling rate (Uyarlar et al. 2018). Numerous studies demonstrated the relationship between hyperketonemia and reduced productivity in dairy cows (Dohoo and Martin 1984; Drackley 1999; Herdt 2000; Ospina et al. 2010b; McArt et al. 2012). Moreover, subclinical ketosis is related with increased incident in puerperal metabolic disorders (Ospina et al. 2010a).

The reproductive performance of dairy cows is closely related to the energy status in dairy cows (Grummer 2007). Oftentimes, delayed onset of luteal activity, decreased conception rate in the first insemination and increased number of the days from calving to conception in dairy cows are occurred in severe negative energy balance (Staples et al. 1990; Reist et al. 2003). Moreover, Rutherford et al. (2016) demonstrated that subclinical ketosis caused impaired reproductive performance by prolonging calving to first standing heat, calving to first insemination and calving to pregnancy intervals. In addition, drenching dextrose, electrolytes and vitamins is a prophylactic administration, when severe dehydration occurs (Enemark et al. 2009). However, nutritionists traditionally suggest oral administration of glucose precursors such as propylene glycol, glycerol, and calcium propionate to dairy cows during the transition period (Overton and Waldron 1996). This treatment aids in decreasing the energy gap, whereas it does not help to alleviate the loss of water, electrolytes and vitamins due to the parturition (Stokes and Goff 2001; Enemark et al. 2009). From this point of view, this study was aimed to determine the effect of orally given fluid-electrolyte and energy supplementation for three days after parturition on fertility parameters in Holstein dairy cows.

MATERIALS and METHODS

Animals and Housing

The study was conducted in a private Holstein Friesian dairy cow farm which were located in Türkiye. The cows had same management and feeding programs and were milked thrice daily. The dry period was separated to the far-off (50-60 days before the expected delivery) and close-up (21 days before the expected days) periods. After parturition, the cows were housed in fresh group (3-30 days postpartum). The individual milk yield of the cows which were taken from an automatic milking system was daily recorded (DairyPlan, GEA, Germany) until next delivery and the lactation milk yield were calculated at the end of study. The cows were grouped according to the individual milk yield following the fresh period.

Experimental Design

A total of 145 Holstein dairy cows immediately after delivery were divided into two groups. Cows in the experiment group (n = 70) were treated by orally given 5 kg fluid-electrolyte and energy supplementation (Polihidra, Polimed, Türkiye) dissolved in 20 litres lukewarm water for three consecutive days, whereas control group (n = 70) received only 20 litres of lukewarm water. The ear tag numbers of all cows used in the study were recorded and the cows were monitored for the reproductive parameters (synchronization rate, pregnancy rate, service number per pregnancy, calving to pregnancy interval and calving interval) until next delivery.

Diets

The animals were fed by same diet which were prepared and served as total mix ration (TMR). Nutrient composition and feedstuff formulation of the diet are presented in Table 1.

Measurement of metabolic profile

Blood samples were collected from coccygeal vein of the cows on the postpartum 4th day. Sera were harvested from blood samples following the centrifugation at 5.000 rpm for 10 min. The serum concentrations of glucose were analyzed by the commercially available reagents of Cobas C111 analyzer (Roche Diagnostic, Mannheim, Germany), whereas betahydroxybutyric acid (βHBA, #RB1008, Randox Laboratories Ltd., Crumlin, UK) and non-esterified fatty acid (NEFA, #FA115, Randox Laboratories Ltd., Crumlin, UK) were measured by ELISA (ChemWell, 2910, Awareness Technology, Inc., Florida, USA).

Monitoring of Reproductive Parameters

The voluntary waiting period was 55-65 days on average. The first postpartum (pp) artificial insemination (AI) was performed by G6G synchronization protocol (Yilmaz et al. 2011) or standing heat observation. When the cows showed standing heat 18-24 days after AI detected by observation and/or pedometer activity, the estrous was confirmed by transrectal ultrasonography (6 MHz, DP 10, Mindray, China) and the cows were re-inseminated. If the cows did not display standing heat, the pregnancy status (pregnant or non-pregnant) was diagnosed by transrectal ultrasonography 33-35 days after AI. Single dose or double dose of prostaglandin $F_{2\alpha}$ 14 days apart or Ovsynch protocol were used for the insemination of non-pregnant cows. All data were daily recorded to the farm computer system. When the cows reached to the next delivery, the data were evaluated for the calculation of the synchronization rate (%), pregnancy rate (%), service number per pregnancy, calving to pregnancy interval (day), and calving interval (day).

Statistics

The distribution of normality of data were analysed by Shapiro-Wilk normality test. The data regarding the concentrations of blood betahydroxybutyric acid, non-esterified fatty acid, glucose, service number per pregnancy, calving to pregnancy interval, and calving interval were evaluated by t-test, whereas synchronization rate, pregnancy rate and embryonic death rate were evaluated by chi-square test (SPSS 26.0, IBM, USA). Statistical significance was set at $p<0.05$.

RESULTS

The blood concentrations of βHBA, NEFA and glucose in the experiment and control groups are given at Table 2. The concentrations of βHBA in the experiment group (0.74±0.34 mmol/l) was lower than those measured in the control group (0.91±0.49 mmol/l) and the difference was statistically significant ($p<0.01$). However, the concentrations of NEFA in the experiment group (0.73±0.39 mmol/l) was slightly lower than those measured in the control group (0.87±0.50 mmol/l) but the difference was not statistically significant ($p>0.05$). The glucose concentrations did not differ between the experiment (53.73±3.71 mg/dl) and control (53.68±3.77 mg/dl) groups ($p>0.05$).

All related reproductive parameters and lactation milk yield in the experiment and control groups are given at Table 3. Accordingly, it was seen that the synchronization rate was lower in the experiment group (50.90 %) than those detected in the control group (70.90 %) and the difference was statistically significant ($p<0.05$). The pregnancy rate was slightly higher in the experiment group (75.70 %) than those measured in the control group (73.30 %) throughout the study but the difference was not significant statistically ($p>0.05$). On the other hand, the service number per pregnancy was displayed a higher incidence in the control group (2.21±0.85) as compared to the experiment (1.68±0.69) group ($p<0.001$). Similarly, calving to pregnancy interval was higher ($p<0.05$) in the control group (137.27±33.25) than those measured in the experiment group (124.22±31.39), whereas calving interval was also high ($P<0.05$) in the control group (415.27±33.25) as compared to the experiment group (402.22±31.39). Finally, the lactation milk yield in the experiment group was 32.90±2.99 litres, whereas the control group had 31.37±3.01 litres lactation milk yield and the difference was statistically significant ($p<0.05$).

DISCUSSION

The data obtained in this study provide evidence that electrolyte and energy supplementation with vitamin support can be beneficial for the improvement of metabolic and fertility parameters. In the present study, it was demonstrated that serum βHBA concentrations on four days in milk (DIM) were the lowest in the experiment group which were treated by orally given 5 kg fluid-electrolyte and energy supplementation dissolved in 20 litres lukewarm water at postpartum first 3 days. Moreover, a non-significant drop was also noticed in NEFA concentrations in the experiment group, whereas the serum glucose level did not differ on four DIM. Blood βHBA level is one of the most reliable parameters for monitoring energy balance and the incidence of ketosis which is characterized by loss of appetite, reduced dry matter intake and body condition loss in transition dairy cows (Grummer 2007; Overton et al. 2017; Lei and Simones 2021). Animals with serum βHBA level higher than 1.2 mmol/l are considered as hyperketonemic and sub-clinically ketotic (Iwersen et al. 2009; Konkol et al. 2009). Subclinical ketosis can occur any day in the first 100 days of lactation (Duffield 2000). However, the first week of lactation is the most critical period for the occurrence of hyperketonemia and the development of subclinical ketosis (McArt et al. 2012). This fact was the reason why the fourth day of lactation was chosen for blood sampling in the present study. It has been well documented that during transition period, all dairy cows experience dramatic changes including in the metabolic, physiological and/or nutritional status which can be resulted in a period of negative energy balance, when the diet do not meet the energy demand (Grummer 2007). Negative energy balance in dairy cattle resulting from insufficient energy intake immediately after parturition is considered the most important problem for dairy cattle nutritionists (Churakov et al. 2021). The increasing βHBA and NEFA or decreasing concentrations of glucose are the expected results during negative energy balance period in dairy cows (Grummer 1993; Drackley 1999; McArt et al. 2012). The present study revealed that the experiment group displayed better energy status with lower βHBA and NEFA concentrations than the control group.

Table 1. Diet Composition

Feedstuffs (DM%)	Far off (Dry Period)	Close Up (Dry Period)	Fresh Period (Lactation)
Corn Silage	26.85	35.43	31.52
Wheat Straw	40.58	15.52	0.00
Alfalfa Hay	11.84	5.58	16.99
Soybean Meal	0.00	5.61	19.39
Sunflower Meal	8.39	7.68	4.11
Corn Steam Flakes	6.72	14.79	18.42
Barley Grain Ground	3.40	7.47	2.66
Rumen Protected Fat[1]	0.00	2.92	2.97
Premix[2]	0.69	1.47	1.79
Limestone	1.15	0.00	1.19
Salt	0.38	0.34	0.72
Rumen Protected Lysine[3]	0.00	0.00	0.09
Rumen Protected Methionine[4]	0.00	0.00	0.15
Magnesium Sulfate	0.00	1.68	0.00
Calcium Chloride	0.00	1.51	0.00
	Nutrients		
CDMI[5]	13.53	11.85	16.65
Crude Protein (DM %)	10.15	12.46	18.28
MP[6] (g/day)	1032.3	1101.5	1895.2
NEl (Mcal/day)	14.78	18.89	28.63
NEl (Mcal/kg)	1.13	1.59	1.72
NFC (DM %)	26.12	37.05	39.36
NDF (DM %)	53.55	35.64	27.53
ADF (DM %)	37.15	23.19	17.68
Ether Extract (DM %)	2.23	5.33	5.49
Ca (DM %)	0.76	0.82	0.62
P (DM %)	0.38	0.37	0.38
Lysine (MP %)	2.45	2.42	6.82
Methionine (MP %)	6.75	6.89	2.51
(NA+K)-(Cl-S)	**17.72**	**-41.3**	**17.15**

[1] Fractionated rumen protected fat (Polimed, Turkiye), Fat 98% (Minimum), Palmitic Acid 85% (Minimum), Stearic Acid 3,5% (Maximum)
[2] Premix composition in 1 kg; 3000000 IU of Vitamin A, 500000 IU of Vitamin D, 9000 mg of Vitamin E,12000 mg of Mn, 5000 mg of Fe, 50000 mg of Zn, 9000 mg of Cu, 400 mg of I, 75 mg of Co, 250 mg of S
[3] Lysipearl (Kemin, Belgium)
[4] Metipearl (Kemin, Belgium)
[5] Calculated Dry Matter Intake (NRC, 2001)
[6] Metabolizable Protein (NRC, 2001).

Table 2. The concentrations of blood betahydroxybutyric acid (βHBA), non-esterified fatty acid (NEFA) and glucose in cows in the experiment (n = 70) and the control (n = 75) groups.

Parameters	**Experiment** (n =70)	**Control** (n =75)	**P**
βHBA (mmol/l)	0.74±0.34	0.91±0.49	0.003
NEFA (mmol/l)	0.73±0.39	0.87±0.50	0.057
Glucose (mg/dl)	53.73±3.71	53.68±3.77	0.937

Experiment group (n = 70) was treated by orally given 5 kg fluid-electrolyte and energy supplementation dissolved in 20 litres lukewarm water, whereas control group (n = 75) received only 20 litres of lukewarm water.

Table 3. Lactation milk yield (l) and reproductive parameters [synchronization rate (%), pregnancy rate (%), service number per pregnancy, calving to pregnancy interval (day), and calving interval (day)] detected in cows in the experiment (n = 70) and the control (n = 75) groups.

Parameters	**Experiment** (n =70)	**Control** (n =75)	**P**
Synchronization rate (%)	50.90 (27/53)	70.90 (39/55)	0.048
Pregnancy rate (%)	75.70 (53/70)	73.30 (55/75)	0.849
Service number per pregnancy	1.68±0.69	2.21±0.85	0.000
Calving to pregnancy interval	124.22±31.39	137.27±33.25	0.038
Calving interval (day)	402.22±31.39	415.27±33.25	0.038
Lactation milk yield (L)	32.90±2.99	31.37±3.01	0.003

Experiment group (n = 70) was treated by orally given 5 kg fluid-electrolyte and energy supplementation dissolved in 20 litres lukewarm water, whereas control group (n = 75) received only 20 litres of lukewarm water.

These findings are in accordance with another study (Enemark et al. 2009) which reported that oral administration of electrolytes improved the energy balance of dairy cows by reducing βHBA and NEFA concentrations in the first days of lactation. It is suggested that the replacement of electrolyte status with the support of orally given glucose throughout three consecutive days may improve the dry matter intake and/or controlled the incidence of metabolic diseases after parturition. It is needed to clarify the hypothesis that consume the relationship between the postpartum prophylactic treatment and the metabolic or reproductive health status. It has been known that various prophylactic treatments are available in the veterinary field to minimize NEB and related postpartum disorders (Studer et al. 1993; Goff and Horst 1994), consequently maintaining the high productive performance (Miyoshi et al. 2001). It was documented that oral administration of electrolytes with a high amount of water as a prophylactic treatment in fresh cows improves the metabolic haemostasis (Stokes and Goff 2001). Accordingly, $MgSO_4$ helps to increase magnesium concentrations in blood and to maintain the calcium homeostasis at calving due to parathormone induced absorption of dietary calcium (Enemark et al. 2009). On the other hand, KCL provides daily potassium supply from the diet is very important in helping to maintain acid-base balance, water retention, and Mg^{2+} absorption (Ammerman and Goodrich 1983). It is known that a cow approximately lost 60 litres of uterine fluid (Doreau et al. 1981) and simultaneously plasma protein due to the colostrum secretion (McLennan and Willoughby 1973) at calving which may result electrolyte imbalances in the dam. Moreover, the filling of the rumen with water may help to avert the displacement of the abomasum by restricting rumen movements (Enemark et al. 2009). Therefore, it is speculated that the administration of postpartum large amounts of fluid may serve the replacing of electrolytes which are lost at calving. In accordance with Enemark et al. (2009), it is suggested that drenching with electrolyte-supplemented water helped to improve energy balance in dairy cows and ameliorated the metabolic status of the cows.

Lucy et al. (1992) states that a relationship exists between positive energy status and the diameter of largest follicle on day 10 postpartum, since the gonadotropic hormones tend to be increased by the improving of energy status. In the present study, the artificial inseminations were more likely to be performed following by the observation of standing heat in the experiment group. In another words, the experiment group needed significantly less synchronization protocols as compared to the control group. In early lactation stage, the delayed resumption of postpartum cyclicity is associated with severe NEB which negatively affects the postpartum reproductive efficiency (Jeong et al. 2015). It has been reported that adequate levels of blood glucose 60 mg/dl on average is appropriate for the cow to get pregnant at first insemination (Garverick et al. 2013). Furthermore, it was noted that the animals with more amount of blood glucose came to heat within 2 months compared to those with lesser blood glucose (Veena 2015). In the present study, the concentrations of blood glucose were 53.73±3.71 and 53.68±3.77 in the experiment and the control groups, respectively. It seems that the concentrations of glucose are in the reference range (Mair et al. 2016). Therefore, it is suggested that the lesser proportions of the synchronized cows might be related not only the glucose concentrations but also the electrolyte and vitamin supplementation. It was reported that there have been differences in the blood concentrations of certain vitamins in dairy cows at the different stages of the oestrus cycle (Ataman et al. 2010). Generally, injections of vitamin A, D, E and C in combinations had no effect on the rate of conception or pregnancy in cows (Likittrakulwong et al. 2022). However, it was stated that the injectable vitamin and trace element combination may improve some metabolic and fertility parameters such as blood NEFA and aspartate aminotransferase (ALT) concentrations and pregnancy rate in dairy cows (Yazlik et al. 2021). Moreover, it has been reported that the mineral supplementation can be used to improve productivity and reproductive well-being (Molefe and Mwanza 2020). In addition, it was reported that under the adequate metabolic conditions the cows showed shorter calving to conception interval and lower insemination index (Çolakoğlu et al. 2020). Although the pregnancy rate did not differ between groups in the present study, the service number, the calving to pregnancy interval and the calving interval were significantly lesser in the experiment group. Therefore, it is suggested that both energy and electrolyte supplementation might be synergistically beneficial to improve the fertility parameters.

The present study showed that the lactation milk yield in the experiment group was significantly higher as compared to the control group. This finding was not accordance with other reports (Enemark et al. 2009) which dextrose supplementation did not exist in the drenching. However, it was reported that the oral supplementation of glucose precursors caused a numeric increase in milk yield (Akhtar et al. 2023), whereas a significant increase in milk yield was observed when the cows had clinical or subclinical ketosis (McArt et al. 2011, Gordon et al. 2013). The discrepancies above mentioned studies might be due to the absence of electrolytes and vitamin supplementation for the prophylactic treatment of the cows.

In conclusion, the present study revealed that orally given electrolyte and energy supplementation dissolved in water at the postpartum three consecutive days induced the decrement in the blood βHBA and NEFA concentrations. Consequently, the decrement in the service number, calving to pregnancy interval and calving interval might be observed in the dairy cows. Therefore, it is suggested that the postpartum short administration of fluid-electrolyte and energy supplementation can be of importance for the improvement fertility and lactation milk yield in the Holstein dairy cows.

Conflict of interest: The authors have no conflicts of interest to report.

Authors' Contributions: Cangir Uyarlar and Oktay Yılmaz contributed to the project idea, design and execution of the study. Cangir Uyarlar and Oktay Yılmaz contributed to the acquisition of and analysed the data, drafted, wrote and reviewed the manuscript critically. All authors have read and approved the finalized manuscript.

Ethical approval: All animal-related procedures were approved by the local ethic committee (approval AKUHADYEK 49533702/24).

Acknowledgement: There is no conflict of interest.

REFERENCES

Akhtar, M. U., Hifzulrahman, Pasha, T. N., Avais, M., Khan, N., Chishti, G. A., Ali, M., Imran, M., Tahir, M. N., & Naveed-Ul-Haque, M. (2023). Effects of peripartal glucose precursor supplementation on lactation performance and metabolic health of primiparous and multiparous dairy cows. Animal Bioscience, 36(6), 869-878.

Ammerman., C. B., & Goodrich, R. D. (1983). Advances in mineral nutrition in ruminants. Journal of Animal Sciences, 57 (Suppl 2), 519-533.

Ataman, M. B., Erdem, H., Bülbül, B., Haliloğlu, S., Çınar, M., & Aköz, M. (2010). Plasma β-carotene, vitamin A and vitamin C levels in cyclic and pregnant cows. Kafkas Üniversitesi Veteriner Fakültesi Dergisi, 16(4), 579-584.

Churakov, M., Karlsson, J., Rasmussen, A. E., & Holtenius, K. (2021). Milk fatty acids as indicators of negative energy balance of dairy cows in early lactation. Animal, 15(7), 100253.

Collard, B. L., Boettcher, P. J., Dekkers, J. C. M., Petitclerc, D., & Schaeffer, L. R. (2000). Relationships between energy balance and health traits of dairy cattle in early lactation. Journal of Dairy Science, 83, 2683-2690.

Çolakoğlu, H., Yazlik, M., Kaya, U., Kurt, S., Bayramoglu, R., Vural, R., & Kuplulu, S. (2020). Relationship between body condition score, blood metabolites and oxidative stress in transition period and reproductive performance of dairy cows. Journal of the Hellenic Veterinary Medical Society, 71(1), 2041-2046.

Dohoo, I. R., & Martin, S. W. (1984). Subclinical ketosis: Prevalence and associations with production and disease. Canadian Journal of Comparative Medicine, 48,1-5.

Doreau, M., Journet, M., Marquis, B., Ollier, A., Flechet, J., LeFaivre, R. & Sornet, R. (1981). Variations de la digestibilité de la ration et de quelques paramétres sanguint chez la vache laitiére á proximité do vélage. Bulletine Technique du CRZV Theix, INRA, 44, 17-22.

Drackley, J. K., Veenhuizen, J. J., Richard, M. J., & Young, J. W. (1991). Metabolic changes in blood and liver of dairy cows during either feed restriction or administration of 1, 3-butanediol. Journal of Dairy Science, 74(12), 4254-4264.

Drackley, J. K. (1999). ADSA Foundation Scholar Award: Biology of dairy cows during the transition period: The final frontier? Journal of Dairy Science, 82, 2259–2273.

Duffield, T. (2000). Subclinical ketosis in lactating dairy cattle. Veterinary Clinics of North America, Food Animal Practice, 16(2), 231-253.

Enemark, J. M. D., Schimidt, H. B., Jcobsen, J., & Enevoldsen, C. (2009). Failure to improve energy balance or dehydration by drenching transition cows with water and electrolytes at calving. Veterinary Research Communications, 33, 123-137.

Goff, J.P., & Horst, R.L. (1994). Calcium salts for treating hypocalcemia: Carrier effects, acid-base balance, and oral versus rectal administration. Journal of Dairy Science, 77, 1451–1456.

Gordon, J. L., LeBlanc, S. J., & Duffield, T. F. (2013). Ketosis treatment in lactating dairy cattle. Veterinary Clinics of North America: Food Animal Practice, 29, 433-445.

Grummer, R. R. (1995). Impact of changes in organic nutrient metabolism on feeding the transition cow. Journal of Animal Science, 73, 2820–2833.

Grummer, R. R. (2007). Strategies to improve fertility of high yielding dairy farms: management of the dry period. Theriogenology, 68, S281-S288.

Garverick, H. A., Harris, M. N., Vogel-Bluel, R., Sampson, J. D., Bader, J., Lamberson, W. R., Spain, J. N., Lucy, M. C., & Youngquist, R. S. (2013). Concentrations of nonesterified fatty acids and glucose in blood of periparturient dairy cows are indicative of pregnancy success at first insemination. Journal of Dairy Science, 96(1), 181–188.

Herdt, T. H. (2000). Ruminant adaptation to negative energy balance. Influences on the etiology of ketosis and fatty liver. Veterinary Clinics of North America: Food Animal Practice, 16, 215–230.

Iwersen, M., Falkenberg, U., Voigtsberger, R., Forderung, D., & Heuwieser, W. (2009). Evaluation of an electronic cowside test to detect subclinical ketosis in dairy cows. Journal of Dairy Science, 92, 2618–2624.

Jeong, J. K., Choi, I. S., Kang, H. G., Hur, T. Y., Jung, Y. H., & Kim, I. H. (2015) Relationship between serum metabolites, body condition, periand post-partum health and resumption of post-partum cyclicity in dairy cows. Livestock Science, 181, 31–37.

Konkol, K., Godden, S., Rapnicki, P., & Overton, M. (2009). Validation of a rapid cow-side test for the measurement of blood beta hydroxybutyrate in fresh cows. In Proceedings 42nd Annual Conference of the American Association of Bovine Practitioners, Omaha, NE. VM Publishing Co., Stillwater, OK , p 190.

Lei, M. A. C., & Simões, J. (2021). Invited review: ketosis diagnosis and monitoring in high-producing dairy cows. Dairy, 2(2), 303-325.

Lucy, M. C., Staples, L. M. & Thatcher, W. W. (1992). Energy balance and size of and number of ovarian follicles detected by ultrasonography in early postpartum dairy cows. Journal of Dairy Science, 74, 473-482.

Mair, B., Drillich M., Klein-Jobstl D. Kanz, P., Borchardt, S., Meyer, L., Schwendenwein, I., & Iwersen, M. (2016). Glucose concentration in capillary blood of dairy cows obtained by a minimally invasive lancet technique and determined with three different hand-held devices, BMC Veterinary Research, 12, 34.

McArt, J. A. A., Nydam, D. V., & Oetzel, G. R. (2012). Epidemiology of subclinical ketosis in early lactation dairy cattle. Journal of Dairy Science, 95(9), 5056-5066.

McArt, J. A. A., Nydam, D. V., Ospina, P. A., & Oetzel, G. R. (2011). A field trial on the effect of propylene glycol on milk yield and resolution of ketosis in fresh cows diagnosed with subclinical ketosis. Journal of Dairy Science, 94, 6011-6020.

McLennan, M. W., & Willoughby, R. A. (1973). Observations of serum protein values of cows during oestrus, lactation, parturition and milk fever Canadian. The Veterinary Journal, 14, 233-242.

Miyoshi, S., Pate J. L., & Palmquist, D. L. (2001). Effect of propylene glycol drenching on energy balance, plasma glucose, plasma insulin, ovarian function and conception in dairy cows. Animal Reproduction Science, 68, 29-43.

Molefe, K., & Mwanza, M. (2020). Effects of mineral supplementation on reproductive performance of pregnant cross-breed Bonsmara cows: An experimental study. Reproduction Domestic Animals, 55, 301-308.

National Research Council (2001). Nutrient requirements of dairy cattle. National Academies Press.

Oetzel, G. R. (2007). Herd-level ketosis–diagnosis and risk factors. In Preconference seminar 7C. Dairy Herd Problem Investigation Strategies: Transition Cow Troubleshooting, American association of bovine practitioners 40th Annual Conference, pp. 67-91, September 19, Vancouver, BC, Canada.

Ospina, P. A., Nydam, D. V., Stokol, T., & Overton, T. R. (2010). Association between the proportion of sampled transition cows with increased nonesterified fatty acids and beta-hydroxybutyrate and disease incidence, pregnancy rate, and milk production at the herd level. Journal of Dairy Science, 93(8), 3595-3601.

Ospina, P. A., Nydam, D. V., Stokol, T., & Overton, T. R. (2010). Associations of elevated nonesterified fatty acids and beta-hydroxybutyrate concentrations with early lactation reproductive performance and milk production in transition dairy cattle in the northeastern United States. Journal of Dairy Science, 93(4), 1596-1603.

Overton, T. R., McArt, J. A. A., & Nydam, D. V. (2017). A 100-Year Review: Metabolic health indicators and management of dairy cattle. Journal of Dairy Science 100(12), 10398-10417.

Overton, T. R., & Waldron, M. R. (2004). Nutritional management of transition dairy cows: strategies to optimize metabolic health. Journal of Dairy Science, 87, E105-E119.

Reist, M., Erdin, D. K., von Euw, D., Tschümperlin, K. M., Leuenberger, H., Hammon, H. M., & Blum, J. W. (2003). Postpartum reproductive function: association with energy, metabolic and endocrine status in high yielding dairy cows. Theriogenology, 59(8), 1707-1723.

Rutherford, A. J. Oikonomou, G., & Smith, R. F. (2016). The effect of subclinical ketosis on activity at estrus and reproductive performance in dairy cattle. Journal of Dairy Science, 99(6), 4808-4815.

Staples, C. R., Thatcher, W. W. & Clark, J. H. (1990). Relationship between ovarian activity and energy status during the early postpartum period of high producing dairy cows. Journal of Dairy Science, 73(4), 938-947.

Stokes, S. R., & Goff, J. P. (2001). Evaluation of calcium propionate and propylene glycol administered into the esophagus of dairy cattle at calving. The Professional Animal Scientist, 17(2), 115-122.

Studer, V.A., Grummer, R. R., Bertics, S. J., & Reynolds, C. K. (1993). Effect of propylene glycol administration on periparturient fatty liver in dairy cows. Journal of Dairy Science, 76, 2931-2939.

Uyarlar, C., Çetingül, S., Gültepe, E. E., Sial, A. R., & Bayram, İ. (2018). Effects of subclinical and clinical ketosis on the incidence of mastitis, metritis, culling rate and some hematological parameters in dairy cows. Kocatepe Veterinary Journal, 11(2), 186-193.

Veena M. P., Gowrakkal, M., Kumar, V. G., Gupta, P., & Swamy, M. N. (2015). Relation of various physiological blood parameters with the postpartum reproductive efficiency in cattle. International Journal of Biomedical Research, 6(10), 780-785.

Likittrakulwong, W., Poolprasert, P., Hanthongkul, W., & Roytrakul, S. (2022). Effects of Intramuscular Injections of Vitamins AD3E and C in Combination on Fertility, Immunity, and Proteomic and Transcriptomic Analyses of Dairy Cows during Early Gestation. Biotech (Basel), 11(2), 20.

Yazlik, M., Vural, M. R., Küplülü, Ş., & Pekcan, M. (2021). Effect of injectable vitamin and trace element administrations during transition period on fertility in brown Swiss dairy cows. Kocatepe Veterinary Journal, 14(1), 113-122.

Yilmaz, C., Yilmaz, O., & Ucar, M. (2011). Effect of PGF2α and GnRH injections applied before ovsynch on pregnancy rates in cows and heifers. Kafkas Üniversitesi Veteriner Fakültesi Dergisi, 17(4), 641-644.