การประเมินดัชนีทางเมตาบอลิซึมและสมรรถนะการสืบพันธุ์ของโคนมที่ไม่กลับสัดหลังคลอด

The Assessment of Metabolic Indices and Reproductive Performance of Dairy Cattle with Postpartum Anestrus

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Received: June 9, 2022/ Revised: June 24, 2023/ Accepted: May 31, 2023

บทคัดย่อ

ระยะเวลาหลังคลอดของโคนมเป็นช่วงเวลาที่มีความสำคัญเนื่องจากมักพบภาวะความผิดปกติของระบบเมตาบอลิซึมและสมดุลพลังงานซึ่งมีผลเชิงลบต่อประสิทธิภาพการสืบพันธุ์ การศึกษานี้มีวัตถุประสงค์เพื่อตรวจสอบดัชนีทางเมตาบอลิซึมและสมรรถนะการสืบพันธุ์ของโคนมที่มีการไม่กลับสัดหลังคลอด ทำการศึกษาในแม่วัววัวพันธุ์ผสมโฮลสไตน์ ฟรีเชียน จำนวน 30 ตัว โดยแบ่งแม่โคออกเป็น 2 กลุ่มตามพฤติกรรมที่แสดงการเป็นสัดภายในระยะ 45-50 วันหลังคลอด ได้แก่กลุ่มที่มีการกลับสัด (E cows; แสดงพฤติกรรมเป็นสัดภายใน 45-50 วันหลังคลอด; n=12) และไม่สามารถกลับสัด (NE cows; ไม่แสดงพฤติกรรมการเป็นสัดภายใน 45-50 วันหลังคลอด; n=18) ผลการศึกษาพบว่าเมื่อเปรียบเทียบข้อมูลกับ E cows, กลุ่ม NE cows มีแนวโน้มให้ผลผลิตนมสูงแต่อัตราการผสมติดครั้งแรกต่ำ ($P>0.05$) และพบยังพบว่ามีระดับเอสโตรเจน ($P<0.05$), โปรเจสเตอโรน ($P>0.05$) และ IGF-1 ($P<0.05$) ต่ำกว่า นอกจากนี้ยังพบว่าในแม่โคกลุ่มนี้มีระดับความเข้มข้นของ ไตรกลีเซอไรด์, HDL และ VLDL ลดลงอย่างมีนัยสำคัญ ($P<0.01$) อีกทั้งยังพบว่าปริมาณ BHB ($P>0.05$) และ NEFA ($P<0.01$) ในซีรัมสูงขึ้น แต่มีระดับของ กลูโคส ($P>0.05$) และ อินซูลิน ($P<0.01$) ที่ต่ำกว่าแม่วัวที่สามารถกลับสัดได้อย่างมีนัยสำคัญ แม้ว่าการศึกษานี้ไม่พบความสัมพันธ์ของ BHB กับฮอร์โมนที่สร้างจากรังไข่และฮอร์โมนอินซูลิน แต่ก็พบว่าความเข้มข้นของ NEFA ที่ตรวจได้จากตัวอย่างเลือดของแม่โคที่ไม่กลับสัด (NE cows) แสดงความสัมพันธ์เชิงลบกับฮอร์โมนเอสโตรเจน ($r=-0.531$, $P<0.05$) และโปรเจสเตอโรน ($r=-0.621$, $P<0.01$) จากข้อมูลเหล่านี้ชี้ให้เห็นว่าแม่โคที่ไม่สามารถกลับสัดได้หลังคลอดอยู่ในภาวะพลังงานติดลบ ซึ่งส่งผลให้เพิ่มความเสี่ยงของความผิดปกติของเมตาบลิซึมการใช้พลังงานของร่างกายและส่งผลทำให้ประสิทธิภาพการสืบพันธุ์ลดลง

คำสำคัญ: ภาวะพลังงานติดลบ, โคนม, ไม่กลับสัดหลังคลอด

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Abstract

Postpartum is a critical period which metabolic abnormality and energy imbalance, can cause negative impact on reproduction capability. The objective of this study was to investigate certain metabolic biochemical parameters and reproductive performance of dairy cows with postpartum anestrus. A total of 30 healthy Holstein Frisian crossbred cows were divided into 2 groups according to their estrus behaviors on 45-50 d postpartum: estrus (E) cows (showed estrus behaviors on 45-50 d postpartum; n=12) and non-estrus (NE) cows (did not show estrus behaviors on 45-50 d postpartum; n=18). The result showed a tendency of higher milk yield, but lower conception rate at first AI in NE group (P>0.05). The NE group was found to have a lower level of estrogen (P<0.05), progesterone (P>0.05) and IGF-1 (P<0.05) as compared to the E group. The NE group also showed a lower level of serum triglyceride, HDL and VLDL concentrations (P<0.01). Additionally, the NE group elicited a lesser level of glucose (P>0.05) and insulin (P<0.01) but increased in BHB (P>0.05) and NEFA (P<0.01) levels. However, BHB concentration of the NE group did not have any correlation to ovarian hormones or insulin, but the NEFA concentration exhibited a negative correlation with estrogen (r=-0.531, P<0.05) and progesterone (r= -0.621, P<0.01) concentration. These results suggested that NE group experienced to NEB which resulting in increased the risk of metabolic disorders and impaired fertility.
Keywords: Negative energy balance, dairy cow, postpartum anestrus

Introduction

The impaired performance of dairy cow reproductive system has become a serious concern to herders' profit (Meadows et al., 2005). A post-calving period is one of the most critical physiological stages as most of the metabolic disorders and related diseases occur (Holtenius et al., 2000; Yáñez et al., 2022), hence, postpartum health is a reliable determinant of the potential and capacity of subsequent reproduction (Royal et al. 2000).

During early lactation, plasma nonesterified fatty acid (NEFA) concentration increases as a consequence of lipid mobilization from adipose tissue (Chilliard, 1999). Although the consequence is a typical adaptive process with high-yielding cows, several metabolic and infectious disorders can occur and affect their productive and reproductive efficiency when cows fail to adapt to this metabolic challenge. One of the most significant factors affecting fertility is negative energy balance (NEB) that occurs in the early postpartum period (LeBlanc et al., 2006; Carvalho et al., 2014; Wiltbank et al., 2014). During NEB, fat mobilization takes place and increases the supply of NEFA and the serum levels of β-hydroxybutyrate acid (BHB). Cows with NEB have reduced capacity to draw essential nutrients to return to normal reproductive cyclicity, as well as the NEB directly diminishes the frequency of luteinizing hormone pulses, serum glucose, insulin-like growth factor type 1 (IGF-1), insulin, and production of estradiol by the dominant follicle that thereby increased the risk of anovulation (Butler, 2000). Consequences of such difficulty often illustrated

through delayed postpartum estrus, silent estrus, delayed ovulation, decreased ovulation rate and increased embryonic mortality (Gutierrez et al., 1997; Roberts et al., 1997).

As mentioned, the postpartum period is the critical phase in cattle reproduction, and the duration of postpartum anestrus has a considerable influence on reproductive performance. Moreover, a delayed in ovulation or expression of estrus is associated with reduced conception rate, pregnancy rate, and increased intervals from calving to conception (Rhodes et al., 2003). Therefore, this study was conducted to investigate certain metabolic biochemical parameters and reproductive performance in non-estrous return dairy cows after calving.

Research Methodology

Animal's protocol

This study complied with all research ethical principles involving animal and experiment was approved by the Ubon Ratchathani Rajabhat University Animal Ethic Committee (Number AN64002). The study was performed at a standardized private dairy farm in Sikhio district, Nakhon Ratchasima province, Thailand. Thirty crossbreed Holstein Friesian cows in their second and third lactation, with a milk yield of 7,675 ± 920l (calculated from 305 days of previous lactation), were enrolled in the study. All cows were clinically healthy, free from internal and external parasites, maintain similar body condition score (BSC), and were enrolled around 2 weeks earlier than expected delivery date. BSC were measured on a five-point scale of 1 (thin) to 5 (obese) and recorded on the 7d of pre- and post-calving date. Cows were fed with a total mixed ration (TMR) diet that adequately meet the nutritional requirements (NRC, 2001). The composition of TMR include 5.80kg dried Napier grass, 1.50kg dried Leucaena leaf meal, 0.50kg soybean meal, 2.40kg cottonseed meal, 1.30kg molasse, 4.00kg cassava chip, 1.25kg corn meal, 0.13kg urea, and 0.15kg mineral premix. Roughage and water were accessible ad libitum.

At 45-50d postpartum, normally the time for the first estrus, an artificial insemination (AI) technician performed estrus detection twice a day for approximately 60 min/d. The estrous behaviors were determined by the fluctuations in the steps count and clinical manifestations of estrus such as standing estrus, mounting activity, the presence of vaginal mucous discharge and/or swollen mucous membranes of the labia (Wang et al., 2022).

Cows were classified into 2 groups according to estrus behaviors on 45-50d postpartum. 12 cows with estrus behaviors were assigned as the estrus (E) group, whereas 18 cows with no significant clinical estrous behavior were assigned as the non-estrus (NE) group. All cows were screened for reproductive abnormalities and dominant follicle size by rectal palpation and transrectal ultrasonography using a real time B-mode scanner with a 7.5 MHz linear array transducer (Tringa liner, Esaotepie medical, Maastricht, The Netherlands). Cows with reproductive abnormalities such as persistent corpus luteum, ovarian cyst, or uterine abnormalities were excluded. All healthy cows were treated with CIDR®; P4 device containing 1.9g P4 (Wazibreed™ CIDR®, Livestock Improvement Association of Japan, Inc, Tokyo, Japan) in vagina for day 14 days for estrous synchronization. 48hr after CIDR® removed, AI was performed by the same inseminator with frozen semen from the same bull. 45 days after AI, pregnancy outcomes were evaluated by rectal palpation and transrectal ultrasonography using a real time B-mode scanner with a 7.5 MHz linear array transducer (Tringa liner, Esaotepie medical, Maastricht, The Netherlands), conception rates at first AI were recorded.

Blood sampling and analysis

Blood samples were collected by jugular venipuncture before feeding (07:00 am), using plastic disposable syringes. Of the 10 milliliters (ml) of blood collected, 4 ml was immediately transferred to sodium fluoride-coated tubes for estimation of glucose, and 6 ml was transferred to a plain tube for the evaluation of all other biochemical parameters. The blood samples were then centrifuged at 2,000g for 10 min, the obtained serum was transported to the laboratory at 4°C and then stored at -20°C until analysis.

Serum estrogen and progesterone concentrations were determined by radioimmunoassay to confirm cycling status, the sample were analyzed at the Laboratory of Veterinary Technology Department, Nakhon Ratchasima Rajabhat University, Thailand. Serum IGF-1 concentrations were evaluated by enzyme-linked immunosorbent assay kit (Bovine IGF1 ELISA Kit, Abcam, UK). Non-esterified fatty acids (NEFA) (Elabscience Biotechnology Inc, USA), β-hydroxybutyrate acid (BHB) (β-Hydroxybutyrate Assay Kit, Abcam, UK.), insulin (Commercial Kit INSULIN Siemens, Italy), glucose, total cholesterol, triglyceride, HDL, and LDL (XL multical, Erba Lachema s.r.o., CZ) were analyzed by a colorimetric method according to the prescribed protocol given by the kit manufacturers. Very low-density lipoprotein (VLDL) concentrations were calculated according the formular of triglyceride/5 (Tietz, 1995).

Statistical analysis of data

The SPSS version 26.0 software (IBM, Armonk, NY, USA) was used for statistical analysis of general clinical information and blood biochemical parameters obtained from the experimental cows via independent t-test. Result data are expressed as mean $\pm$ standard deviation (SD). The Pearson's test was used to assess significant correlations. P value below 0.05 indicated significant differences.

Results

General clinical information of cows

As shown on Table 1, clinical characteristic of each cow within the group did not show any statistical difference. There was, however, a tendency of higher milk yield, but lower conception rate at first AI observed with NE cows (P>0.05).

Reproductive hormones

Table 2 illustrates the analytical result of reproductive hormones that included estrogen, progesterone, and IGF-1 in both groups. The size of dominant follicle observed in NE cows is slightly smaller than E cows (p=0.593), NE cows also had a significant lower level of serum estrogen and IGF-1 compared to E cows (P<0.05), whereas the mean serum progesterone for all animals was not significantly different (P> 0.05).

Table 1 Clinical characteristic in dairy cows with estrus (E) and non-estrus (NE) behavior after calving.

Parameter	E cows	NE cows	P value
Body weight (kg)	718.58 $\pm$ 34.48	722.50 $\pm$ 35.12	0.765

BSC			
7d- before calving	3.13 ± 0.25	3.15 ± 0.31	0.418
7d-after calving	2.88 ± 0.14	2.77 + 0.27	0.404
Parity	2.50 ± 0.58	2.67 ± 0.78	0.531
Average milk yield (kg/d)	33.03 ± 3.24	34.39 ± 2.96	0.245
Dominant follicle size (mm)	7.90 ± 1.12	5.69 ± 1.38	0.593
Conception rate at first AI (%)	58.44 ± 51.39	44.44 ± 51.13	0.634

All data presented as mean ± SD.

*. Indicates statistical significance between the group (P<0.05).

Table 2 Assessment of serum estrogen, progesterone, and IGF-1 in dairy cows with estrus (E) and non-estrus (NE) behavior after calving.

Parameter	E cows	NE cows	P value
Estrogen (pg/ml)	23.13 ± 7.36	8.82 ± 0.92	0.015*
Progesterone (ng/ml)	4.92 ± 1.02	2.08 ± 0.76	0.608
IGF-1(ug/l)	46.06 ± 1.87	33.19 ± 3.43	0.010*

All data presented as mean ± SD.

*. Indicates statistical significance between the group (P<0.05).

Blood biochemical indices

Table 3 shows blood biochemical parameters in both experimental cow groups. As compared to E group, biochemical analysis result of NE group's lipid parameters showed significantly lower value of triglyceride and VLDL (P<0.01) whereas serum total cholesterol and LDL concentrations of all cows were comparable (P>0.05).

NE cows had slightly lower level of serum glucose, but they showed a greater level of BHB (P>0.05) and NEFA (P<0.05) concentrations as compared to E cows. A significant decreased in serum insulin concentration was also observed (P<0.01).

Correlation between reproductive hormones and blood biochemical parameters in E cows is demonstrated on Table 4. The result

Table 3 Assessment of blood biochemical parameters in estrus (E) and non-estrus (NE) cows.

Parameter	E cows	NE cows	P value
Total cholesterol (mg/dl)	153.78 ± 9.32	137.33 ± 10.94	0.869
Triglyceride (mg/dl)	25.59 ± 3.15	22.12 ± 2.63	0.003**
HDL (mg/dl)	90.84 ± 7.48	66.74 ± 4.04	0.001**
LDL (mg/dl)	26.36 ± 4.28	30.06 ± 6.00	0.076
VLDL (mg/dl)	5.12 ± 0.63	4.22 ± 0.57	0.003**
Glucose (mg/dl)	79.83 ± 3.09	71.50 ± 5.16	0.064
Insulin (μIU/ml)	17.09 ± 1.45	13.32 ± 2.19	0.000**
BHB	0.66 ± 0.83	0.99 ± 1.06	0.374
NEFA (mmol/l)	0.18 ± 0.04	0.63 ± 0.09	0.002**

All data presented as mean ± SD.

*. Indicates statistical significance between the group (P<0.05).

**. Indicates statistical significance between the group (P<0.01).

showed the progesterone levels had a significant negative correlation with triglyceride level (r=-0.582, P<0.05) and VLDL (r=-0.582, P<0.05).

Table 4 Correlation analysis between reproductive hormones and biochemical parameters in estrus (E) cows.

	E2	P4	IGF-1	TC	Tg	HDL	LDL	VLDL	Glu	BHB	Ins	NEFA
E2	1	-0.380	0.251	0.200	0.466	-0.186	0.311	0.466	0.033	-0.552	-0.126	0.259
P4	-0.380	1	-0.119	0.198	-0.582*	0.516	-0.147	-0.582*	0.085	0.526	0.532	-0.094
IGF-1	0.251	-0.119	1	0.221	0.456	-0.050	0.217	0.456	-0.381	-0.319	-0.388	-0.229
TC	0.200	0.198	0.221	1	-0.026	0.513	0.646*	-0.026	-0.333	0.188	-0.250	-0.167
Tg	0.466	-0.582*	0.456	-0.026	1	-0.012	-0.166	1.000**	-0.001	-0.709**	-0.206	0.375
HDL	-0.186	0.516	-0.050	0.513	-0.012	1	-0.312	-0.012	-0.079	0.049	0.447	0.101
LDL	0.311	-0.147	0.217	0.646*	-0.166	-0.312	1	-0.166	-0.294	0.266	-0.631*	-0.326
VLDL	0.466	-0.582*	0.456	-0.026	1.000**	-0.012	-0.166	1	-0.001	-0.709**	-0.206	0.375
Glu	0.033	0.085	-0.381	-0.333	-0.001	-0.079	-0.294	-0.001	1	0.067	0.384	0.340
BHB	-0.552	0.526	-0.319	0.188	-0.709**	0.049	0.266	-0.709**	0.067	1	0.175	-0.604*
Ins.	-0.126	0.532	-0.388	-0.250	-0.206	0.447	-0.631*	-0.206	0.384	0.175	1	0.195
NEFA	0.259	-0.094	-0.229	-0.167	0.375	0.101	-0.326	0.375	0.340	-0.604*	0.195	1

*. Correlation is significant at the 0.05 level.

**. Correlation is significant at the 0.01 level.

E2=estrogen, P4=progesterone, TC= total cholesterol, Tg= triglyceride, HDL= high-density lipoprotein, LDL= low-density lipoprotein, VLDL= very low-density lipoprotein, Glu= glucose, BHB= β-hydroxybutyrate acid, Ins= insulin, NEFA= non-esterified fatty acid.

Insulin concentrations also showed a significant negative correlation with LDL (r=-0.631, P<0.05). BHB showed a significant negative correlation with triglyceride (r=-0.709, P<0.01), VLDL (r=-0.709, P<0.01) and NEFA (r=-0.604, P<0.05).

On the other hand, estrogen and IGF-1 did not elicit statistical correlation with all blood biochemical parameters (P>0.05). A significant positive correlation was observed between estrogen and progesterone (r=0.555, P<0.05) in the NE group. IGF-1 and insulin concentrations did not significantly associate with any blood biochemical parameters. NEFA concentration was significant and negatively associated with estrogen (r=-0.531, P<0.05) and progesterone (r=-0.621, P<0.01) concentration (table 5).

Table 5 Correlation analysis between reproductive hormones and blood biochemical parameters in non-estrus (NE) cows.

	E2	P4	IGF-1	TC	Tg	HDL	LDL	VLDL	Glu	BHB	Ins	NEFA
E2	1	0.555*	-0.225	-0.162	0.184	0.146	-0.224	0.184	0.422	-0.128	-0.200	-0.531*

P4	0.555*	1	-0.342	-0.072	-0.244	-0.201	0.039	-0.244	0.096	0.066	-0.383	-0.621**
IGF-1	0-.225	-0.342	1	-0.136	0.288	0.011	-0.184	0.288	0.069	0.286	0.287	0.285
TC	-0.162	-0.072	-0.136	1	-0.060	0.279	0.901**	-0.060	0.077	0.140	0.286	0.111
Tg	0.184	-0.244	0.288	-0.060	1	0.153	-0.202	1.000**	0.293	-0.026	-0.015	-0.098
HDL	0.146	-0.201	0.011	0.279	0.153	1	-0.117	0.153	0.104	-0.077	0.180	0.219
LDL	-0.224	0.039	-0.184	0.901**	-0.202	-0.117	1	-0.202	0.061	0.138	0.205	0.082
VLDL	0.184	-0.244	0.288	-0.060	1.000**	0.153	-0.202	1	0.293	-0.026	-0.015	-0.098
Glu	0.422	0.096	0.069	0.077	0.293	0.104	0.061	0.293	1	0.285	-0.278	-0.057
BHB	-0.128	0.066	0.286	0.140	-0.026	-0.077	0.138	-0.026	0.285	1	-0.402	0.007
Ins	-0.200	-0.383	0.287	0.286	-0.015	0.180	0.205	0-.015	-0.278	-0.402	1	0.313
NEFA	-0.531*	-0.621**	0.285	0.111	-0.098	0.219	0.082	-0.098	-0.057	0.007	0.313	1

*. Correlation is significant at the 0.05 level.

**. Correlation is significant at the 0.01 level.

E2=estrogen, P4=progesterone, TC= total cholesterol, Tg= triglyceride, HDL= high-density lipoprotein, LDL= low-density lipoprotein, VLDL= very low-density lipoprotein, Glu= glucose, BHB= β-hydroxybutyrate acid, Ins= insulin, NEFA= non-esterified fatty acid.

Discussion

The physiological changes occurred due to energy and reproductive requirements after calving, such as the process of physical recovery from the latest delivery and colostrum production. Additionally, the physiological changes occurred during the peak of milk production when compared with the dry period, which the changes correlate to the need of nutrients and energy required for maternal maintenance and milk production that could affect the next reproductive cycle. Furthermore, more than 75% of diseased affecting cows often occurred within the first month after delivery (Ohtsuka et al., 2001; LeBlanc et al., 2006). During the period of this study, clinical characteristic data from both groups did not significantly differ. For the NE group, the average milk yield was slightly higher, but the conception rate at first AI was lower than the E group (Table 1).

The change of progesterone level in blood found in this study illustrated the return to normal postpartum ovarian cyclicity, which coincide with the study of Mekonnin et al. (2017) that also reported cows with progesterone level of 1 ng/ml or above indicate functional ovary and return normal ovarian cyclicity after calving. This study found a serum progesterone concentration of the E group to be slightly higher than NE group. In addition, the concentrations of serum progesterone obtained ranged between 1.10-6.00 ng/ml (data not shown), indicating the return of normal cyclicity at the observation date. The increased level of estrogen in blood is an indicator of follicles development in the ovary, with the highest level of estrogen occurs around the 24-hr preovulatory period. The estrogen increase during this period affects estrus behavior, which changes the reproductive system, especially the uterus for implanting the embryo after fertilization (Miller and Moor, 1976). Furthermore, the increased serum estrogen induces FSH/LH receptor expression in granulosa cells (Richards et al., 1976) and stimulate FSH action on aromatase activity to support normal cycle of ovulation (Zhuang, 1982). Although the concentration of serum progesterone indicated the return to normal cyclicity, no NE cow elicited significant estrus behavior. The ultrasonic images of dominant follicle confirmed E cows developed more follicles in the ovary more than NE cows, which correlates to the analysis of more estrogen circulating in the blood of E cows over NE cows ($P<0.05$). This development in E cows affected estrus and reproductive behaviors observed, and it influenced natural ovulation that increase higher rate of successful insemination than NE cows ($P>0.05$). However, the measured dominant follicles in E group revealed a higher average than the NE group, but the conception rate did not statistically differ ($P>0.05$). These results suggested E cows might encounter delayed the growth of follicles in the ovary, which caused a disturbance in the production and secretion of progesterone, then affect the increased secretion of LH pulses in pituitary gland and increased concentration of estradiol. Such effect caused follicles to grow, but there was no ovulation, and eventually decrease the insemination rate (Wiltbank et al., 2012; Vasconcelos et al., 2013).

IGF-1 influences the function of ovarian cells, including stimulate granulosa cell mitogenesis, stimulate the production and release of progesterone from luteal cells, as well as stimulate the production of androgen of theca cells in the ovary (Spicer et al., 1993). Therefore, an increase of serum IGF-1 concentration indicates the quality of ovarian function.

The result of the study revealed E cows had a significantly higher IGF-1 concentration in serum as compared to NE cows ($P<0.05$). This evidence was supported by previous studies in dairy and beef cattle, suggested higher level of serum IGF-1 level found in cattle that initiated early estrous cyclicity when compared to anestrus cattle during the postpartum period (Thatcher et al., 1996; Roberts et al., 1997). The characteristics mentioned could occurred by the physiological effects of IGF-1 influencing paracrine action of the ovary by complementing gonadotropin hormones and activate cells in the ovary to produce estrogen and progesterone via steroidogenesis action. The reduction of IGF-1 in postpartum cows could impair the growth and development of follicles in the ovary, eventually influence more risk of inactive ovary, cystic ovary, or more nonfunctional of corpus luteum. These issues could extend the time to ovulate and further affect the continuity of the next insemination (Lucy, 2000). The analysis of estrogen, progesterone, and IGF-1 obtained from this study suggested E cows have more ability than NE cows to produce estrogen and progesterone that directly control the behavior of female cow reproductive system.

During the first week of lactation, energy intake in high-yielding dairy cows is not sufficient to meet requirements for milk production and maintenance. This induces spontaneous and excessive lipolysis of adipose tissues resulting in elevated free fatty acids (FFA) concentrations in plasma. FFA are taken up by the liver where transform into triglyceride (TG) and further exported as VLDL (Vernon, 2005). Grummer (1993) reported diminishing VLDL secretion rate as liver TG elevated, which further modulate the development of fatty liver. Our study showed, the significantly lower of serum triglyceride and VLDL ($P<0.01$). This evidence supported by prior studies which demonstrated the postpartum high lactating cows had a low level of serum triglyceride and higher phospholipid as compared to medium to low lactating cows (Fiore et al., 2018; Imhasly et al., 2015). In addition to insufficient hepatic VLDL secretion, the drop in serum triglycerides and concomitant rise of phosphatidylcholines around parturition can be due to an increase in mammary lipoprotein lipase activity (Van den Top et al., 2005).

The serum glucose analysis of both groups revealed that all cows are within the 50-75mg/dl physiological range. NE cows tended to show a slightly lower level of glucose (P>0.05) and a significantly lower level of insulin than E cows (P<0.05). Our finding is supported by previous studies that exemplified a significant hypoglycemic condition that was generally observed in high-yielding ketogenic dairy cows during postpartum period (Djoković et al., 2014). The diminishing blood glucose level during postpartum period may be due to reallocation to an increased mammary gland activity in lactose synthesis, as well as to interrupt hepatocyte activity to synthesize glucose through gluconeogenesis under lipid-mobilization and lipogenesis in the liver (Overton and Waldron, 2004; Dann et al., 2005).

It is commonly accepted that postpartum cows showing serum BHB between 1.2 to 2.9 mmol/l or NEFA that is higher or equal to 0.26 mmol/l) can develop subclinical ketosis (Oetzel, 2004; Wang et al., 2016, Brunner et al., 2019). In addition, Huzzey and colleagues (2011) demonstrated that cows with postpartum serum NEFA and BHB concentration greater than 0.60 mEq/l and 10 mg/dl, respectively, have 4 times higher risk for developing some postpartum disease such as metritis, endometritis, retained fetal membranes, displacement abomasum or clinical ketosis. This study exemplified serum BHA and NEFA level of NE cows is significantly higher than E cows. The result inferred that NE cows may at be at risk to develop these mentioned postpartum diseases. In addition, some researchers reported a noticeable decline of blood insulin concentration in ketogenic cows with raising blood NEFA levels (Delic´ et al., 2020).

Moreover, it is generally accepted that serum NEFA above 0.6 mmol/l indicates a negative energy balance (NEB) condition in cattle. Our finding showed a serum NEFA concentration of NE cows ranged between 0.48-0.79 mmol/l, whereas E cows ranged between 0.11-0.22 mmol/l. This data implied that NE group encountered NEB and may further develop to clinical ketogenic condition.

NEFA values obtained from NE cows exhibited a negative correlation between estrogen and progesterone concentrations (P<0.05), this evidence revealed that NEFA negatively affect the ovarian hormones. While this study did not find any correlation between insulin and NEFA, a significantly lower level of blood insulin and higher NEFA in NE cows were observed (P<0.01). This evidence suggested that during the observing period, the NE cows had reduced anabolic effect of insulin on lipid metabolism that resulted in immediate uncontrolled mobilization of NEFA from the body reserve (Butler et al., 2003).

Conclusion

The result of this study suggested the NE cows experienced NEB that resulted in the increased risk of metabolic disorders such as fatty liver and ketosis that consequently impaired fertility, which affect the rate of success of AI or delay postpartum estrus and silent estrus. Based on our findings, further study will focus on the development of insulin sensitivity that may be beneficial to monitoring metabolic health in high-yielding cattle with postpartum anestrus.

Acknowledgment

We extend our appreciation to the NRRU Research Institute for the financial support of this study.

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