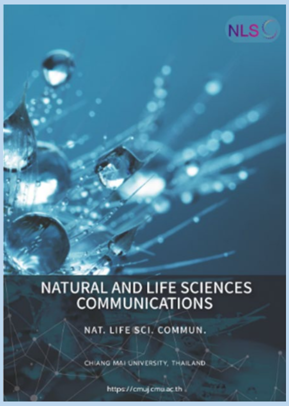
Research article



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Effects of Vitamin A Supplementation on Reproductive Function and Estrus Synchronization Response of Kamphaeng Saen Heifers Raised under Restrictive Feeding

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ABSTRACT

During the dry season in tropical areas, green pastures are limited. Heifers or cows fed primarily hay or rice straw are at risk of insufficient ß-carotene (provitamin A). Vitamin A (VitA) is crucial for reproductive functions. This study investigated the influence of VitA supplementation on the reproductive performance of Kamphaeng Saen beef heifers raised under restrictive feeding (n = 18; body condition score [BCS] = 3.5) over 48 days. The animals were equally divided into two groups: 1) the control group (fed a basal diet) and 2) the VitA group (fed the basal diet plus 100,000 IU of VitA per head per day). After 28 days of VitA supplementation, all heifers underwent estrus synchronization protocol. Reproductive tract and ovarian components were examined using ultrasonography to evaluate the size of the cervix, uterine horn, ovaries, follicles, and corpus luteum (CL) on Days 13 and 20 of the estrus synchronization protocol. Blood samples were collected to assess VitA and progesterone concentrations in serum. The results indicated that heifers supplemented with VitA tended to have more compact CL (P = 0.06). However, VitA did not significantly improve overall reproductive tract components or blood parameters between the control and VitA groups (P > 0.05). These findings suggest that VitA supplementation can enhance CL quality with short-term supplementation, but long-term VitA supplementation requires further investigation.

Keywords: Beef cattle, Kamphaeng Saen Heifers, Reproductive performance, Restrictive feeding, Vitamin A

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INTRODUCTION

The beef cattle farming industry has expanded significantly in recent years, leading to the development of various technologies aimed at enhancing the efficiency of beef cattle production. Reproductive technologies in female cattle, including artificial insemination (AI) and hormone-based estrus and ovulation induction, can potentially raise farm productivity (Velazquez, 2023). Proper estrus and ovulation are essential for managing the estrous cycle and facilitating rapid herd breeding management. In Thailand, embryo transfer (ET) and AI have been used to disseminate the genetic material of superior bulls and cows (Techakumphu et al., 2001). Several factors affect the success of ET or AI, including semen quality, embryo viability, the reproductive health status of the heifer/cow/recipient, AI techniques, transfer methods, and nutrient management in the breeding herd (Muhaidat et al., 2023). Nutrient intake plays an important role in the reproduction of cows and heifers, with the efficiency of the reproductive system relying on both macronutrients (protein and energy) and micronutrients (vitamins and minerals) (Ikeda et al., 2005; Cho et al., 2014; NASEM, 2016).

In tropical regions, green pasture is scarce during the dry season, necessitating the preservation of forages such as silage, hay, or rice straw for ruminant animals. Thailand is a major producer and exporter of rice, ranking sixth globally in production (Sowcharoensuk, 2024). Consequently, rice straw, a by-product of rice production, serves as a primary roughage during the summer. However, the quality of rice straw is poor, characterized by low digestibility and nutrient content (Wanapat et al., 2009; NASEM, 2016; Srisaikham and Lounglawan, 2020). Additionally, rice straw and other dried forages contain low levels of ß-carotene (Arikan and Rodway, 2001; Katamoto et al., 2003; Thongun et al., 2021), which is essential for synthesizing vitamin A (VitA) in cattle (Mitsuishi and Yayota, 2024).

VitA is vital for embryonic development and reproductive functions, existing in the form of carotenoids (ß-carotene) in green forage tissues (Haliloglu et al., 2002; Mitsuishi and Yayota, 2024). This fat-soluble vitamin supports reproductive system health in preparation for mating (Clagett-Dame and Knutson, 2011). Numerous studies have reported that retinoids play a role in oocyte maturation, follicular growth, fertilization, and early embryonic development. The level of VitA in ovarian follicular fluid may reliably indicate follicle health, with healthy follicles strongly correlated with elevated estradiol levels. Follicular disorders are indicated by low serum VitA levels (Brown et al., 2003; Abouzaripour et al., 2018). Thus, VitA is crucial for the reproductive system, stimulating and promoting the development of the corpus luteum (CL) by enhancing *StAR* gene transcription, which increases cholesterol availability for progesterone synthesis in luteal cells (Wickenheisser et al., 2005; Manna et al., 2015). Progesterone facilitates implantation and gestation, playing a critical role in preparing the uterine endometrium for embryo implantation (Hafez et al., 2000; Clagett-Dame and Knutson, 2011; Halasz and Szekeres-Bartho, 2013). Many reports indicate that CL is rich in VitA, which is essential for steroidogenesis and CL formation (Arıkan and Rodway, 2001; Haliloglu et al., 2002; Wickenheisser et al., 2005; Pasquariello et al., 2022; Mitsuishi and Yayota, 2024). The low serum VitA concentrations in cows are associated with increased uterine horn diameter and infertility (Ganguly and Mukherjee, 1988; Raila et al., 2017). Furthermore, the quality of embryos in the cows receiving VitA supplements has been shown to improve (Shaw et al., 1995).

In breeding programs or ET procedures, the preparation of the heifer/cow or recipient is essential. A healthy cow or heifer with good fertility must receive adequate nutrition and maintain a favorable BCS. For ET or fixed-time AI, synchronization programs are required to prepare the uterine environment for pregnancy after ET or to induce ovulation (Bó and Baruselli, 2014; Mebratu, 2020). The effects of VitA or ß-carotene on the reproduction of cows or heifers have been suggested (Haliloglu et al., 2002; Ikeda et al., 2005; Cho et al., 2014; Gouvêa et al., 2018; Mitsuishi and Yayota, 2024). However, the impact of low VitA or ß-carotene consumption on crossbred heifers or cows raised in tropical conditions has not been reported. Therefore, this study aims to determine the effect of VitA supplementation in the diet on the reproductive function of Kamphaeng Saen heifers fed a low VitA or ß-carotene diet under restrictive feeding in tropical conditions in Thailand.

MATERIALS AND METHODS

Study area, animal housing and management

This study was conducted at a beef cattle farm, Department of Animal Science, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Nakhon Pathom, Thailand. Eighteen heifers (440 ± 47 kg body weight, average age 23 months) were individually housed in open-air pens (2.5 × 5 m) with a concrete floor, experiencing average temperatures of 24–34 °C during the rainy season (August to October). The animals were selected based on uniformity of BCS >3.5 and were examined via ultrasonography for a normal reproductive system from the replacement heifers of the Kamphaeng Saen beef cattle herd (BCS: emaciated = 1, thin = 2, ideal = 3, fat = 4, overfat = 5, scaled according to the method described by Edmonson et al., 1989). Kamphaeng Saen beef cattle is the first synthetic breed developed in Thailand (*Bos taurus*; 50% Charolais and *Bos indicus*; 25% Brahman × 25% Thai native) (Sawanon et al., 2011). The animals were treated for intestinal and external parasites with Aben-15® (150 mg Albendazole; F.E. Pharma Company Limited®, Bangkok, Thailand), Ivomec® Plus (1% Ivermectin and 10% Clorsulon, Merial® Inc., Duluth, GA, USA), and vaccinated against foot-and-mouth disease. The animals were restrictively fed 3 kg/head/day of rice straw as roughage (low VitA or provitamin A content) and a concentrate diet (16% CP) at 3 kg/head/day, twice daily (7:00 a.m. and 6:00 p.m.). All animals had free access to fresh water. The animal study protocol was approved by The Animal Usage and Ethics Committee of Kasetsart University, Thailand (ACKU64-AGK-013).

Experimental design

Heifers were randomly assigned to two experimental groups: 1) control (n = 9), heifers fed a basal diet and 2) VitA group (n = 9), heifers fed a basal diet supplemented with 100,000 IU of VitA per head per day (Vitamin A acetate 1000; 1,000,000 IU/g, Zhejiang NHU Co., Ltd., Zhejiang ICP, China). VitA was supplemented using a top-dressing method (50 mg/head/meal) for each meal throughout the experiment. The quantity of supplementation was recommended in the Guidelines for OVN Optimum Vitamin Nutrition® in ruminants, DSM, 2022. This experiment was conducted from August to October, with a 14-day adaptation period before treatment commenced.

Feed chemical composition analysis

The nutrient compositions and ß-carotene concentrations of the diets are shown in Table 1. The concentrate diet and rice straw were analyzed for crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) using the Association of Official Analytical Chemists (AOAC) procedures (2016). The concentration of ß-carotene (provitamin A) in the diet was analyzed using high-performance liquid chromatography (HPLC) following the In-house method WI-TMC-11, as described in Munzuroglu et al. (2003).

Table 1. Chemical compositions (%DM) and ß-carotene concentration of concentrate diet[/1] and rice straw.

Items	Concentrate	Rice straw
Dry matter (%)	89.08	91.30
Crude protein	16.89	5.90
Ether extract	4.61	1.50
Neutral detergent fiber	26.51	74.00
Acid detergent fiber	14.05	44.50
Ash	7.11	12.10
Calcium	0.85	0.53
Phosphorus	0.73	0.12
ß-carotene (µg/100 g)	61.30	<LOQ

Note: [/1]Commercial concentrate diet in powder form containing 16% crude protein.
LOQ is the limit of quantification.

Estrus synchronization (ES) and detection

All heifers underwent synchronization using procedures described in the Operational Manual for Bovine Embryo Transfer Procedures (Bureau of Biotechnology in Livestock Production, 2013), as scheduled in Figure 1. On Day 0, the ovaries were examined via rectal ultrasonography (SonoScape S2 ultrasonography, B-mode 2-16 MHz rectal probe, Medical Corp, China). The dominant follicles were destroyed using a GnRH hormone treatment (Receptal®, 0.004 mg/mL buserelin acetate, Merck & Co., Inc., Rahway, NJ, USA) via a 2.5 mL intramuscular injection, followed by the insertion of a progesterone hormone device (Controlled internal drug release device; CIDR®, 1.38 g progesterone/device, Zoetis, 10 Sylvan Way Parsippany, NJ, USA) into a vagina. On Day 10, $PGF_{2\alpha}$ (Estrumate®, 500 mcg cloprostenol, Merck & Co., Inc., Rahway, NJ, USA) was administered via a 2 mL intramuscular injection in the morning. On Day 11, the progesterone device was removed in the morning and the ovaries were examined via rectal ultrasonography. On Day 13, the behavior and signs (such as clear mucus discharge from the vulva, mounting, and standing heat) and the ovaries (examined via rectal ultrasonography) of the heifers were recorded.

A penile translocation bull was used to detect estrus behavior of the hiefers twice daily (6:00–7:00 a.m. and 4:00–5:00 p.m.).

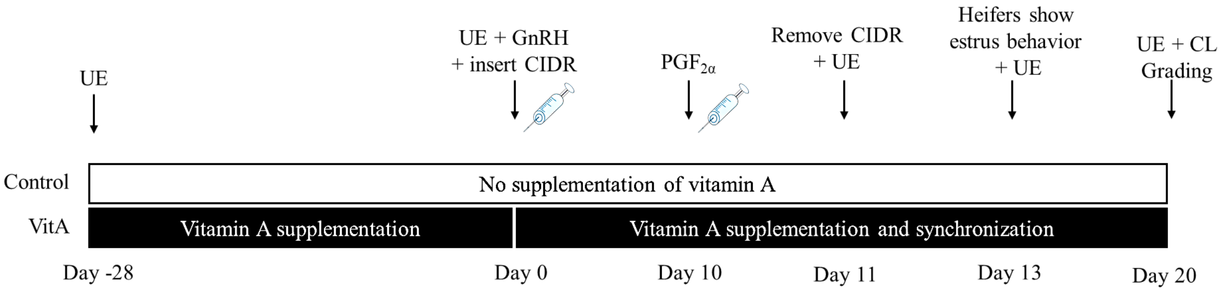

Figure 1. Vitamin A supplementation and synchronization protocol schedule. UE = Ultrasonography evaluation, GnRH = GnRH injection, $PGF_{2\alpha}$ = $Prostaglandin_{2\alpha}$ injection, P4 = Progesterone, CIDR = Controlled internal drug release device.

Evaluation of reproductive parameters

Reproductive tract and ovarian components were measured (ultrasonography evaluation; UE) as scheduled in Figure 1 using ultrasound (SonoScape S2 ultrasonography, B-mode 2-16 MHz rectal probe, Medical Corp, China) via a transrectal probe via a B-mode screen to determine the diameter of the cervix, uterine horn, preovulatory follicle, and CL on Days 0, 11, 13 and 20 of the ES protocol. CL quality was graded using the method described in Vieira et al. (2014). On Day 20 of the ES protocol, CL grades were classified as follows: 1) grade A: CL represents 75% of the ovary volume, 2) grade B: CL represents 50% of the ovary volume, and 3) grade C: CL represents 25% of the ovary volume.

Blood collection and hormone assay

Blood samples were collected to assess the concentrations of progesterone and ß-carotene in serum. The collection was via the jugular vein using vacuum blood collection tubes containing a clot activator (two tubes with 4 mL each). All blood samples were allowed to clot for 2 hours at room temperature, then centrifuged for 15 minutes at 1,000×g. The serum was transferred into sterilized 2 mL microtubes and stored at -80°C. The concentration of VitA in serum was measured using the HPLC method (Kanďár et al., 2014). The concentration of progesterone in serum was measured using an enzyme immunoassay method (Brown et al., 2004).

Statistical analysis

The student's t-test was applied to evaluate the effects of VitA supplementation on the diameter and area of the ovary, dominant follicle preovulatory follicle (POF), and CL. Normal distribution was checked using descriptive statistics, including mean, minimum, maximum, frequency, and standard deviation. Data on estrus expression and insemination rates were analyzed using Fisher's exact test with SAS® on Demand for Academics (SAS Institute, 2023). Significance was declared at a *P*-value of < 0.05, with tendencies declared at *P*-values between 0.05 and 0.10.

RESULTS

Vitamin A and ß-carotene intake of heifers

The ß-carotene concentration in the concentrate diet and rice straw (Table 1) indicated that the concentrate diet contained ß-carotene 1,839 µg/100 g of the diet. In contrast, the rice straw contained ß-carotene below the limit of quantification (40 µg/100 g). Therefore, the heifers ingested the primary source of VitA from the treatment, while the control group received ß-carotene only from the basal diet.

Effect of vitamin A on estrus expression and ovulation rate of Kamphaeng Saen heifers on Day 13 of synchronization

This study investigated the impact of VitA supplementation on estrus expression and ovulation rate in Kamphaeng Saen heifers. The results are shown in Table 3. They indicated no significant difference between the control and the VitA groups.

Table 2. Effect of vitamin A on estrus expression and ovulation rate of Kamphaeng Saen heifers on Day 13 of the synchronization protocol.

Items	Control	Vitamin A	SEM	*P*-value
Number of heifers (n)	9	9		
Estrus expression (%)	89 (8/9)	100 (9/9)	0.11	0.33
Standing heat after removing a CIDR (%)	78 (7/9)	89 (8/9)	1.18	0.92
Ovulation rate (%)	78 (7/9)	89 (8/9)	1.18	0.92

Note: SEM = Standard error of mean; in parentheses () is the number of animals expressing estrus/total number of animals. CIDR = Controlled internal drug release device

Effect of vitamin A on the diameter of the reproductive tract and ovarian components on Day 13 of the estrus synchronization protocol in Kamphaeng Saen heifers

The measurements of the diameters and areas of the reproductive tract and ovarian components using ultrasonographic images (Figure 2) on Day 13 of the ES protocol in Kamphaeng Saen heifers are shown in Table 4. There were no significant (P > 0.05) differences in the diameters of the cervix, uterine horn, and ovary, or the areas of the ovary and POF between the groups.

Table 3. Effect of vitamin A on the diameter of the reproductive tract and ovarian components on Day 13 of estrus synchronization protocol in Kamphaeng Saen heifers.

Items	Control	Vitamin A	SEM	*P*-value
Number of heifers (n)	9	9		
Diameter of the cervix (ipsilateral POF, cm)	1.95	2.02	0.31	0.82
Diameter of uterine horn (ipsilateral POF, cm)	1.18	1.16	0.16	0.90
Diameter of ovary (ipsilateral POF, cm)	2.37	2.30	0.17	0.67
Area of ovary (ipsilateral POF, cm^2)	3.19	2.91	0.39	0.47
Diameter of POF	1.25	1.20	0.14	0.79
Area of preovulatory follicle (ipsilateral POF, cm^2)	0.89	0.99	0.11	0.39

Note: SEM = Standard error of the mean; POF = preovulatory follicle

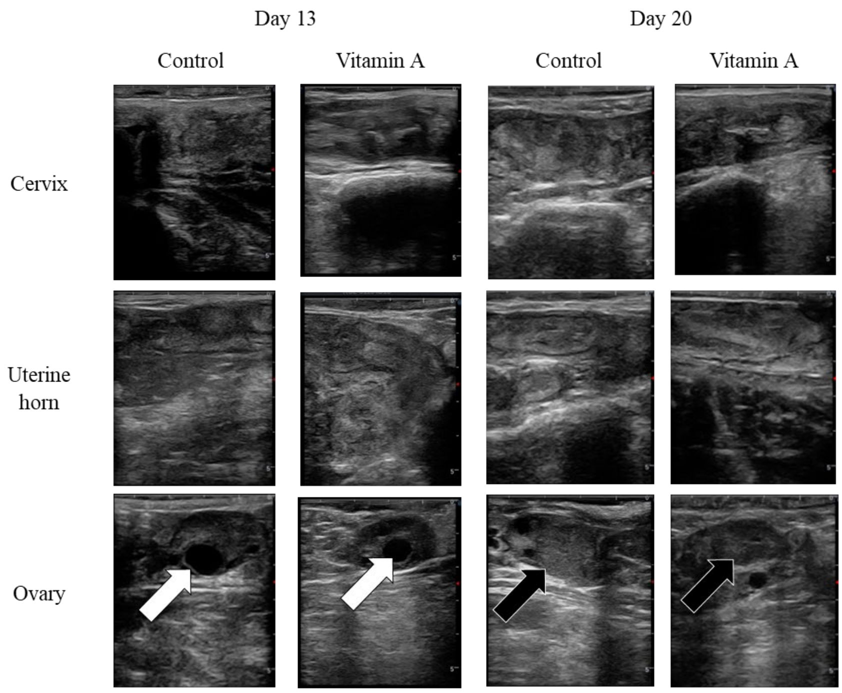

Figure 2. Ultrasonographic images of Kamphaeng Saen heifer reproductive tract on Days 13 and 20 of the ES protocol. White arrows indicate the ovary. Black arrows indicate the CL.

Effect of vitamin A on the diameter of the reproductive tract and ovarian components on Day 20 of the estrus synchronization protocol in Kamphaeng Saen heifers

The measurements of the diameters and areas of the reproductive tract and ovarian components using ultrasonographic images (Figure 2) on Day 20 of the ES protocol in Kamphaeng Saen heifers are shown in Table 5. There were no significant ($P > 0.05$) differences in the diameter of the cervix, diameter of the uterine horn, diameter of the ovary, area of the ovary, diameter of the CL, and area of the CL between the groups on Day 13. However, during this period, the CL developed after the ovulation of the dominant follicle.

Table 4. Effect of vitamin A on the diameter of the reproductive tract and ovarian components on Day 20 of the estrus synchronization protocol in Kamphaeng Saen heifers.

Items	Control	Vitamin A	SEM	*P*-value
Number of heifers (n)	9	9		
Diameter of the cervix (ipsilateral CL, cm)	1.61	1.73	0.17	0.51
Diameter of uterine horn (ipsilateral CL, cm)	1.12	1.06	0.18	0.73
Diameter of ovary (ipsilateral CL, cm)	1.71	1.79	0.13	0.55
Area of ovary (ipsilateral CL, cm^2)	3.34	3.86	0.48	0.30
Diameter of CL (cm)	1.69	1.64	0.12	0.68
Area of CL (cm^2)	1.64	1.75	0.35	0.76

Note: SEM = Standard error of the mean; CL = corpus luteum.

Effect of vitamin A in synchronization protocol on heifer ovulation

The percentage of the ovulated Kamphaeng Saen heifers was evaluated. The results indicate no significant (*P* = 0.84) difference in the percentage of heifers ovulating between the control and VitA groups (78% vs. 89%, respectively). In the present study, although the differences in ovulation were not observed between the control and VitA groups, the quality of CL was detected after ovulation as shown in Table 7.

Table 5. Effects of dietary vitamin A supplementation on Kamphaeng Saen heifers ovulation on Day 20 of the estrus synchronization protocol.

Items	Control	Vitamin A	SEM	*P*-value
Number of heifers (n)				
Diameter of the cervix (ipsilateral CL, cm)	9	9		
Diameter of uterine horn (ipsilateral CL, cm)	1.61	1.73	0.17	0.51
Diameter of ovary (ipsilateral CL, cm)	1.12	1.06	0.18	0.73
Area of ovary (ipsilateral CL, cm^2)	1.71	1.79	0.13	0.55
Diameter of CL (cm)	3.34	3.86	0.48	0.30
Area of CL (cm^2)	1.69	1.64	0.12	0.68

Note: SEM=Standard error of the mean; CL = corpus luteum.

Effects of vitamin A on quality classes of the corpus luteum in Kamphaeng Saen heifers on Day 20 of the synchronization protocol

The quality and type of CL in Kamphaeng Saen heifers are shown in Table 7. There were no significant (*P* > 0.05) differences in CL quality between the control and VitA groups. The distribution of CL grades in the control versus VitA group was as follows: Grade A; 44% vs. 56%, Grade B; 22% vs. 33%, Grade C; 11% vs. 0%, respectively. These results suggest a positive effect of VitA supplementation. Regarding CL type, there was no significant (*P* > 0.05) difference in the presence of cavity CL between the control and VitA groups, with 56% vs. 22%, respectively. However, the occurrence of compact CL (Figure 3) tended to be higher (*P* = 0.06) in the VitA group compared to the control group (67% vs. 22%, respectively).

Table 6. Effects of dietary vitamin A supplementation on corpus luteum (CL) quality in Kamphaeng Saen heifers on Day 20 of the estrus synchronization protocol.

Treatments	Corpus luteum grade (%) [/1]			Compact CL (%)	Cavity CL (%)
	A	B	C		
Control	33.33 (3/9)	22.22 (2/9)	11.11 (1/9)	22.22 (2/9)	55.56 (5/9)
Vitamin A	55.56 (5/9)	33.33 (3/9)	0.00 (0/9)	66.67 (6/9)	22.22 (2/9)
P-value	0.66	0.62	0.33	0.06	0.16

Note: [/1] Corpus luteum (CL) grade: Grade A = CL represents 75% of the ovary volume, Grade B = CL represents 50% of the ovary volume, and Grade C = CL represents 25% of the ovary volume. In the parenthesis () is the number of detected animals/total number of animals.

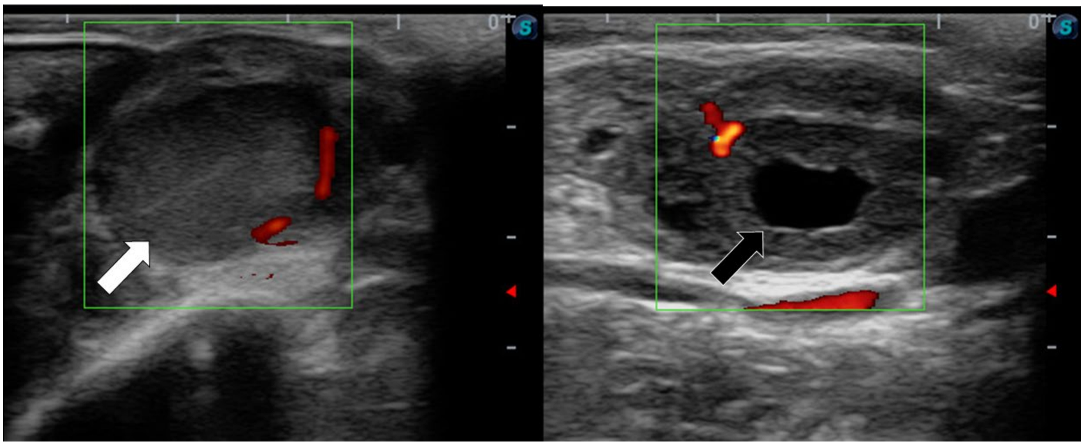

Figure 3. **Ultrasonographic images of Kamphaeng Saen heifer ovary showing compact corpus luteum (white arrow) and cavity corpus luteum (black arrow).**

Effect of vitamin A on serum progesterone concentration of Kamphaeng Saen heifers in ES protocol

The concentrations of serum progesterone are shown in Figure 4. There was no significant (P > 0.05) difference in serum progesterone levels between the control and the VitA groups at various time points. On Day 0 of the protocol, the concentrations were 3.48 ng/mL and 4.60 ng/mL, respectively (P = 1.49). On Day 10, the concentrations were 4.83 ng/mL and 4.86 ng/mL, respectively (P = 1.08). On Day 13, the concentrations were 1.82 ng/mL and 1.06 ng/mL, respectively (P = 0.51). On Day 20, the concentrations were 3.73 ng/mL and 4.33 ng/mL, respectively (P = 1.29).

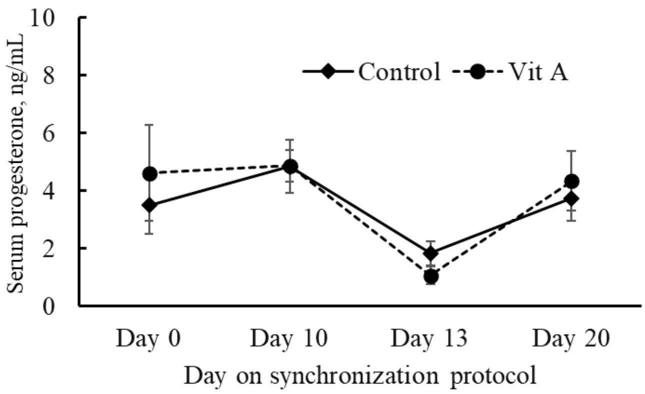

Figure 4. **Serum progesterone concentration of Kamphaeng Saen heifers during estrus synchronization**

Effect of vitamin A on vitamin A concentration in serum of Kamphaeng Saen heifers at Day 20 of the synchronization protocol

The concentrations of serum VitA of the hiefers were measured. The results indicated no significant (P = 0.05) difference between the control group and VitA groups (0.35 vs. 0.4 mg/L, respectively).

DISCUSSION

This experiment was conducted on Kamphaeng Saen heifers raised in an intensive program under low VitA or ß-carotene consumption and restrictive diets. This situation often occurs in summer when green pasture is limited. Farmers preserve roughages such as grass or corn silage, hay, or rice straw for the long dry season. Particularly, rice straw is a valuable by-product of rice production. However, this material contains low levels of provitamin A or ß-carotene, which has an essential role in reproductive health. The present study shows heifers that ingested VitA or ß-carotene from both supplementation and the basal diet but in the control group, they ingested only from the basal diet (Table 1). Nonetheless, the basal diet contained a very low ß-carotene. Kawashima et al. (2010) reported that cows fed synthetic β-carotene (2,000 mg/day) for three weeks before calving exhibited apparent luteal activity within three weeks postpartum compared to cows without β-carotene supplementation. β-carotene may also play a crucial role in CL development to maintain pregnancy (Mitsuishi and Yayota, 2024). However, in the present study, the basal diet and roughage contained very low levels of VitA or ß-carotene. In cases of insufficient VitA or provitamin A in a herd, supplementation is essential. VitA activates the *StAR* and cytochrome P450 genes, accumulating in the form of the retinoic acid receptors and retinol X receptors, which promote *StAR* gene expression and directly induce transcription, facilitating cholesterol transport for progesterone production (Lee et al., 1999; Suwa et al., 2016). VitA also induces the expression of the cytochrome P450 11α-hydroxylase (*CYP11*) gene, which is involved in producing the P450scc enzyme and converting cholesterol into pregnenolone, thereby increasing the synthesis in luteal cells (Wickenheisser et al., 2005; Manna et al., 2015). Although the results in these studies did not show significant effects on reproduction, animals supplemented with VitA had higher serum VitA concentrations on Day 20 of the ES protocol.

In the present study, on Day 13, the reproductive tract of Kamphaeng Saen heifers supplemented with VitA did not show significant effects either. Previous studies reported that the diameter of the cervix ranged from 1.5 to 2 cm, the uterine horn from 2 to 3 cm, and the ovary approximately 1.5 cm in crossbred heifers during estrus (Gupta, 1962; Anderson et al., 1991). Although the roles of VitA on the cervix or uterine horn responses have not been extensively reported, the present study found no significant differences in reproductive tract measurements between the control and VitA groups. VitA supplementation was expected to play an important role in estrus expression in heifers. The percentage of estrus expression in the VitA group was higher than in the control group on Day 13. VitA may influence the diameter and area of POF. This vitamin potentially activates and enhances estrogen and progesterone synthesis, regulated by *Aldh1a1* and *Aldh1a2* genes. These functions improve ovarian and steroid hormone synthesis by increasing the expression of *MESP2*, which targets *StAR* and *CYP11a1* genes to control the proliferation of ovarian granulosa cells through the PI3K-Akt-hedgehog pathway (Shuang et al., 2023).

The VitA supplementation group exhibited greater serum progesterone concentrations. Rode et al. (1990) and Weiss (1998) reported that VitA is degradable in the rumen by rumen microbes. Therefore, adding VitA to the diet is essential to address the insufficiency of VitA or provitamin A in the cattle herd, especially during the dry season. In the present study, supplementation of 100,000 IU/head/day for 48 days resulted in serum progesterone levels of 4.33 ng/mL. In contrast, Tharnish and Larson (1992) reported that supplementation of VitA in gestating cows at 100,000 vs. 1,000,000 or 2,000,000 IU/head/day for less than three months resulted in serum progesterone levels of 9.89 vs. 8.92 or 6.35 ng/mL, respectively. They also reported that longer supplementation (>5 months) with similar treatments resulted in serum progesterone levels of 11.06 vs. 10.35 or 9.46 ng/mL, respectively. These findings indicate that long-term VitA supplementation could elevate and maintain progesterone levels. The size and type of CL influence progesterone production, making CL quality essential for maintaining gestation. The size and type of CL are determined by POF size, which directly affects CL development. After ovulation, larger POFs lead to better CL quality (size and compactness) (Robinson et al., 2006; Vasconcelos et al., 2001). The development of CL could be categorized into two types: compact and cavity-type. The cavity-type has more quick development than the compact-type. However, the development of cavity-type CL may be limited by blood flow to the CL (Alila and Hansel, 1984; Wiltbank, 1994; Grygar et al., 1997; Jaśkowski, 2019; Jaśkowski et al., 2021). Perez-Marin (2009) reported that the fertilization rate of cows or heifers with cavity CL was lower than that of cattle with compact CL (42.9% vs. 57.1%, respectively). Although the size of the cavity CL ranges from medium to large, it does not adversely affect progesterone production (Kastelic et al., 1990; Gábor et al., 2004; Jaśkowski, 2019; Jaśkowski et al., 2021). As previously mentioned, VitA may enhance the reproductive performance of beef cattle. To prepare heifers or cows as recipients for ET or the breeding season, sufficient daily VitA intake is necessary. During periods of insufficient green pasture, VitA supplementation in the diet could effectively maintain reproductive system potential.

CONCLUSION

Supplementation of VitA in Kamphaeng Saen beef heifers tends to improve CL quality compared to no VitA supplementation. However, further studies are needed to determine the successful transfer or conception rates of either ET or insemination programs in the production herd, particularly during insufficient green pasture.

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AUTHOR CONTRIBUTIONS

S. Leelachayakun: Original manuscript preparation and revision; sample collection and processing; formal analysis; data collection, curation, analysis and interpretation.

S. Majarune: Study conceptualization and design; methodology; formal analysis; data collection, curation, analysis and interpretation; manuscript writing and revision.

W. Maitreejet: Methodology; formal analysis; data collection.

C. Borirak: Data collection.

T. Rongthong: Data collection.

A. Khongpradit: Data collection, curation and analysis.

S. Sawanon: Resources and supervision

P. Boonsaen: Study conceptualization and design; methodology; formal analysis; results; interpretation supervision; project administration; fund acquisition; writing -review and editing the article; critical revision.

All authors approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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