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Effect of energy sources on ovarian follicular dynamics and oestrous activity of Holstein cows

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Abstract

The objective of the study was to evaluate the effects of three nutritional treatments, which differed in energy level and source, on preovulatory follicles, number of follicles and oestrous activity in dairy cows. Twenty two Holstein multiparous cows from the Elsenburg herd were used in this study. After parturition, cows were kept on kikuyu-ryegrass pastures on an *ad libitum* basis, and allocated to various levels and types of concentrate supplements, which differed in starch and fat contents. The control group received 7 kg/day of a control concentrate, and the treatment groups each received 12.6 kg/day of concentrate. The concentrates contained high starch-low fat (HSLF) and high starch-low fat/low starch-high fat (HSLF-LSHF) levels. The supplement in treatment HSLF was a glucogenic concentrate using maize as the energy source. The supplements in treatment HSLF-LSHF were a combination of a glucogenic concentrate, which was offered for the first 60 days in milk (DIM), similar to treatment HSLF, followed from 61 DIM by a lipogenic concentrate using wheat bran and calcium (Ca) salts of long-chain fatty acids as the energy sources. At 80 ± 10 DIM, cows were synchronized with an Ovsynch protocol without being inseminated before the ultrasonography observation. While they were detained in a shaded neck clamp, cows were assessed individually with an ultrasound scanner every three days for ovarian measurements and follicular activity until the subsequent oestrus. Results showed that ovarian and follicular measurements and the numbers of follicles in various follicle size classes were similar between nutritional treatments. However, the total ovarian follicular counts were significantly higher in cows that received the HSLF and HSLF-LSHF treatments, compared with their counterparts in the control group (i.e. 7.23 ± 0.22, 7.21 ± 0.14 and 6.53 ± 0.19, respectively), through possible improvement in nutritional status. Further research is required to investigate various energy levels and sources that enhance the viability and the quality of the oocyte ovulating from the dominant follicle and improve the intensity and length of the oestrous expression in dairy cows.

Keywords: Energy nutrients, follicular numbers, heat expression, preovulatory follicle, ruminant
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Introduction

Optimizing fertility in dairy herds requires cows to calve annually to maximize the economic returns from milk production (Roche *et al*., 2000). However, several studies have reported a worldwide decline in fertility of dairy cows (Lucy, 2007; Butler, 2003; Walsh *et al*., 2011). The peripartum negative energy balance (NEB) in dairy cows could last for 10 to 12 weeks postpartum (Butler, 2003) and suppresses the somatotropic axis and gonadotropins on the ovaries (Beam & Butler, 1999), resulting in extended anoestrous periods (Butler, 2000; Mellouk *et al*., 2017). Roche *et al*. (2000) stressed that anovulatory oestrus in dairy cows is not owing to an absence of follicular development, but to the failure of a dominant follicle to ovulate, because of an NEB. Anoestrus is caused by low levels of metabolites (i.e. glucose) and hormones (i.e. insulin, insulin-like growth factor-I (IGF-I), luteinizing hormone (LH) and oestradiol) in the bloodstream (Beam & Butler, 1999; Diskin *et al*., 2007). The decline in these metabolic and endocrinal signals reduces the diameter and growth rate of the dominant follicle (Beam & Butler, 1997), prevents or delays it from ovulating (Butler, 2003; Webb *et al.* 2004), and eventually decreases the weight of the corpus luteum after ovulation

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(Beam & Butler, 1999). Also, ovaries of cows that were exposed to adverse conditions of NEB were reported to be small in size and low in follicular counts (Ireland *et al*., 2008), they produced low levels of oestrogens and progesterone (P_4) after ovulation (Leroy *et al*., 2008a), and they were associated with poor endometrial development (Jimenez-Krassel *et al*., 2009). Wathes *et al*. (2007) reported that inferior oocytes were produced in dairy cows at the start of the breeding season when ovarian follicles underwent their earlier stages of maturation during the NEB nadir. Additionally, studies in which dairy cows were monitored using milk P_4 profiles found that NEB was associated with greater incidence of irregular oestrous cycles (Taylor *et al*., 2003; Wathes *et al*., 2003). Consistent with these arguments, NEB has been shown to have adverse long-term carry-over effects on fertility through ovarian dysfunction and reduced oocyte competence (Britt, 1994; Lucy, 2007; Leroy *et al*., 2008a; 2008b; Garnsworthy *et al*., 2008a). Thus the ultrasound imaging technique offers a way to investigate bovine follicular dynamics to understand the ovarian function on fertility in dairy cows (Sakaguchi *et al*., 2004; Mossa *et al*., 2012). Real-time ultrasonographic imaging allows non-invasive visual assessment of changes in ovarian structures over time (Fricke, 2002). Therefore, the objective of this study was to evaluate the effects of inclusion level (low versus high) and type of energy supplements (starch versus fat) on preovulatory follicles, numbers of follicles and oestrous activity in Holstein cows grazing on kikuyu-ryegrass pastures following oestrous synchronization.

Materials and Methods

Ethical clearance for this study was obtained from Western Cape Department of Agriculture (WCDA, Project AP/BR/D/CM31).

The trial was conducted at Elsenburg Research Farm of the WCDA, located approximately 50 km east of Cape Town, at an altitude of 177 m, longitude of 18° 50' and latitude of 33° 51' in the winter rainfall region of South Africa. The area receives an average annual rainfall of 650 mm, and has a typical Mediterranean climate with short cool wet winters and long warm dry summers.

In this study, the ovarian follicular dynamics and oestrous activity of 22 multiparous Holstein cows (i.e. 3.89 ± 0.40 years old in their second and third parities) were assessed over an oestrous period (± 21 days). Following parturition, cows were allocated to three isonitrogenous nutritional treatments , namely control (n = 10), high starch-low fat (HSLF) (n = 6) and high starch-low fat/low starch-high fat (HSLF-LSHF) (n = 6), according to calving date, LW at calving and milk yield during the previous lactation. Cows were offered unrestricted access to cultivated kikuyu-ryegrass pastures, and were supplemented with pelleted concentrates, which differed in energy level and type. Pastures were irrigated, as required, using a permanent irrigation system. Cows followed a rotation programme to ensure an *ad libitum* dry matter (DM) intake under normal conditions. The control group received 7 kg/day of a control concentrate, while the treatment groups were fed concentrates at 12.6 kg/day. Half of the concentrate allowance was fed after each milking. Cows were milked twice a day at 05:30 and 15:00. Representative samples of pastures and concentrates were collected weekly, then bulked monthly, and analysed for chemical composition. Neutral detergent fibre (NDF) was determined according to Van Soest *et al*. (1991), using sodium sulphite anhydrous and amylase to decrease nitrogen and starch contamination in the NDF determination. AOAC (1990) official methods were used to determine the concentrations of DM, fat, crude protein (CP) and nitrogen (N x 6.25) in the diet. Diets were formulated on a DM basis with the CPM-Dairy software programme (2006), using an intake level of 25 kg DM/day per animal and consisted of the various levels and types of concentrate offered among treatments, with the rest of the intake being supplied by pastures. Starch and metabolizable energy (ME) of diets were estimated using the CPM-Dairy software programme (2006).

The control concentrate consisted of maize as the energy source. It contained a low level of energy content, and was fed from calving until the end of the trial. The control diet provided 10.3 MJ ME/kg DM and 457, 104 and 40 g/kg NDF, starch and fat on a DM basis, respectively. The supplement in the HSLF treatment was a glucogenic concentrate containing maize as the energy source, and was fed from calving until the end of the trial. The HSLF diet contained a high energy content (11.3 MJ ME/kg DM) and offered 341, 242 and 35 g/kg of NDF, starch and fat on a DM basis, respectively. This HSLF diet was formulated to increase the circulating glucose and insulin levels to improve the postpartum EB status and encourage ovarian activity in cows. The supplements in the HSLF-LSHF treatment were combinations of a glucogenic concentrate, which was offered for the first 60 DIM per treatment HSLF, followed from 61 DIM until the end of the trial by a lipogenic (LSHF) concentrate using wheat bran and Ca salts of long-chain fatty acids (Megalac rumen bypass fat, Volac International Ltd., UK) as the energy sources. In the HSLF-LSHF combination treatment, the high starch-based diet was fed to achieve the same objective as the HSLF treatment during the first 60 DIM. The LSHF diet contained a high level of energy (11.3 MJ ME/kg DM) and was formulated to provide 388, 137 and 58 g/kg of NDF, starch and fat on a DM basis, respectively. The LSHF diet was fed from 61 DIM to decrease plasma insulin and improve plasma cholesterol to increase ovulatory follicular competence.

At 80 ± 10 DIM, 22 cows were synchronized using an Ovsynch protocol without subsequent insemination. Cows were handled individually with care, while restrained in a sheltered neck clamp. The synchronization procedure began in early August 2014, and followed this pattern: i) at 11:00 on day 0, cows received an intravaginal progesterone (P_4) controlled internal drug release device (CIDR) (Pfizer Laboratories Pty Ltd, Sandton, South Africa), which contained 1.9 g of P_4, together with an intramuscular injection of 2 mL of Cidirol (Pfizer Laboratories Pty Ltd, Sandton, South Africa), which contained 1 mg/mL of oestradiol benzoate in 100 mg/mL of benzyl alcohol as preservative; ii) on day 7, animals received an intramuscular injection of 1 mL prostaglandin-$F_{2\alpha}$ ($PGF_{2\alpha}$) Estrumate, containing 0.1% m/v of chlorocresol as preservative and 263 µg of cloprostenol sodium, equivalent to 250 µg of cloprostenol (Schering-Plough Animal Health, Isando, South Africa) at 16:00; iii) on day 8, CIDR devices were removed at 07:00 and heat detectors (Kamar® Heatmount Detectors, Kamar Products Inc., Zionsville, USA) were attached to the tail-head area; iv) on day 9, a dose of 1 mL of $PGF_{2\alpha}$ was administrated by an intramuscular injection; and v) on day 10, cows were not inseminated, but the positive heat detectors were removed. Ovaries were then subjected every three days at 15:00 to an ultrasonographic evaluation for the next 21 ± 3 days until the subsequent oestrus. To avoid ultrasonographic procedures on Sundays, an interval of four days was sometimes used. The diagnostic imaging was performed with an ultrasound scanner (SonoScape A6, SonoScape Medical Corp., Shenzhen, China), with a linear transrectal probe and standardized settings. Settings were 5-6 MHz for the frequency, 80% for power and 62 mm for depth. This procedure was conducted by one trained veterinarian for all cows to minimize variation. The Kamar detectors were attached to cows on day 9 from the start of the ultrasound observation, and cows were artificially inseminated when oestrous detection was positive. The Kamar detector was positive when cows showed typical oestrous behaviours (i.e. mucous vaginal discharge, mounting and sniffing). The trial was completed after a pregnancy diagnosis by rectal palpation by the veterinarian at 35–40 days after artificial insemination (AI).

Ovarian follicles, which were observed as black circular fluid-filled structures, were identified on all digitized images (Fricke, 2002). An electronic calliper was used to measure the diameters of the ovaries and follicles. The relationship between the measured dimensions on the digitized images and the calculated dimensions on the ultrasound was determined with established linear regression ($y = 0.392\ x$, $R^2 = 0.84$). All visible follicles were counted on both ovaries following each ultrasound observation and the total number of follicles per animal was noted, as were the areas (eclipses) of the ovaries over time. The area of the eclipse was measured as area = 3.142 x short radius x long radius, with short and long radiuses being half of their diameters. Follicles were categorized in relation to the long diameter in size classes according to Garnsworthy *et al.* (2008c). Small-sized follicles were < 5 mm, medium-sized 5 to 10 mm and large-sized > 10 mm in diameter.

Data were analysed using the PROC MIXED of SAS enterprise guide (SAS, 2012). The statistical model included the treatment (*T*) effect, time (*t*) effect of observations and interaction effect between treatment and time (*Tt*) as fixed effects, while animal effect within treatments was specified as a random effect. The measured variables obtained in an ultrasound observation during the trial were regarded as repeated observations of a particular block. The statistical model was defined as follow:

$Model = \mu + T_i + t_j + (tT)_{ij} + \delta_{(ij)k} + \varepsilon_{ijk}$, where

μ = overall mean

T_i = the fixed effect of the i^{th} treatment (i.e. control, HSLF and HSLF-LSHF)

t_j = the fixed effect of the j^{th} time of ultrasound observation (i.e.1 to 7)

$(tT)_{ij}$ = the interaction between levels i^{th} treatment and j^{th} time of ultrasound observation

$\delta_{(ij)k}$ = the variable effect of the k^{th} block effect in the i^{th} treatment (repeated statement)

ε_{ijk} = the random experimental error

All effects were used to analyse the ovarian dimensions. The follicular counts were analysed without the repeated statement ($\delta_{(ij)k}$) in the statistical model. The area of the preovulatory follicle at the first ultrasound observation was analysed without the repeated statement ($\delta_{(ij)k}$) and interactions between treatment and time of ultrasound observation using the treatment as the only fixed effect in the statistical model. Statistical assumptions were described as fixed effects and their interactions were equal to zero with $\delta_{(ij)k} \sim N(0, \sigma_e^2)$, varying independently of ε_{ijk}. Differences in means and standard error (SE) of means between treatments were obtained using the pairwise comparison of the Bonferroni t-test and significance was declared at $P < 0.05$. Interactions were reported as NS (not significant) if $P > 0.05$.

Results and Discussion

The effects of concentrate supplementation that differed in energy level and type in a pasture-based system on ovarian and follicular dimensions, follicular counts and proportions of dairy cows that were ovulating or pregnant are presented in Table 1. Changes in total ovarian follicles of dairy cows over time of observation until the subsequent oestrus are showed in Figure 1.

Table 1 Effects of nutritional treatments that differed after calving in energy levels and sources on ovarian and follicular dimensions, follicular counts (means ± SE) and proportions of Holstein cows ovulating and pregnant after an Ovsynch protocol, recorded during a 21 ± 3-day period

	Concentrate feeding			*P*-values		
Parameters	**Control**	**HSLF[1]**	**HSLF-LSHF[1]**	***T*[2]**	***t*[2]**	***Tt*[2]**
Number of cows	10	6	6			
Ovarian and follicular dimensions						
Short diameter of the ovary (mm)	16.2 ± 0.42	16.3 ± 0.43	16.1 ± 0.32	0.22	0.76	NS
Long diameter of the ovary (mm)	33.1 ± 0.62	33.4 ± 0.79	33.7 ± 0.81	0.32	0.87	NS
Ovarian area (mm^2)	426 ± 15	437 ± 19	445 ± 20	0.29	0.73	NS
Area of preovulatory follicles at first observation (mm^2)	115 ± 15	167 ± 21	131 ± 19	0.16	-	-
Follicular counts						
Number of small follicles	3.16 ± 0.36	3.46 ± 0.42	3.58 ± 0.30	0.19	0.09	NS
Number of medium follicles	2.04 ± 0.17	2.48 ± 0.22	2.16 ± 0.24	0.42	0.08	NS
Number of large follicles	1.30 ± 0.08	1.29 ± 0.11	1.44 ± 0.11	0.58	0.07	NS
Number of total follicles	6.53^a ± 0.19	7.23^b ± 0.22	7.21^b ± 0.14	0.03	0.09	NS
Proportion of cows ovulating and pregnant[3]						
Proportion of cows ovulating after Ovsynch	1.00	0.83	1.00	-	-	-
Proportion of cows showing oestrous expression	0.50	0.66	0.83	-	-	-
Pregnancy rate after AI	0.40	0.50	0.50	-	-	-

[1]HSLF: high starch-low fat, HSLF-LSHF: high starch-low fat/low starch-high fat
[2]*T*: treatment, *t*: time of ultrasound observation, *Tt*: Interaction between treatment and time of ultrasound observation
[3]Not statistically analysed due to the small dataset (n = 22)
[a, b, c] Row means with different superscripts differ significantly at $P < 0.05$

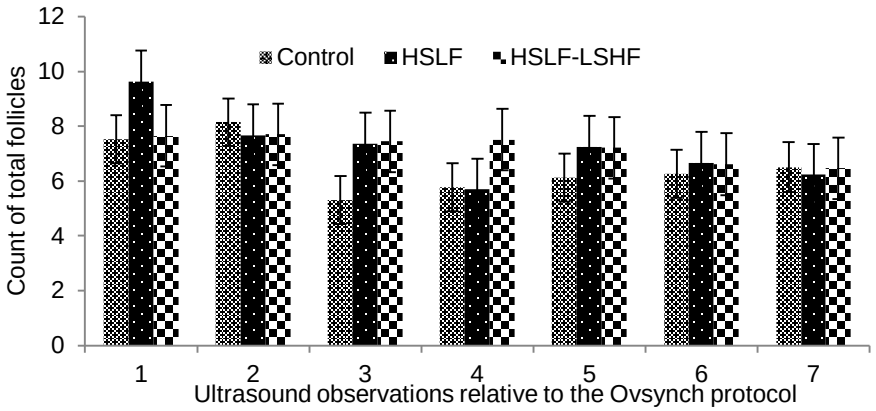

Figure 1 Effects of nutritional treatments differing after calving in energy levels and sources on the total number of ovarian follicles (mean ± SE) following an Ovsynch protocol, recorded during a 21 ± 3 day period [HSLF: high starch-low fat, HSLF-LSHF: high starch-low fat/low starch-high fat]

The mean (± SE) area of the bovine ovaries between nutritional treatments was 436 ± 18 mm^2, with a long diameter of 33.5 ± 0.74 mm and a short diameter of 16.2 ± 0.39 mm. The ovarian dimensions of grazing Holstein cows were similar between nutritional groups that differed in inclusion level and type of concentrate supplements. No interaction effects between the treatment and the time of observation were detected on the ovarian dimensions of cows. Furthermore, the area of the preovulatory follicle, regarded as the largest follicle at the first ultrasound observation of cows, was similar among nutritional treatments. Energy levels and types among treatments did not affect the number of follicles in the various follicle size classes of dairy cows. However, cows that received 12.6 kg concentrate per cow per day in the HSLF and HSLF-LSHF treatments increased ($P < 0.05$) the number of total follicles, both amounting to 15% more follicles, in comparison with cows that received only 7 kg concentrate/cow/day in the control.

Although the molecular mechanisms that are involved in the acquisition of competence are not well known, the size of the preovulatory follicle is an important parameter, which influences oocyte competence (Blondin *et al*., 2012). Lonergan *et al*. (1994) and Blondin & Sirard (1995) studied *in vitro* the effect of follicular size on the quality and viability of oocytes in dairy cows, and found that oocytes that emerged from smaller follicles (< 3 mm) had reduced or no developmental competence. These researchers reported that these oocytes appear to have been recovered too early and therefore probably lacked some follicular factors that would have signalled the oocytes to acquire their full competence. These factors, consisting of maternal messenger RNA and protein molecules, are synthesized and stored in a time, space and dose-dependent manner in the nucleus and ooplasma during oocyte growth and maturation (Blondin *et al*., 2012). Barnes *et al.* (1993) reported that an oocyte that ovulates from a larger dominant follicle has higher developmental competence in quality and viability. This competence is the ability of the oocyte to complete maturation, undergo successful fertilization, reach the blastocyst stage and yield a healthy embryo (Watson, 2007). Vasconcelos *et al*. (2001) studied the effect of follicle aspiration and gonadotropin-releasing hormone treatment on the size of follicles and corpus luteum of lactating dairy cows using an in vivo system. These researchers reported that increased follicle sizes in non-aspirated cows had advantageous effects on the corpus luteum function and its secretion of oestrogen and P_4, compared with the aspirated group. In other investigations, no differences were recorded in the preovulatory follicle diameter (area = 3.412 x R^2, with R being the radius) when isocaloric diets, which differed in increasing levels of starch and fat, were fed to lactating Holstein dairy cows (Garnsworthy *et al*., 2008b; 2008c). In the current study, the areas of the preovulatory follicle were 45% and 14% larger in lactating dairy cows fed the HSLF and HSLF-LSHF treatments compared with those fed the control diet, respectively. However, the recorded difference was not significant (*P = 0.16*), possibly owing to the small number of cows used in the study (n=22).

In the current study, the number of follicles in the follicle size classes was similar between dairy cows fed various energy levels and types of concentrate. However, total follicular counts were improved (*P < 0.05*) in dairy cows fed HSLF and HSLF-LSHF concentrates compared with those that received the control concentrate, being 7.23 ± 0.22, 7.21 ± 0.14 and 6.53 ± 0.19, respectively. Mellouk *et al*. (2017) found no differences in numbers of small (3–5 mm), medium (> 5 and ≤ 7 mm) and large (> 7 mm) follicles of dairy cows when fed diets containing high and low energy levels. Adamiak *et al.* (2005) studied the follicular growth in 20-month-old beef x dairy heifers fed 5 or 10 MJ ME/kg of metabolic LW per day. These researchers found no effect on the total number of visible follicles, but an increased number of medium-sized (4–8 mm) and large (> 8 mm) follicles and improved growth rate and maximum diameter of the dominant follicles when feeding the high metabolizable energy (ME) diet compared with the low ME diet. On the other hand, Gong *et al.* (2002a) studied the ovarian follicular responses to follicle stimulating-hormone (FSH) treatment in Hereford x Friesland heifers fed on a DM basis 2 kg of hay + 2.5 kg of concentrates at maintenance level and 2 kg of hay + 6.5 kg of concentrates at twice maintenance level. They found that feeding heifers at twice the maintenance level resulted in a significant increase in the number of small (2–4 mm) and large (> 9 mm) follicles. Lucy *et al.* (1991a) found that the energy balance (EB) status in early lactation influenced early changes in follicular populations of lactating dairy cows and reported decreasing numbers of small (< 5 mm) ovarian follicles and increasing numbers of large (> 8 mm) follicles, with increasing days postpartum. In addition, lactating dairy cows supplemented with lipids in early lactation in an attempt to improve the EB status were found to have follicles with larger diameters (Lucy *et al*., 1991b). Beam & Butler (1997) studied the influence of EB status and level of dietary fat on the dominant follicle development and function in lactating Holstein cows. They found that follicles that emerged after the NEB nadir, rather than before the nadir, showed improved follicular growth and diameter, enhanced oestradiol production, and were more likely to participate in ovulation. Although insulin and IGF-I levels were not measured in this study, these hormones are signals that mediate critical changes in EB status. These hormonal improvements in postpartum dairy cows support the number, differentiation and maturation of follicles (Roche *et al*., 2011), thereby increasing oestradiol production (Gong *et al*., 2002b) and the chance of dominant follicles ovulating in response to the LH surge (Beam & Butler, 1997; 1999; Lucy, 2003). Supporting this argument, NEB, which is a physiological state of undernutrition, has been related to the inability of the pituitary gland to sustain high-frequency LH pulses to the ovaries, resulting in the prevention of ovulation (Schillo, 1992; Jolly *et al*., 1995). Several reports indicated that energy sources could be manipulated via ingredients in the diet to prevent or treat peripartum NEB and improve the fertility of dairy cows (Staples *et al*., 1998; Gong *et al*., 2002b; Van Knegsel *et al*., 2007; Garnsworthy *et al*., 2008b; 2008c; Santos *et al*., 2008). Increasing the supply of glucogenic contents in the diet of dairy cows exerts a stimulating effect at ovarian level (Letelier *et al*., 2008), encouraging folliculogenesis (Scaramuzzi *et al.*, 2011) and early resumption of oestrous cycles (Gong *et al*., 2002b). However, excessive insulin and IGF-I levels from high starch-based diets may overstimulate the ovarian developmental competence of oocytes and thus yield inferior oocytes in dairy cows (Armstrong *et al*., 2001; Laskowski *et al*., 2016). The decrease in

the quality and viability of oocytes occurs as the synthesis and accumulation of maternal messenger RNA and protein molecules are uncoupled in an insulinogenic condition during oocyte growth and maturation (Leroy *et al*., 2008b). In contrast, feeding high fat diets after a voluntary waiting period, such as in the HSLF-LSHF treatment, increased the number and size of follicles and the oestradiol secretion of the preovulatory follicle (Lucy *et al.,* 1991b; Beam & Butler, 1997; Moallem *et al*., 2007), probably via the induction of high cholesterol levels in the follicular fluid and the corpus luteum (Van Knegsel *et al*., 2007; Santos *et al*., 2008). In the present pasture-based study, HSLF and HSLF-LSHF treatments were designed to provide isocaloric concentrates that differed in starch and fat contents at an inclusion level of 12.6 kg/day, compared with 7 kg/day of concentrate in the control group. The total number of ovarian follicles (Figure 1) showed a positive relationship with the inclusion levels (high versus low) of concentrates, demonstrating increased ovarian follicular growth in dairy cows. Such enhanced follicular dynamism suggested improvements of specific receptors, metabolites and hormones that signal follicular growth in dairy cows. This positive effect in the current study could be attributed to the levels of concentrate supplements provided in the HSLF and HSLF-LSHF treatments, improving the nutritional status (i.e. total digested nutrients and ME) compared with the control. This nutritional improvement had possibly optimized the hypothalamic-pituitary-ovarian axis and the energy carry-over effect into follicular and ovarian function, compared with the control.

The proportions of cows showing oestrous expression in the subsequent oestrus were 0.50, 0.66 and 0.83 for the control, HSLF and HSLF-LSHF, respectively (Table 1). The proportion of cows that were pregnant after AI during the subsequent oestrus were 0.40, 0.50 and 0.50 for the control, HSLF and HSLF-LSHF, respectively (Table 1). Although they were not compared statistically in the current study, several physiological events influence the expression of oestrus and pregnancy rates in dairy cows. The root cause of poor oestrous expression (poor intensity and duration) is related to low circulating oestradiol levels, owing to its high metabolic clearance rate (Sangsritavong *et al*., 2002) and low plasma LH, insulin, and IGF-I levels induced by the NEB (Diskin *et al*., 2007; Garnsworthy *et al*., 2008a) and stress (Dobson *et al*., 2008). Coupled with poor oestrous expression, an inability to detect oestrus easily could impede AI from being performed at the correct time (Walsh *et al*., 2011; Thatcher, 2017). Whether the energy sources and intake levels affected fertility outcomes (Butler, 2000; 2003; Gong *et al.*, 2002a; 2002b; Garnsworthy *et al*., 2008b; 2008c; Santos *et al*., 2008) remains to be investigated under field conditions and a longer postpartum period (i.e. 305 DIM).

Conclusion

In this study, no differences were observed between nutritional treatments in the dimensions of the ovaries and the preovulatory follicles and numbers of follicles in various classes in dairy cows. However, grazing cows that received high (12.6 kg/day) levels of concentrates in the HSLF and HSLF-LSHF treatments recorded a higher number of total follicles, compared with those on the low (7 kg/day) level of concentrate in the control group. This response was related to the increase in total nutrient intake and ME, which supported an improvement of ovarian follicular growth in the HSLF and HSLF-LSHF treatments in comparison with the control. Future research needs to investigate the influence of various inclusion levels and sources of energy nutrients that enhance the viability and the quality of the oocyte ovulating from the dominant follicle and improve the intensity and length of the oestrous expression in dairy cows. Also, effects of types and levels of dietary energy nutrients on pregnancy rates with a large number of cows under field conditions and a longer postpartum period (i.e. 305 DIM) require further investigation.

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Authors' Contributions

Conception and design of the trial: BAU and CJCM; data collection and analysis: BAU; drafting of paper: BAU; critical revision: CJCM; final approval of version to be published: CWC.

Conflict of Interest Declaration

The authors certify that they have no affiliations with any organization or entity with any financial or non-financial interest in the subject matter or materials discussed in this manuscript.

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