# อิทธิพลของการใช้ถั่วเหลืองไขมันเต็มในสูตรอาหาร ต่อการพัฒนาของฟอลลิเคิล และความเข้มข้นของฮอร์โมนโปรเจสเตอโรน ในโคนมพันธุ์ผสมหลังคลอด

นางสาวสุภลักษณ์ ตันประยูร



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# INFLUENCES OF FULL FAT SOYBEAN DIETS ON OVARIAN FOLLICULAR DEVELOPMENT AND CONCENTRATION OF PROGESTERONE IN THE POSTPARTUM CROSSBRED DAIRY COWS

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A Thesis Submitted in Partial Fulfillment of the Requirements
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| Thesis Title | INFLUENCES OF FULL FAT SOYBEAN DIETS ON OVARIAN FOLLICULAR DEVELOPMENT AND CONCENTRATION OF PROGESTERONE IN THE POSTPARTUM CROSSBRED DAIRY COWS |
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สุภลักษณ์ ต้นประยูร : อิทธิพลของการใช้ถั่วเหลืองไขมันเต็มในสูตรอาหาร ต่อการพัฒนาของฟอลลิเคิล และความเข้มข้นของฮอร์โมนโปรเจสเตอโรน ในโคนมพันธุ์ผสมหลังคลอด. (INFLUENCES OF FULL FAT SOYBEAN DIETS ON OVARIAN FOLLICULAR DEVELOPMENT AND CONCENTRATION OF PROGESTERONE IN THE POSTPARTUM CROSSBRED DAIRY COWS) อ.ที่ปรึกษาวิทยานิพนธ์หลัก : ศ.น.สพ.สมชาย จันทร์ผ่องแสง, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม : ศ.น.สพ.ดร.ณรงค์ ศักดิ์ ชัยบุตร 62 หน้า.

การวิจัยครั้งนี้มีวัตถุประสงค์เพื่อศึกษาอิทธิพลของการใช้ถั่วเหลืองไขมันเต็มในสูตรอาหาร ต่อการพัฒนาของฟอลลิเคิล และระดับความเข้มข้นของฮอร์โมนโปรเจสเตอโรน ในโคนมพันธุ์ผสมหลังคลอด สัตว์ทดลองเป็นแม่โค จำนวน 6 ตัว และโคสาว จำนวน 9 ตัว ซึ่งเป็นพันธุ์ผสมฟรีเชียน ระดับสายเลือด 87.5% จะถูกสุ่มเข้าการทดลองซึ่งมีการจัดแบบ 3 x 2 แฟคทอเรียล อาหารมี 3 สูตร ได้แก่ อาหารที่ไม่ใช้ถั่วเหลืองไขมันเต็ม (กลุ่มควบคุม) อาหารที่ใช้ถั่วเหลืองไขมันเต็มระดับ 18 และ 24% ของวัตถุแห้ง โดยอาหารทั้ง 3 สูตรนี้มีระดับโปรตีนและระดับพลังงานเท่ากัน มีระดับไขมันเท่ากับ 1.4 4.5 และ 5.9% ตามลำดับ เริ่มทำการวิจัยตั้งแต่คลอดถึง 8 สัปดาห์หลังคลอด โคทุกตัวที่ใช้ในการวิจัยครั้งนี้ได้รับอาหารผสมรวมที่มีอัตราส่วนของ ต้นข้าวโพดหมัก:อาหารข้น เท่ากับ 39:61 การวิจัยครั้งนี้ได้ทำการบันทึกหาปริมาณการกินได้ ปริมาณน้ำนม คะแนนความสมบูรณ์ของร่างกาย และน้ำหนักตัว มีการเก็บอย่างน้ำนม เพื่อนำไปวิเคราะห์หาองค์ประกอบน้ำนม การเก็บตัวอย่างของเหลวในกระเพาะรูเมน เพื่อวิเคราะห์หากรดไขมันระเหยได้ มีการเก็บตัวอย่างน้ำเลือด เพื่อนำไปวิเคราะห์หาระดับความเข้มข้นของกลูโคส non esterified fatty acid (NEFA) และฮอร์โมนโปรเจสเตอโรน มีการวัดการพัฒนาของฟอลลิเคิลโดยใช้เครื่องอัลตร้าซาวนด์

จากการศึกษาครั้งนี้พบว่า โคที่ได้รับอาหารในแต่ละสูตร มีค่าเฉลี่ยปริมาณการกินได้ คะแนนความสมบูรณ์ของร่างกาย น้ำหนักตัว องค์ประกอบน้ำนม ค่า pH ของของเหลวในกระเพาะรูเมน และกรดไขมันระเหยได้ ไม่มีความแตกต่างกัน ($p>0.05$) แต่การใช้ถั่วเหลืองไขมันเต็มที่ 24% ของวัตถุแห้ง สามารถเพิ่มปริมาณน้ำนม ($p<0.05$) ในช่วงเดือนที่สองหลังคลอด และตลอดการทดลองเมื่อเปรียบเทียบกับกลุ่มควบคุม และกลุ่มที่ใช้ถั่วเหลืองไขมันเต็มที่ 18% ของวัตถุแห้ง โดยค่าเฉลี่ยปริมาณน้ำนมของกลุ่มควบคุม และกลุ่มที่ใช้ถั่วเหลืองไขมันเต็มที่ 18% และ 24% ของวัตถุแห้ง ในช่วงเดือนที่สองหลังคลอด และตลอดการทดลอง เท่ากับ 16.8 17.8 เปรียบเทียบ 20.9 และ 16.3 17.1 เปรียบเทียบ 19.4 กก/วัน ตามลำดับ แม่โคที่ได้รับถั่วเหลืองไขมันเต็มที่ 18% ของวัตถุแห้ง มีระดับสมดุลพลังงานเป็นลบเพิ่มขึ้น ($p<0.05$) เปรียบเทียบกลุ่มควบคุม ระดับความเข้มข้นของกลูโคส และ NEFA ในพลาสม่า และการพัฒนาของฟอลลิเคิลไม่มีความแตกต่างกัน ($p>0.05$) แต่ระดับโปรเจสเตอโรนในพลาสม่าเพิ่มขึ้น ($p<0.01$) ในกลุ่มควบคุม และกลุ่มที่ใช้ถั่วเหลืองไขมันเต็มที่ 18% ของวัตถุแห้ง เปรียบเทียบการใช้ถั่วเหลืองไขมันเต็มที่ 24% ของวัตถุแห้ง

การใช้ไขมันในรูปเมล็ดน้ำมันจากถั่วเหลืองไขมันเต็ม เพื่อเป็นแหล่งพลังงานนั้น ไม่มีผลต่อการพัฒนาของฟอลลิเคิล ในโคนมพันธุ์ผสม แต่การใช้ถั่วเหลืองไขมันเต็มที่ 24% ของวัตถุแห้ง ทำให้ปริมาณน้ำนมเพิ่มขึ้นซึ่งนำไปสู่ภาวะสมดุลพลังงานเป็นลบเพิ่มขึ้น ทำให้ระดับโปรเจสเตอโรนในพลาสม่าลดลง

ภาควิชาสัตวบาล ลายมือชื่อนิสิต.....

สาขาวิชาอาหารสัตว์ ลายมือชื่ออ.ที่ปรึกษาวิทยานิพนธ์หลัก.....

ปีการศึกษา 2551 ลายมือชื่ออ.ที่ปรึกษาวิทยานิพนธ์ร่วม.....

## 4875572931 : MAJOR ANIMAL NUTRITION
KEYWORDS : FULL FAT SOYBEAN / MILK YIELD / ENERGY BALANCE / CONCENTRATION OF PROGESTERONE /FOLLICULAR DEVELOPMENT

SUPALAK TUNPRAYOON : INFLUENCES OF FULL FAT SOYBEAN DIETS ON OVARIAN FOLLICULAR DEVELOPMENT AND CONCENTRATION OF PROGESTERONE IN THE POSTPARTUM CROSSBRED DAIRY COWS. ADVISOR : PROF. Dr. SOMCHAI CHANPONGSANG, PROF. Dr. NARONGSAK CHAIYABUTR, Ph.D., 62 pp.

An experiment was studied to investigate influences of full fat soybean (FFS) diets on follicular development and concentration of progesterone in the postpartum crossbred dairy cows. Six multiparous and nine primiparous 87.5% crossbred Friesian cows were assigned randomly to a 3 x 2 factorial arrangement to evaluate 3 groups of diets which began after calving to 8 weeks postpartum. Cows received total mixed ration consisted of a 39:61 corn silage:concentrate ratio with 0, 18 or 24% of DM diet as FFS. All diets which were isonitrogenous and isoenergetic and contained 1.4, 4.5 or 5.9%EE, respectively. Dry matter intake (DMI), milk yield, body condition score (BCS) and body weight (BW) were recorded. Milk samples and rumen fluid were collected to analyze milk composition and volatile fatty acids (VFA). Blood plasma was analyzed for glucose, non esterified fatty acid (NEFA) and progesterone (P4) concentrations. Follicular development was monitored by ultrasonography.

Results of the experiment showed that average DMI, BCS, BW, milk composition, ruminal pH and VFA concentration were not significantly different ($p>0.05$) among dietary groups. Average milk yield during the 2$^{nd}$ month postpartum and all experimental period were significant difference ($p<0.05$) among dietary groups. Average milk yield of 0, 18%FFS versus 24%FFS groups were 16.8, 17.8 versus 20.9 and 16.3, 17.1 versus 19.4 kg/d, respectively. Average EB was more negative ($P<0.05$) in multiparous cows fed 18%FFS diet than multiparous cows fed 0%FFS diet. The concentration of plasma glucose, NEFA and follicular development did not differ ($p>0.05$) among dietary groups. However the concentration of plasma P4 in 0 and 18%FFS groups were greater ($p<0.01$) than 24%FFS group.

Augmentation fat supply in form of oilseed from FFS did not influence on follicular development in crossbred Friesian cows but supplementation FFS at 24% of diet apparently led to increase milk yield while it enhanced negative EB (NEB). This greater NEB led to reduce plasma P4 significantly when compared to cows fed 0 and 18% FFS diets.

Department : Animal Husbandry ..... Student's Signature .....

Field of Study : Animal Nutrition ..... Advisor's Signature .....

Academic Year : 2008 ..... Co-Advisor's Signature .....

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# CONTENTS

III. MATERIALS AND METHODS



IV. RESULTS

# LIST OF TABLES

# LIST OF FIGURES

# ABBREVIATIONS

| | | |
|---|---|---|
| ADF | = | acid detergent fiber |
| BCS | = | body condition score |
| BW | = | body weight |
| C2 | = | acetate |
| C3 | = | propionate |
| C4 | = | butyrate |
| C5 | = | valerate |
| CP | = | crude protein |
| d | = | day |
| DM | = | dry matter |
| DMI | = | dry matter intake |
| DMI (%BW) | = | dry matter intake as percentage of body weight |
| EB | = | energy balance |
| EE | = | ether extract |
| FA | = | fatty acids |
| FCM | = | fat corrected milk |
| FFS | = | full fat soybean |
| FMPP | = | the first month postpartum |
| GnRH | = | Gonadotropin releasing hormone |
| HDL | = | high density lipoprotein |
| kg/d | = | kilogram per day |
| LDL | = | low density lipoprotein |
| LH | = | Luteinizing hormone |
| Mcal/kg | = | megacalorie per kilogram |
| mg/dl | = | milligram per daciliter |
| mm | = | millimeter |
| mm/d | = | millimeter per day |
| ml | = | milliliter |
| µmol/l | = | micromole per liter |
| NADPH | = | nicotinamide adenine dinucleotide phosphate |

| | | |
|---|---|---|
| NDF | = | neutral detergent fiber |
| NE | = | net energy |
| $NE_L$ | = | net energy for lactation |
| NEB | = | negative energy balance |
| NEFA | = | non esterified fatty acid |
| overall | = | average values of the whole period of experiment |
| P4 | = | progesterone |
| SMPP | = | the second month postpartum |
| SNF | = | solid not fat |
| TG | = | triglycerides |
| TMR | = | total mixed ration |
| VFA | = | volatile fatty acid |
| VLDL | = | very low density lipoprotein |
| WI | = | water intake |
| WI/DMI | = | water intake per dry matter intake |

# CHAPTER I

# INTRODUCTION AND AIMS

In the early stage of lactation after parturition, a deficiency of negative energy balance (NEB) might occurs due to inadequate dietary energy intake, which is relative to the energy utilized for milk production. As a result, the mobilization of energy body reserves as energy resources will be occurred to support lactation (Butler and Smith, 1989; Lubojacka et al., 2005; Konigsson et al., 2008) and consequent loosing of body weight (BW). Body condition score (BCS) is a management tool indicating energy status in cow. Cows in NEB status will accompany with a loose in BCS. The degree for a loss of BCS in animal depends on the feeding management and milk yield. BCS is believed to be a good indicator for energy status in dairy cow. (Butler and Smith, 1989; Gransworthy and Webb, 2002).

NEB can cause changes in many bodily functions, for examples, decreases in the plasma concentrations of insulin and glucose and mobilization of body adipose tissue resulting in elevation of the plasma nonesterified fatty acids (NEFA) concentrations (Reist et al., 2003; Accorsi et al., 2005). All of these metabolic parameters can affect reproductive performance (Konigsson et al., 2008) which is one of the major factors influencing the profitability of dairy herds (Wiltbank et al., 2006). The manifestations for the effect of NEB have also been reported to occur at the level of the hypothalamus with a reduction of gonadotropin releasing hormone (GnRH) pulse frequency in accompanying with a reduction in pulsatile pituitary luteinizing hormone (LH) and inhibition of follicular development. The ovulation does not occur despite ovarian follicular development, because growing follicles do not mature. Alternatively, a reduced rate of progesterone (P4) rise following ovulation also reduced fertility (Beam and Butler, 1999; Butler, 2000; Diskin et al., 2003; Montiel and Ahuja, 2005; Wiltbank et al., 2006).

Due to a cow will not produce milk until animal has produced her calf (Gransworthy and Webb, 2002). A strategy to increase the energy requirements for lactation has been suggested for management of dietary concentration ratio. However, overfeeding of concentration can result in negative effects on digestion, milk

composition, and health (Elliott et al., 1995; Staples et al., 1998). Supplementation of fat in the diet during the early stage of lactation has been performed to increase the energy density of the diet in attempt to meet the energetic demands of lactation (Staples et al., 1998). According to physical and chemical properties of fat, which can be classified into three categories, namely, plant oils for highly unsaturated, animal fats for highly saturated and protected fats that for commercial use. Some supplemental fats especially plant oils with a high degree of unsaturation, disturb ruminal fermentation, decrease fiber digestibility, and lower milk fat test (Coppock and Wilks, 1991). However, the commercial fat and oilseed have been reported to use for preventing these disadvantageous effects. A number of studies have been reported for available commercial fat supplementation in animals, which showed little effect on ruminal fermentation and highly digestible postruminally (Coppock and Wilks, 1991). Whereas oilseeds (e.g., full fat soybean (FFS), whole cottonseed) comparing to animal fats, oil seeds are more consistency in quality, more readily available and have superior handling characteristics (Ruegsegger and Schultz, 1985). The oilseeds can be fed without observable ruminal inhibition, probably because of a slow release of the oil into ruminal contents (Coppock and Wilks, 1991).

FFS containing 40% crude protein (CP) and 17 to 20% ether extract (EE) are of interest as a source of protein and energy in the ration of high yielding dairy cows especially during early lactation (Chouinard et al., 1997). Higher milk yield after FFS supplementation has been demonstrated previously (Ruegsegger and Schultz, 1985; Faldet and Satter, 1991; Knapp et al., 1991; Chouinard et al., 1997; Dhiman et al, 1999; Abu-Ghazaleh et al., 2002[a]), but there is limited information on the effects of FFS supplementation on reproductive performance. Ruegsegger and Schultz (1985) reported that FFS supplementation (12.9% of dry matter) positively influenced the reproductive status of dairy cow including reduced days open and services per conception (115 versus 109 day and 2.1 versus 1.8 times, respectively). Therefore, the hypothesis of the present study was the increasing levels of FFS in diet could increase energy density to improve reproduction performance. The objectives of this experiment were; to study the effects of FFS supplementation diet on production, energy balance and reproductive performance in the postpartum crossbred dairy cows.

# CHAPTER II

# BACKGROUND INFORMATION

## 2.1 Role of nutrients in ruminants

In ruminants, the digestive process is a complex process. There are many types of micro-organisms in rumen. These microorganisms break down plant material to provide the energy and protein required for their growth. They digest complex carbohydrates, including cellulose-based carbohydrates, can produce volatile fatty acids (VFA) such as acetate (C2), propionate (C3), butyrate (C4) and ammonia as their waste product. The VFA provide the cow with the source of energy and carbon skeletons to synthesize milk compositions. C2 is the major precursor for synthesis of milk fat. The cow can use C3 that is the main energy substrate to convert to glucose in the liver (Boland et al., 2001). The glucose is precursor for lactose synthesis and necessary for the pentose-phosphate pathway. This pathway is a metabolic system with two important consequences. The first is production of nicotinamide adenine dinucleotide phosphate (NADPH) for biosynthesis, and the second is production of ribose-5-phosphate for nucleotide and coenzyme biosynthesis (Matthews et al., 1997). The ammonia is converted to urea in the liver and either excreted in the urine or recycled via the saliva and then urea will be utilized for microbial protein synthesis in the rumen. Microbial protein is made available to the cow when the microorganisms pass out of the rumen for digestion in abomasum and small intestine (Garnsworthy, 2002).

## 2.2 Early postpartum energy balance

Energy requirements for maintenance and milk production are expressed in net energy for lactation ($NE_L$). The general relationship between dietary energy intake and energy utilization is defined as energy balance (EB) and described by following equation: daily EB = NE (consumed) – NE (required). NE requirement includes both maintenance and production components (Butler and Smith, 1989; NRC, 2001; Van Knegsel et al., 2005). In early lactation, Dry matter intake (DMI) increase slower than

does milk yield, leading to NEB (NRC, 2001) which has been found to occur in 80% of cows (Butler and Smith, 1989). NEB begins a few days (d) before calving and usually reaches its most negative level (nadir) about 2 weeks later (Butler and Smith, 1989). NEB prior to peak milk production occurs because most dairy cows are not able to meet the energy requirements for growth, maintenance and milk production (Waltner et al., 1993). Generally, NEB will last for 10–12 weeks since parturition (Butler, 2003).

The dairy cattle in early lactation usually have a limit in DMI. Not only the feedstuffs are not adequate, but also the quality of the diet is more variable. Apart from that some degree of stress, (physiological, environmental or psychological) can decrease DMI. It is no doubt these results have the negative effect on EB. These result in the mobilization of body reserves. Mobilized body reserves are mostly body fat and to a lesser extent body protein. Mobilization of body fat results in elevated blood NEFA levels, which stored in the liver as triglyceride (TG) through esterification possibly causing fatty liver or can be oxidized to carbon dioxide to provide energy and partially oxidized to Acetyl-CoA. The production of Acetyl-CoA from C2, C4 and fatty acids (FA) from body reserves is high whilst at the same time glucose and glucogenic amino acids, are driven towards lactose. Because of the high milk production in early lactation requires a high lactose production which results in decreased glucose and insulin levels. Consequently, the ratio of oxaloacetate to Acetyl-CoA is out of balance. The availability of citrate to form ATP in the Krebs cycle is decreased. Acetyl-CoA is diverted to the production of ketone bodies, acetone, acetoacetate and $\beta$-hydroxybutyrate, resulting in a status of ketosis (Stich and Berlan, 2004; Van Knegsel et al., 2005; Konigsson et al., 2008).

## 2.3 Effects of energy balance on body condition score, body weight and blood metabolites

An indicator of EB status is BCS. Loss of BCS is correlated with fat mobilization (Edmonson et al., 1989; Komaragiri et al., 1998). Therefore BCS might be used as an indicator of EB during early lactation (De Vries and Veerkamp, 2000). Poor nutritional status of cows were observed as evident from the decreased BCS and BW ($p<0.0001$)

after calving and later (Shrestha et al., 2005). Also BCS decreased ($p<0.001$) by 0.35 to 0.5 points in the cows fed the low energy diet from parturition to 14 weeks postpartum (Schei et al., 2005). A substantial decrease in DMI is initiated in late pregnancy and continues into early lactation (Ingvarten and Andersen, 2000). However DMI depression did not start until d 2 before calving and extend of depression was 40% of DMI on d 3 then DMI increased exponentially. In addition the start of lactation was characterized by a reduction in plasma glucose level ($p<0.05$), a subsequent gradual increase in glucose concentration were observed (Vazquez-anon et al., 1994). Low blood glucose also seems to be a critical factor in the etiology of ketosis and perhaps of fatty liver (Grummer and Carroll, 1991). The 2 weeks before parturition, serum glucose concentrations were 59.5 ± 1.1 mg/dl and decreased sharply after parturition, indicating that cows suffered some degree of NEB (Rukkwamsuk et al., 2008). Clark et al. (2005) reported that the best prediction model for energy balance was plasma glucose which was positively correlated ($r=0.79$) with EB ($p<0.05$). Additionally plasma NEFA values were highest at 1 week postpartum about 600 – 800 μmol/l (Schei et al., 2005). NEFA levels increased until 10 d after calving and then rapidly declined ($p<0.05$) until the fourth month of lactation (Accorsi et al., 2005). Also the plasma NEFA concentrations decreased ($p<0.01$) with week postpartum (Ponter et al., 2006). Rukkwamsuk et al. (2008) reported serum NEFA concentrations increased after parturition and remained as cows entering a period of high energy requirement. Likely plasma concentration of NEFA increased ($p<0.0001$) at 3 weeks after calving, indicating greater lipolysis and NEB (Shrestha et al., 2005).

## 2.4 Effects of negative energy balance on reproduction

The nutrition, NEB, BCS, milk yield, management factors and other environmental factors influence the duration of postpartum anestrus (Montiel and Ahuja, 2005). However after calving almost every cow experiences a period with high energy requirement related to milk production, frequently associated to an insufficient feed intake. This situation leads to the well known NEB (Konigsson et al., 2008), that interfere with the ability of the hypothalamus – hypophyseal axis to develop the pulsatile LH pattern necessary for ovarian follicular development and ovulation (Butler and Smith, 1989). The principal defect caused by NEB occurs at the level of the hypothalamus,

manifested by reduced GnRH pulse frequency. The NEB results in a parallel reduction in pulsatile pituitary LH release, with consequent compromised follicular steroid output and anovulation. In addition, follicular responsiveness to gonadotropin stimulation is blunted by the circulating hormonal and metabolite environment of the NEB state (Beam and Butler, 1999; Butler, 2000; Diskin et al., 2003; Wiltbank et al., 2006). Apart from that low levels of blood glucose, insulin and insulin-like growth factor-I (IGF-I) restrain estrogen production by dominant follicles (Butler, 2000; Butler, 2003). Incapable of producing sufficient oestradiol can induce ovulation failure due to reduced LH pulse frequency (Roche, 2006). The lower LH acts synergistically to promote ovarian follicular development (Lucy, 2000), which may lead to an increase incidence of inactive ovaries, ovarian cysts and non-functional corpora luteal in the postpartum cows, resulting in a prolonged interval to first ovulation (Shrestha et al., 2004) to 40 or 50 d after calving (Beam and Butler, 1997).

Opsomer et al. (2000) performed an epidemiological study and concluded the risk factors for delayed cyclicity were periparturient disorders, postpartum diseases and NEB. NEB that occur in lactating, postpartum beef and dairy cows decreases LH secretion, delay the time of first ovulation, delay return to estrus (Lucy et al., 1992; Butler, 2000; Butler, 2003). NEB also adversely affects the number and size of large ovarian follicles (Lucy et al., 1991; Beam and Butler, 1997), reduces serum P4 concentrations and fertility (Spicer et al., 1990; Butler, 2000; Butler, 2003). All these finding was the most important factors affecting the reproductive efficiency after calving (Konigsson et al., 2008), including low conception rate (Butler, 2003; Roche, 2006). Information on factors affecting the development of follicles, oestrous behaviour, ovulation and corpus luteum development and regression may be used to devise methods for improved reproductive performance.

## 2.5 Negative energy balance prevention

Nutritional requirements shift abruptly at parturition as milk production rapidly increases and cows enter NEB. The severity and duration of NEB is primarily related to DMI which is related to BCS at calving (Butler, 2000). Because of over conditioned

cows (BCS > 3.5) will result in reduced DMI post calving, lose excess BW and lose excess BCS, which are undesirable (Roche, 2006). To prevent the occurrence of NEB there are several tools to be considered. To begin with, BCS of cows were monitored before and after calving. It would help to identify cows with poor nutritional status and improve nutritional management (Shrestha et al., 2005). To achieve this important target, when BCS loss is at or below 0.5 unit during the transition period and cows should be maintained at a BCS of 2.5–3.0 in association with maintenance of proper rumen function through adequate dietary fiber, shortening the dry period (6–8 weeks maximum), reduction in the incidence of metabolic disorders, including hypocalcemia, ketosis and fatty liver and minimizing mobilization of body reserves in the early postpartum (Roche, 2006).

Secondly, the NRC (2001) recommended that a diet containing approximately 1.25 Mcal/kg of $NE_L$ should be fed from dry off until approximately 21 d before calving, and that a diet containing 1.54 to 1.62 Mcal/kg of $NE_L$ be fed during the last 3 week preceding parturition.

Thirdly, it is possible that changes in other dry cow management practices (grouping strategies, avoiding overcrowding, heat abatement, etc.) could have carry over effects and enhance EB, reproduction, or both, but further research is needed, unfortunately, improving EB is very difficult to achieve (Grummer, 2007).

Then, altering diet to increase energy density by feeding more concentrates consist of high-starch diets, which are intensively fermented by the microbial ecosystem in the rumen. It results in high production of VFA. Under normal conditions, lactate concentrations in the rumen are low. When animals are fed high-starch diets can lead to an accumulation of lactate, which exacerbates the decline in pH and is considered as the major cause of rumen acidosis. This rumen dysfunction affects rumen microbes and results in less efficient digestion, thereby decreasing feed intake and exacerbating the energy deficit in the cows (Jouaney, 2006).

Finally, alternatively increasing dietary energy density when various forms and amounts of fat are included in the diet (Palmquist and Jenkins, 1980) could improve the

limitation of consumption in cow whose physical capacity constrains greater feed volume and better meet the energetic demands of lactation (Coppock and Wilks, 1991; Butler, 2000; Butler, 2003). Theoretically, supplementation of fat could have some of the following advantages:

First of all, to increase the energy density of the diet because fat contains three times more net energy of lactation than protein and carbohydrate-rich feeds. Second, improve the energetic efficiency by reduced loss of energy as heat, methane, and urine and because the dietary FA are incorporated directly into milk fat by the mammary gland. Third, reduce the risk of rumen acidosis and a decrease in milk fat percent induced by feeding high levels of cereal grains in the diet. Lastly, alter the milk fat composition by increasing long chain unsaturated FA, conjugated linoleic acids, and decreasing saturated FA to obtain dairy products more beneficial for human health (Chilliard et al., 2003).

## 2.6 Fat sources

Many sources of supplemental fat have been fed to beef and dairy cattle under experimental conditions. Some of these include blends of animal and vegetable fat, tallow, yellow grease, fishmeal, cottonseeds, soybeans, rapeseeds, canola seeds, peanut hearts, safflower seeds, sunflower seeds, flaked fat, prilled fat, hydrogenated fat, calcium soaps of fat, medium-chain TG and FFA (Staples et al., 1998; Functon, 2004)

The lipid content of concentrates is usually higher than that of forages, and the majority is present in the form of TG (Bauman et al., 2003). The major FA in the most seed lipid is linoleic acid (18:2; *cis*-9, *cis*-12), whereas linolenic acid (18:3; *cis*-9, *cis*-12, *cis*-15) is the predominant FA in the most forage lipid (Staples et al., 1998; Bauman et al., 2003). Linoleic and linolenic FA are classified as essential FA, called polyunsaturated FA because the double bonds between the delta 9 carbon and the terminal methyl group of FA cannot be inserted by mammalian biological systems. Therefore, they must be supplied in the diet (Staples et al., 1998).

## 2.7 Disadvantages of fat supplementation

The diet of lactating dairy cows typically contains 4 to 5% fat. The general recommendation is that total dietary fat should not exceed 6 to 7% of dietary dry matter (Jenkins, 1993; NRC, 2001). Higher levels may adversely effect to ruminant digestive system. The ruminal microbes are inhibited by the $C_8$ to $C_{14}$ FA, the unsaturated long chain FA (Doreau and Chilliard, 1997) and vegetable oils (Jenkins, 1993). Next this inhibition of microbial function can decrease fiber digestion (Coppock and Wilks, 1991; Jenkins, 1993; Doreau and Chilliard, 1997). Then, fat supplementation in the diet of cows may reduce feed intake (Coppock and Wilks, 1991; Hayirli et al., 2002). After that, under some condition, supplemental fat decrease the protein percentage of milk (Coppock and Wilks, 1991; Faldet and Satter, 1991; Doreau and Chilliard, 1997). Fat supplementation has variable effect on milk fat, depending on the source of dietary lipids (Doreau and Chilliard, 1997). Thus maximal using of supplemental fats suggest feeding oil seed which is alternative source of fat to be used. Oil seed will slowly release of oil into rumen (Coppock and Wilks, 1991).

## 2.8 Full fat soybeans

FFS which are oil seed, contain 15 to 22% EE and 33 to 44% CP on a dry matter (DM) basis (Ruegsegger and Schultz, 1985; Ishler and Varga, 2000). Then they are as a source of protein and energy content with the highest amount of 18:2; *cis*-9, *cis*-12 (Dhiman et al., 1999; Agazzi et al., 2004). They have been developed that are referred to as “protected fats” or “bypass fat” (Gransworthy, 2002). Due to some deleterious substances in raw soybean they can be processed through an extruder to rupture the seeds (Dhiman et al., 1999) and to inactivate trypsin inhibitor and other enzymes. Thus this process prevents problems that are associated with feeding large amount of raw soybeans to dairy cattle (Ruegsegger and Schultz, 1985). This product resistant to microbial protein degradation in the rumen and is available for absorption in the small intestine. As a result it can supply the extra energy, amino acids (Faldet and Satter, 1991; Faldet et al., 1992) and unsaturated FA (Garnsworthy, 2002).

## 2.9 Lipid metabolism in the rumen

The action of dietary fat is highly dependent on the animal species. Due to digestive and, to a lesser degree, metabolic utilization, the relationships between fat in the diet and fat in the products are different in single-stomached animals and in ruminants. The digestive utilization of fats by ruminants is characterized by events in the rumen before they are absorbed in the intestine. During their stay in the rumen, fats are transformed so that the amount and composition of fat leaving the rumen differ from intake (Doreau and Chilliard, 1997). There are three steps in the rumen, lipolysis, biohydrogenation and microbial fatty acids synthesis (Jenkins, 1993; Doreau and Chilliard, 1997).

### Lipolysis

The esterified plant lipids are found in the form of TG, phospholipids, and glycolipids which are consumed and entered the rumen. Next the most of dietary lipids is hydrolyzed by rumen bacteria (of which the best known is *Anaerovibrio zipolytic*), with little evidence for a significant role by rumen protozoa and fungi, or salivary and plant lipases. Then the constituent fatty acids are released from glycerol backbone. Finally the glycerol and sugars which are liberated are readily metabolized by the rumen bacteria (Jenkins, 1993; Doreau and Chilliard, 1997; Bauman et al., 2003).

### Biohydrogenation

Unsaturated free FA have relatively short half lives in ruminal contents because they are rapidly hydrogenated by microbes to more saturated free FA and products (Figure 1). First of all, biohydrogenation is an isomerization reaction that converts the *cis* – 12 double bond in unsaturated fatty acids to a *trans* – 11 isomer by action of the isomerase. The requirement of a free carboxyl group establishes lipolysis as a prerequisite for biohydrogenation. Next, hydrogenation of the *cis* – 9 bond in $C_{18:2}$ occur by microbial reductase. Then the *trans* – 11 $C_{18:1}$ that is hydrogenated to $C_{18:0}$, depends on conditions in the rumen. Hydrogenation is also adversely affected when excessive unprotected lipid is present in the diet because of either the coating of feed particles or a direct toxic effect on the rumen microorganisms, thus protected lipids are needed that

effectively resist biohydrogenation without interfering with ruminal fermentation or intestinal lipid absorption (Jenkins, 1993).

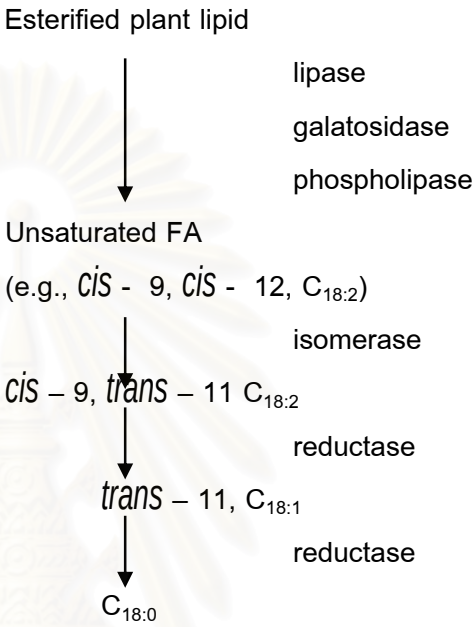


Figure 1. Key steps in the conversion of esterified plant lipid to saturated FA by lipolysis and biohydrogenation in ruminal contents (Jenkins, 1993)

### Microbial fatty acids synthesis

A portion of the FA is found in the rumen are phospholipid components of microbial membranes. The rumen microorganisms derive these from de novo synthesis (mainly $C_{16:0}$ and $C_{18:0}$), which is endogenous source and the uptake of preformed FA from diet, that is exogenous source. The contribution of each source depends on bacterial species and lipid content of diets (Jenkins, 1993).

## 2.10 Digestion and absorption of lipids in small intestine

Dietary TG are hydrolyzed in intestine by lipases to form FA, glycerol, and monoglycerides. However, the out flow of lipids from rumen is predominantly free FA and differences in the digestibility of individual FA in the small intestine are negligible.

Then these FA are absorbed and re-synthesized back into TG in the intestine. Thus, the composition of FA absorbed in the small intestine is similar to the composition of FA leaving the rumen (Matthews et al., 1997; Bauman et al., 2003).

FA reaching the duodenum is mainly adsorbed on feed particles, bacteria and perhaps desquamated cells. Desorption occurs with bile salts and lysolecithins which allow their solubilization in a micellar phase. These micelles allow lipid absorption at the jejunum. In epithelial cells of the small intestine, FA are esterified and reconverted to TG which are incorporated with phospholipids, cholesterol and apoprotein into lipoprotein (Doreau and Chilliard, 1997). TG have relatively low solubility in the plasma, and therefore are transported as lipoprotein (Matthews et al., 1997), which are transported into the lymph and back into the blood stream near the heart (Doreau and Chilliard, 1997). After that the blood is oxygenated through the lungs, the lipoprotein particles are delivered to various organs of the body such as the mammary gland, muscle and heart that can use the TG. TG in lipoprotein are broken down to free FA by enzyme called lipoprotein lipase that is found in the capillaries of these tissues. Then the free FA enter the cells where they can be form back into TG (such as milk) or burned to release energy that can fuel cell functions (Drackley, 2007). Lastly, there is transport of energy release from storage in adipose tissue to the rest of body in the form of FA that are bound to serum albumin (McGarry, 1997).

## 2.11 What classes of lipoproteins transport lipid?

The classes of lipoproteins include chylomicrons, which transport dietary fat, and very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL), which transport endogenous fats. These lipoproteins vary in size, density, relative composition of TG, phospholipids, cholesterol, cholesterol ester and proteins (Matthews et al., 1997).

Chylomicrons are formed in the intestine to function in absorption and transport of dietary triglyceride, cholesterol, and fat soluble vitamins to tissues for fat storage, milk fat production, or for oxidation to produced energy (Bauchart, 1993). Next liver synthesizes VLDL and FA from TG in VLDL are taken by adipose tissue and other

tissues. In the process VLDL are converted to LDL (McGarry, 1997). In addition to VLDL delivering TG to tissues, LDL can deliver cholesterol to peripheral tissues, thereby delivering cholesterol to tissue that can not make cholesterol (Matthews et al., 1997). HDL that are the major plasma lipoproteins in ruminant, synthesized and secreted by liver and the small intestine. The main functions of HDL are to deliver cholesterol to tissues for primary steroidogenesis (liver, ovary, testis and adrenal gland) or membrane synthesis and for transport of cholesterol away from tissues to the liver (Bauchart, 1993).

## 2.12 What is progesterone?

P4 is a kind of steroid hormones. These hormones include mineral corticoids, glucocorticoids (cortisol) and testosterone. These are produced in the adrenal glands and the gonads. The main organ which synthesizes P4 is ovarian luteal cell. P4 production increases at the beginning of the cycle (d 3 – 12 in the cow) and then remains constant until d 15 – 16, starting to decline when regression (luteolysis) begins unless fertilization occurs. P4 does not only prepare the lining of the uterus for implanting of embryo but also maintain pregnancy by providing nourishment to the conceptus (Matthews et al., 1997; Staples et al., 1998; Squires, 2003).

Increased concentrations of plasma P4 have been associated with improved conception rates of lactating ruminants. Similarly, P4 concentration prior to AI has been associated with greater fertility (Staples et al., 1998). P4 is cleared from blood through a variety of pathways. The liver is the primary site of P4 metabolism, and P4 metabolites are excreted in feces, urine, and milk (Parr, 1992). There is an association between low P4 and infertility. Thus P4 is importance, which is required for pregnancy (Lucy, 2001).

Cholesterol is the precursor of steroid hormone (Figure 2.). Cholesterol is an alicyclic compound. Then de novo biosynthesis of cholesterol occurs in virtually all cells, this capacity is greatest in liver, intestine, adrenal cortex, and reproductive tissues including ovaries and testes (Glew, 1997). The amount of cholesterol synthesized by the body can be two to three times or more compared to the amount ingested. Cholesterol is not an essential nutrient and can be made in the body from simple compounds via

Acetyl-CoA (Matthews et al., 1997). Acetyl-CoA can be obtained from several source; 1) the $\beta$ oxidation from FA; 2) the oxidation of ketogenic amino acids such as leucine and isoleucines; and 3) the pyruvate dehydrogenase reaction (Glew, 1997).

Cholesterol synthesized de novo is transported from liver and intestine to peripheral tissue in the form of lipoprotein such as LDL and HDL. Then the cholesterol derived from LDL or HDL to serves as a precursor to the steroid hormones in specialized tissues such as the adrenal gland and ovary (Glew, 1997). Nevertheless cholesterol storage and delivery is controlled by a number of LDL receptors. The tissue rich in LDL receptors are liver, adrenal gland, ovaries, testes and others that convert cholesterol to importance metabolic product. Finally if cellular cholesterol levels are high so there is a decrease in the number of receptors to decrease cholesterol delivery to that tissue (Matthews et al., 1997).

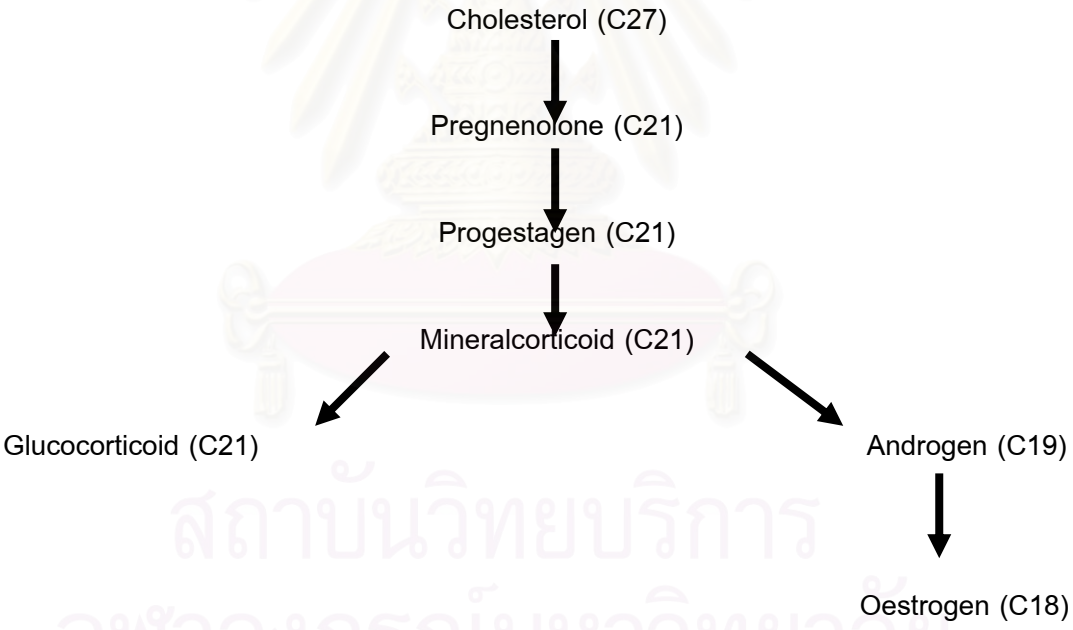


Figure 2. The pathways of steroid hormone synthesis (Squires, 2003)

## 2.13 Follicular dynamics in cattle

Daily ultrasonic monitoring of individually identified follicles was used (Grinther et al., 1989). The process of continue growth and regression of antral follicles that leads to the development of the preovulatory follicle is known as “follicular dynamics.” Ovarian follicular growth in cows occurs in waves. One to four waves of follicular growth and development occur during a single estrous cycle of cattle (Sakagushi et al., 2004). However, most estrous cycles in cows consist of 2 or 3 waves of follicular growth and development (Figure 3 and 4). A wave of follicular growth involves the synchronous development of a group of follicles. One of these becomes dominant, achieves the greatest diameter and suppresses the growth of subordinate smaller follicles. The 3 wave interovulatory intervals differed from 2 wave intervals in: 1. earlier emergence of the dominant follicles 2. longer in length, and 3. shorter interval from emergence to ovulation. The mean length of the 2 wave interovulatory intervals (19.8 ± 0.6 d, n=4) was significantly ($p<0.05$) shorter than that of 3 wave ineterval (22.5± 0.8 d, n=10). The first wave in cows consisted of 2 wave and the first or second wave in cows consist of 3 wave: the development of dominant anovulatory follicles comprised three phases: growing, static, and regressing phases. The follicular waves are first detectable as 4 – 5 mm follicles approximately d 0 and 10 for 2 wave interovulatory intervals and on approximately d 0, 9, and 16 for 3 wave interovulatory intervals (Noseir, 2003). For each wave, the follicles which became dominant versus subordinate did not differ in diameter on the first day of the wave, but the dominant follicle was significantly larger than the subordinates on the following day. On the average, the subordinates ceased growing 4.4 d after the origin of a wave. The dominant follicle of the anovulatory wave grew linearly (1.8 ± 0.1 mm/d) to an average of 15.8 ± 0.5 mm, remained static for a mean of 6 d and then regressed linearly (1.0 ± 0.1 mm/d). The dominant ovulatory follicle grew slower ($p<0.0001$) (linear slope, 1.2 ± 0.1 mm/d) than the dominant anovulatory follicle. The diameter of the ovulatory follicle on the day before ovulation (16.2 ± 0.4 mm) was not different from the diameter of the dominant anovulatory follicle during the static phase. The numbers of growing, static and regressing 4 to 6 mm identified follicles did not differ between anovulatory and ovulatory waves. Ninety-five percent of the growing identified follicles were assignable to a wave (follicles emerging within 2 d of each other) and each wave emerged during a consistent and narrow time

period (anovulatory wave, d -1, 0, or 1; ovulatory wave, d 8, 9, 10, or 11). It was concluded, therefore, that the formation of waves was a well-controlled phenomenon. There was a consistent temporal relationship between emergence of the ovulatory wave and onset of regression of the dominant follicle of the anovulatory wave (length of interval from beginning of ovulatory wave to beginning of regression of anovulatory follicle, approximately 3 d). Perhaps, therefore, the mechanism that caused regression of the subordinate follicles of the ovulatory wave also caused regression of the large, static, dominant follicle of the anovulatory wave (Grinther et al., 1989).

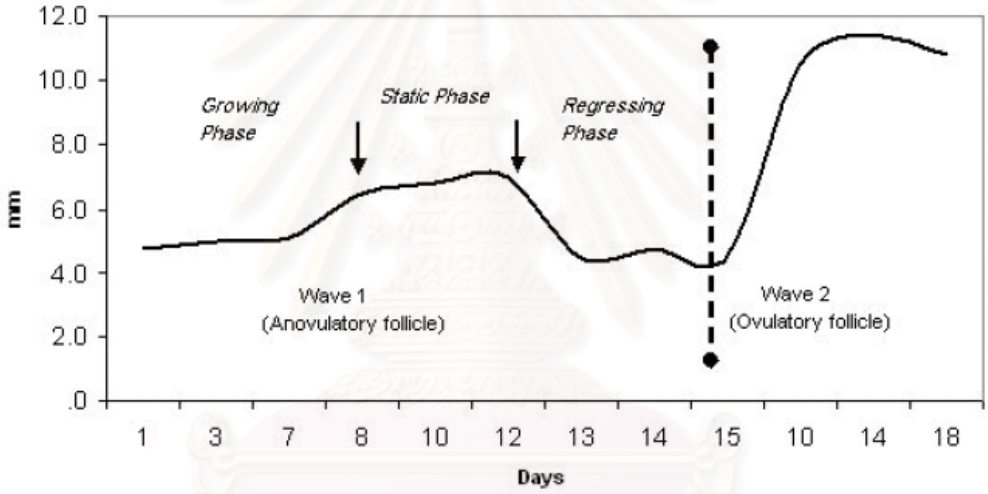


Figure 3. Profile of the dominant follicles during 2 wave interovulatory intervals (Noseir, 2003)

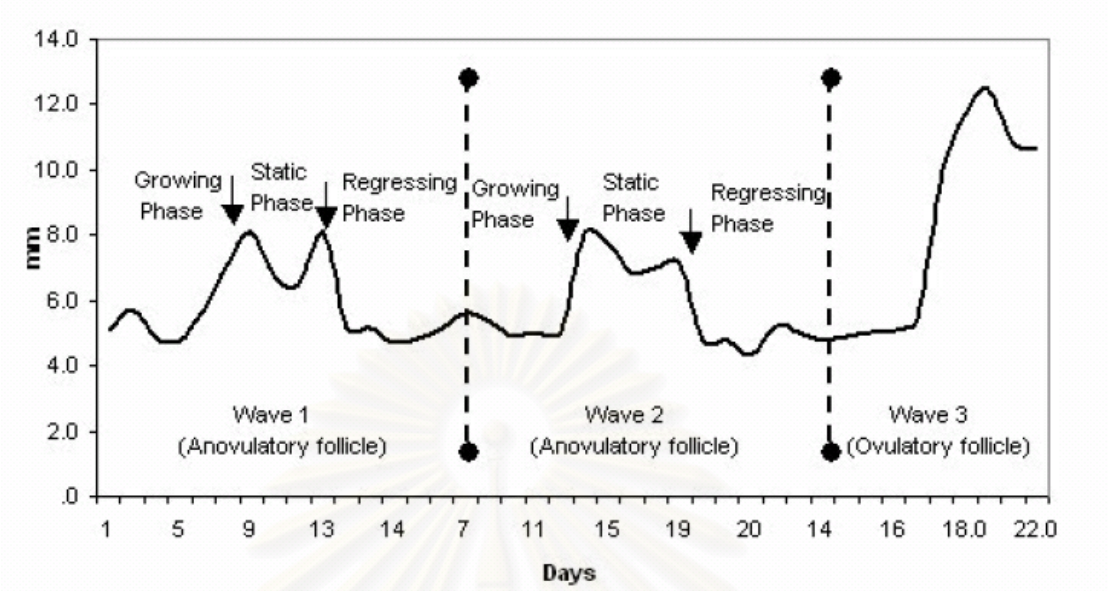


Figure 4. Profile of the dominant follicles during 3 wave interovulatory intervals (Noseir, 2003)

## 2.14 Water for dairy cattle

Cattle consume large amount of water every day (NRC, 2001). Water constitutes 60 to 70 percent of body mass of livestock. Moreover it is necessary for maintaining body fluids and proper ion balance; for digesting, absorbing and metabolizing nutrient; for eliminating waste material and excess heat from the body; for providing a fluid environment for the fetus; and for transporting nutrients to and from body tissues (Waldner and Looper, 2002). Sources of water include drinking or free water intake (WI), feed, and water produced by the body's metabolism of nutrients (Murphy, 1992; NRC, 2001). The amount of water a cow will drink depends on her size and milk yield, quantity of DM consumed, temperature and relative humidity of the environment, temperature of the water, quality and availability of the water, and amount of moisture in her feed (Waldner and Looper, 2002). Then water is an especially important nutrient during period of heat stress. The physical properties of water are important for the transfer of heat from the body to the environment (NRC, 2001). After that water loss occurs via saliva, urine, feces, and milk; and by evaporation from body

surfaces and the respiratory tract. The amount of water lost from the body of cattle is influenced by the activity of animal, air temperature, humidity, respiratory rate, water intake, feed consumption, milk production and other factors (Waldner and Looper, 2002). Finally water availability and quality are extremely important for animal health and productivity. Limiting water availability to cattle will depress production rapidly and severely (NRC, 2001).

## 2.15 Effects of fat supplementation on concentration of blood progesterone

Fat supplementation increased not only plasma cholesterol but also FA profile which served as a precursor for the synthesis of P4 by ovarian luteal cell (Staples et al., 1998). In addition to study of Hawkins et al. (1995) reported that cows fed 6.2%Megalac had greater ($P<0.05$) concentration of serum P4 than cows fed 0%Megalac (12 versus 6 ng/ml) on d 12 to 13 of the estrus cycle. Because of intracellular lipid that increased, would be expected to provide increased precursor for P4 biosynthesis and may partially explain the increase in serum concentrations of P4 in cow consuming high fat diets. Lipid droplets are thought to be cholesterol esters and thus represent excess, stored precursor for steroidogenesis. Similarly study of Spicer et al. (1993) reported that cows fed 1.8%calcium salts of long chain FA had greater concentration of plasma P4 than cows fed 0%calcium salts of long chain FA (8 versus 4 ng/ml) on d 10 to 11 of first estrus cycle. In contrast Carroll et al. (1990) reported that multiparous Holstein cows fed 5%prilled long chain FA (DM basis) had lower ($p<0.05$) mean plasma P4 than multiparous Holstein cows fed 0%prilled long chain FA during the $8^{th}$ d of the first estrous cycle. Similar to Robinson et al. (2002) reported cows consumed an isoenergetic containing control diet (2.7%EE) or dietary fat supplemented with linseed (5.0%EE) or FFS (5.0%EE). Both dietary fats diet had lower ($p<0.001$) plasma P4 than control diet during the $3^{rd}$ through $8^{th}$ day after ovulation. In contrast Webb et al. (2001) reported concentration of plasma P4 did not differ ($p>0.05$) between cows fed 0.83%rice bran and cow fed 0%rice bran. Similarly, De Fries et al. (1998) reported during the first estrous cycle no differences serum P4 concentration in multiparous Brahman cows was found when received either 8.3%rice bran (5.2%EE of diet) or 0%rice bran (3.7%EE of diet). Due to these effects of dietary fat occurred later for the postpartum period or because of rice bran's hypocholesterolemic properties.

## 2.16 Effects of fat supplementation on follicular development

Not only fat supplementation improved reproductive performance of lactating dairy cows but also affected the development of ovarian follicle during the postpartum (Staples et al., 1998). De Fries et al. (1998) reported that cow fed 8.3%rice bran had grater ($p<0.05$) numbers of follicles (medium and large size) than cow fed 0%rice bran during the 3 week before the first normal estrus cycle. Rice bran supplementation seemed to enhance follicular development by stimulating a greater number of small follicles to move into larger follicular sizes as time of the first normal estrous cycle approached. This effect on ovarian follicular populations might be beneficial because there is a substantial increase in the number of potential ovulatory follicles. Similarly, Robinson et al. (2002) reported that cow fed 2.8%FFS had greater ($p<0.05$) number of follicles (5-10 mm) than cow fed 0%FFS. Similarly, Thomas et al. (1997) reported that cows received 4%soybean oil had greater ($p<0.05$) numbers of medium sized (4.0-9.9 mm) follicles than cows received control diet during the first 10 d of the estrus cycle. Because of linoleic acid which was a major constituent of soybean oil, enhanced rumen production of propionate. That was an important precursor for gluconeogenesis in the liver. Increased gluconeogenesis produced a rapid rise concentration of insulin in serum that stimulated granulose cells to proliferate. Therefore soybean oil supplementation had affect on medium sized follicle population. In contrast Webb et al. (2001) reported that the follicular development in multiparous Brahman cows received either increasing level of fat in diet or no inclusion of fat (0 versus 8.3%rice bran; 3.7 versus 5.2%EE of diets) did not differ ($P>0.05$) during the $35^{th}$ d postpartum. Because of dietary fat may affect metabolites and metabolic hormones that act on the hypothalamic-pituitary-ovarian axis. Therefore rice bran supplementation did not enhance follicular development.

# CHAPTER III

# MATERIALS AND METHODS

## 3.1 Animals and managements

Six multiparous and nine primiparous of 87.5% crossbred Friesian cows were used in the experiment which began after calving to 8 weeks postpartum. They were assigned randomly to a 3 x 2 factorial arrangement to evaluate 3 groups of diet and 2 groups of cow. Thereby each group of diet included 2 multiparous and 3 primiparous cows. Initial BCS of each cow was from 3 to 3.5. All cows were housed in a tie-stall barn and open sides.

Diets were formulated to meet NRC (1989) requirements. All animals received diet in the form of total mixed ration (TMR). TMR was composed of corn silage and concentrate. The forage to concentrate ratio was 39:61 (DM basis). Ingredient compositions in three groups of diet which was isonitrogenous and isoenergetic, are shown in Table 1.

This experimental study was approved by Animal Care and Use Committee of the Faculty of Veterinary Science, Chulalongkorn University.

## 3.2 Feed and water intake measurement and feed analysis

The DMI of each individual animal was measured daily from calving to 8 weeks postpartum. Diets were fed for ad libitum intake allowing for 10% feed refusals. TMR was fed twice daily at 0700 and 1500 h. The amount of feed offered and orts were weighed daily. Orts were removed in the morning before next feeding. Samples of feed offered were collected and pooled before freezing at -20 °C for further analysis. Samples were thawed, dried for 48 h at 55°C in a forced-air oven, ground through a 1 mm screen in a Wiley mill (cyclotec 1093 sample mill). Duplicate samples were analyzed for absolute neutral detergent fiber (NDF), acid detergent fiber (ADF), DM, CP, and EE. NDF and ADF were analyzed according to Van Soest et al. (1991). CP and EE were analyzed by proximate analysis according to AOAC (1990). Water was available

ad libitum. The WI of each cow was recorded individually on 2 consecutive days in the last period of experiment.

Table1. Active ingredients of TMR (DM basic)

| | Diet | | |
|---|---|---|---|
| Composition | 0% | 18% | 24% |
| Corn silage | 39 | 39 | 39 |
| FFS | 0 | 18.0 | 24.0 |
| Cassava | 25.4 | 19.3 | 17.8 |
| Soybean meal | 22.1 | 7.1 | 2.1 |
| Soy hull | 11.0 | 14.1 | 14.6 |
| Mono-dicalcium | 1.0 | 1.0 | 1.0 |
| Limestone | 0.9 | 0.9 | 0.9 |
| Premix* | 0.2 | 0.2 | 0.2 |
| Sodium chloride | 0.2 | 0.2 | 0.2 |
| Potassium chloride | 0.2 | 0.2 | 0.2 |
| $NE_L$, Mcal/kg** | 1.63 | 1.65 | 1.66 |

*Premix 1 kg* : Vitamin A 2,400,000 IU, Vitamin $D_3$ 5000,000 IU, Vitamine E 500 IU, Vitamine $B_{12}$ 2 mg, Mn (Maganese) 8 g, Zn (Zinc) 8 g, Fe (Iron) 10 g, Cu (copper) 2 g, Mg (Magnesium) 26.4 g, Co (Cobalt) 400 mg, I (Iodine) 400 mg, Se (selenium) 40 mg*

** Calculation based on NRC (1989)

## 3.3 Body weight and body condition score measurement

BW of each cow was recorded at calving and weekly throughout the experiment. BCS of each cow was performed by using 1-5 score system where 1 = emaciated to 5 = overly fat (Wildman et al., 1982) starting from calving and every week till the end of the experiment.

## 3.4 Energy balance determination

EB of experimental cows receiving each diet were calculated by using following equation (NRC, 1989).

Where

EB = NE consumed - NE required

NE consumed = $NE_L$/kg DM x DM intake (kg)

NE lactation = 0.74(milk (kg) x 0.4 + milk fat (kg) x 15)

NE required = $BW^{0.75}$ (0.08) + NE lactation

For 2nd lactation cows

NE required = $BW^{0.75}$ (0.08)1.1 + NE lactation

## 3.5 Milk yield and milk compositions measurement

Cows were milked twice daily (0700 and 1600 h) and the milk yield was recorded from calving to 8 week postpartum. Milk samples were collected weekly during successive AM and PM milking for eight weeks. Then the milk samples were kept at -20 °C for milk composition analysis. Percentage milk fat, protein, lactose, and solid not fat (SNF) and total solids were determined using Milko-Scan 133B (N.Foss Electric, Denmark).

## 3.6 Blood sample collection and analysis

Blood samples (10 ml) of individual cows were collected weekly by caudal venipuncture into tube containing heparin, for nine weeks, starting from prepartum to the 8th week postpartum. Blood sample then was centrifuged at 3,000g for 10 minutes. Plasma sample was decanted and stored frozen at -20 °C for further determinations of NEFA and glucose concentration. Plasma samples were used to determine NEFA as described by Wang et al. (2004) and glucose by using enzymatic colorimetric test (Human®).

After ovulation was detected by ultrasonography, blood samples (5 ml) from each cow were collected daily by caudal venipuncture into heparinised tubes for 10 d after ovulation. The blood was centrifuged at 3,000xg for 10 min. Plasma sample was

decanted and stored frozen at -20°C until subsequent analysis. The concentration of plasma P4was determined in all samples, using Enzyme Linked Immuno - Sorbent Assay (ELISA) test kit (Human®).

## 3.7 Rumen fluid collection and determination

The 1st and 2nd months postpartum, the oral – ruminal intubation was used for rumen fluid collection after 3 hours feeding in the morning. Rumen content was sucked by the air pump. The rumen content was strained immediately using two layers of cheesecloth and pH of the rumen fluid was measured using pH meter (pH Scan 2). A 60 ml aliquot of the filtered rumen fluid was preserved by adding 3 ml of 6 N of hydrochloric acid and kept at -20 °C. Rumen fluid was analyzed by the method modified from Erwin (1961).

## 3.8 Measurement of follicular development

One month after calving, estrous cycle of individually cow was synchronized with CIDR®. After 10 days CIDR® was removed and measurement of follicular development was performed by same operator using a 7.5 MHz ultrasonographic probe and a dynamic imaging ultrasonograph. The number and the diameter of follicles were noted daily starting from the day of CIDR® removal to the 10th d after ovulation. All follicles which were found, grouped on criteria of their diameter. There were three groups according Spicer et al. (2004) including small size (3.0 – 5.9 mm), medium size (6.0 – 9.9 mm) and large size (over 10 mm).

Technique of ultrasonography in the cow was as described by Kahn et al. (1994). Briefly, a plastic sleeve over the probe was pull and filled with gel to exclude any air bubbles which might cause undesirable reflections in affecting the image quality. No application of any coupling gel between the plastic sleeve and the rectum was performed, since the rectum's natural contractility and moist contents both provide favorable conditions for the exclusion of air between the probe' s screening surface and the rectal wall. The sonographic image of bovine ovarian follicles was observed for the characteristic of the round shape of follicles with the anechoic, circular area of follicular

lumen. The wall of follicle was identified by hyperechoic ovarian stroma. The thin follicular wall was determined by separated from the ovarian parenchyma by a very narrow, hyperoechoic. The counting ovarian follicles by ultrasonography had a tendency to count 10 to 30% fewer follicles in the size order of 3 to 10 mm for actually present. The vesicles with a diameter of less than 2 to 3 mm were not detectable.

## 3.9 Statistical analysis

Treatments were arranged in a 3 x 2 factorial with 3 groups of diet (0, 18 and 24% FFS) and 2 groups of cow (multiparous and primiparous cows). All data was expressed as least squares means. Data was analyzed as 3 x 2 Factorial Experiments in Completely Randomized Design using the general linear model. The model included diets, cows and diet x cow interaction and the error was residual error mean square. The mathematical model, with interaction, could be written as follows;

$$Y_{ij} = \mu + diet_i + cow_j + (\text{diet x cow interaction})_{ij} + e_{ijk}$$

Where;

Yij; = the observed value for the $j^{th}$ replicate of the $i^{th}$ treatment (where i=1 to t and j=1 to b).

$\mu$; = the overall mean for observed value.

$diet_i$; = the treatment effect for the $i^{th}$ treatment; the treatment effects may be either fixed or random.

$cow_j$; = the block effect for the $j^{th}$ block; the block effect may be either fixed or random, however if treatments are fixed then random blocks are required for exact tests of treatment hypotheses.

diet x cow $\text{interaction}_{ij}$; = the interaction effect which can be estimated only when > 1 determinations per cell are available

$e_{ijk}$; = the random error associated with the $Y_{ij}$ experimental unit.

The mean differences between diets were tested by least significant difference. Significant differences were declared at $p<0.05$.

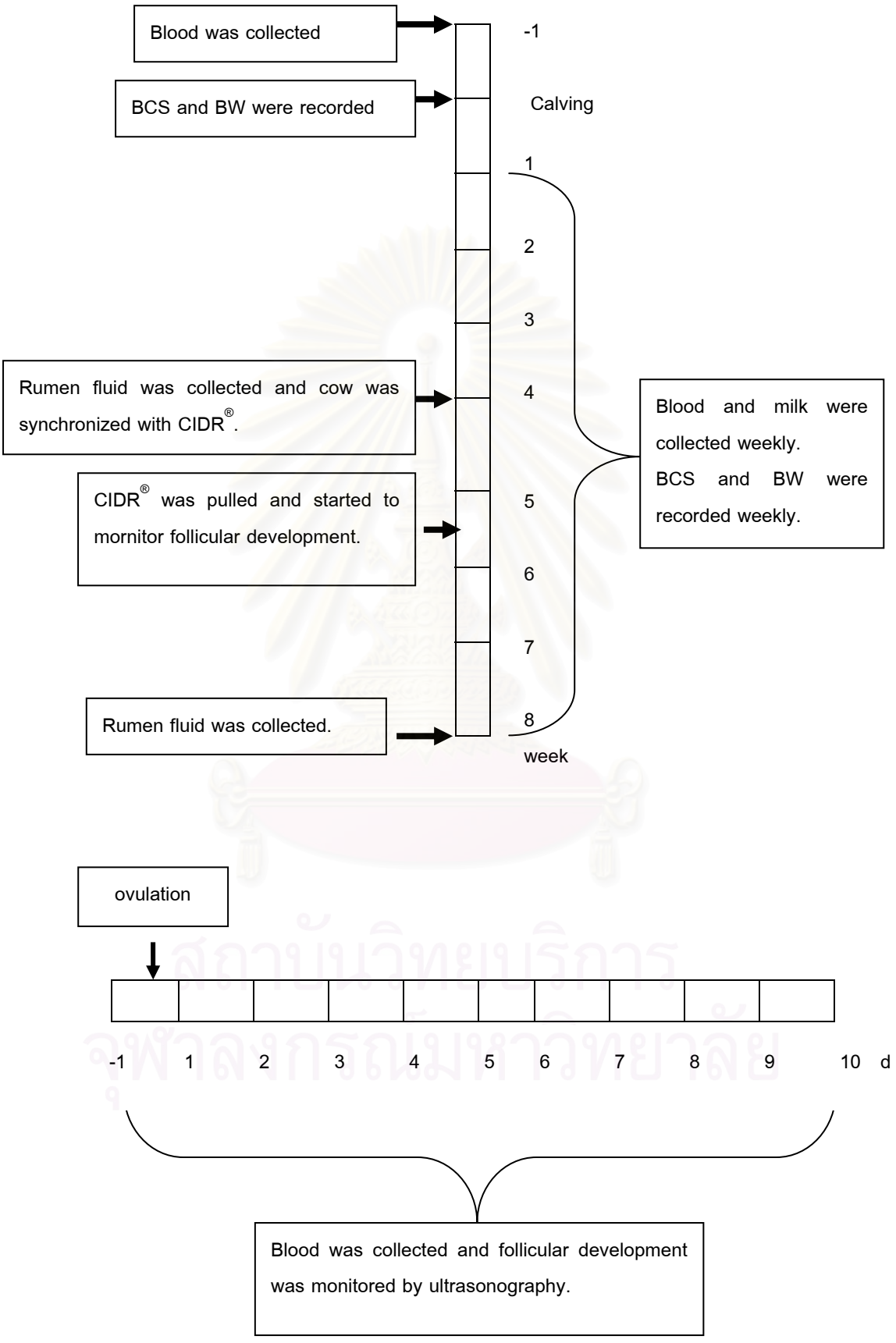


Figure 5. Diagram of sample collection

# CHAPTER IV

# RESULTS

The nutrient compositions of experimental diets are shown in Table 2. All diets were similar for most of nutrient compositions except EE. 0%FFS diet consisted of the lowest level of EE, while 24%FFS diet consisted of the highest level of EE.

## Table 2. The chemical analysis of nutrient compositions of experimental diets (DM basis)

| Nutrient (%) | Percentage of FFS (DM basis) | | |
|---|---|---|---|
| | 0% | 18% | 24% |
| CP | 16.2 | 16.1 | 16.1 |
| EE | 1.4 | 4.5 | 5.9 |
| ADF | 28.9 | 29.0 | 30.2 |
| NDF | 45.4 | 45.6 | 47.1 |

## Effects of FFS supplementation on feed intake, energy balance and water intake of crossbred Friesian cows during the first 2 month postpartum

Average DMI of cows in each group of diet and two types of cows during the first 8 week postpartum are shown in Figure 6 and 7, respectively. Average DMI, DMI as percentage of BW (%BW), EB, WI and WI per DMI (WI/DMI) during the first month postpartum (FMPP), the second month postpartum and the whole period of experiment (overall) are shown in Table 3. During all periods of experiment, no diet x cow interaction ($p>0.05$) was observed for average DMI and DMI (%BW). Average DMI was not significant difference ($p>0.05$) among dietary groups but multiparous cows had greater ($p<0.05$) average DMI than primiparous cows. During all periods average DMI (%BW) was not affected ($p>0.05$) by diets and cows.

During the FMPP, diet x cow interaction was observed ($p<0.05$) for average EB. Thus in this period treatment combinations (dietary treatment) for average EB is summarized in Table 5. During the FMPP multiparous and primiparous cows fed 0%FFS diet had more average EB than ($p<0.05$) multiparous cows fed 18%FFS diet. During the SMPP and overall period, no diet x cow interaction was observed ($p>0.05$) for average EB. Cows fed 0%FFS diet tended to increase EB but there was no difference ($p>0.05$) with diet. In both periods primiparous cows had more average EB than ($p<0.05$) multiparous cows.

No diet x cow interaction ($p>0.05$) was observed for WI and WI/DMI. Although cows received 18 and 24%FFS diets tended to consume higher level of water than cows received 0%FFS diet however no difference was found on WI and WI/DMI. Types of cows had also no influence on WI and WI/DMI. Multiparous and primiparous cows fed different diet consumed similar amount of water.

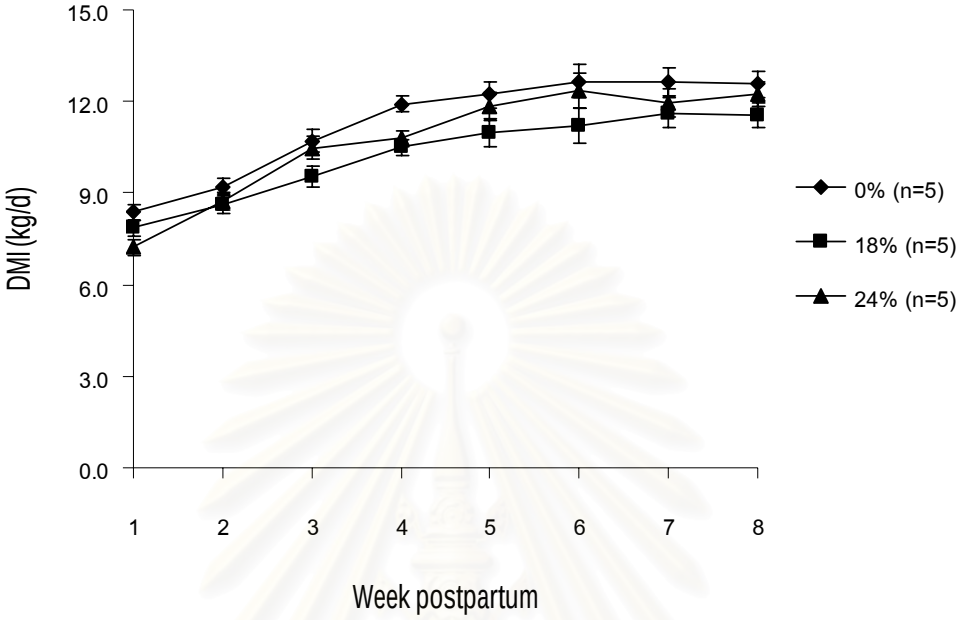


Figure 6. Average DMI for cows fed each diet (0, 18 and 24%FFS) during the first 8 week postpartum. Error bars indicated pooled standard errors.

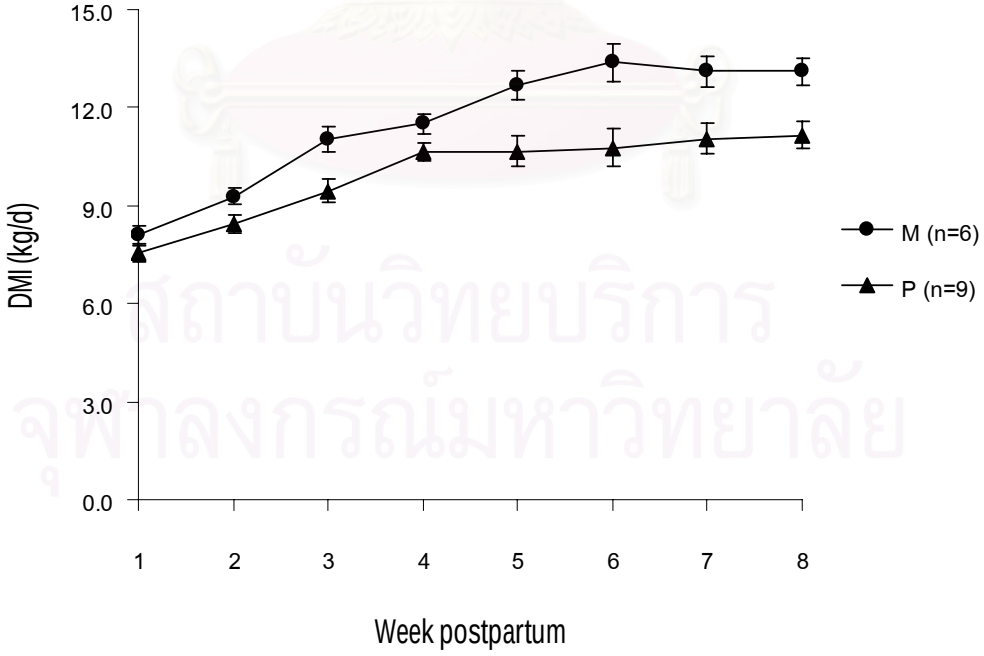


Figure 7. Average DMI for cow (M = multiparous cow and P = primiparous cow) during the first 8 week postpartum. Error bars indicated pooled standard errors.

Table 3. Effects of FFS supplementation on average DMI, DMI (%BW), EB, WI and WI/DMI of crossbred Friesian cows during the first 2 month postpartum

| | Diet[1] | | | Cow[2] | | Pooled |
|---|---|---|---|---|---|---|
| Items | 0% | 18% | 24% | M | P | SE |
| Number of cows | 5 | 5 | 5 | 6 | 9 | - |
| Average DMI (kg/d) | | | | | | |
| FMPP[3] | 10.1 | 9.1 | 9.3 | 10.0* | 9.0 | 0.23 |
| SMPP[4] | 12.5 | 11.4 | 12.1 | 13.1* | 10.9 | 0.46 |
| Overall[5] | 11.3 | 10.2 | 10.7 | 11.5** | 10.0 | 0.31 |
| Average DMI (%BW) | | | | | | |
| FMPP | 2.35 | 2.31 | 2.26 | 2.34 | 2.32 | 0.08 |
| SMPP | 2.91 | 2.56 | 2.68 | 2.67 | 2.76 | 0.07 |
| Overall | 2.63 | 2.48 | 2.40 | 2.48 | 2.54 | 0.07 |
| Average EB (Mcal/d) | | | | | | |
| FMPP[6] | -2.84 | -4.95 | -4.82 | -5.93 | -2.47 | 0.67 |
| SMPP | -1.68 | -1.55 | -2.30 | -2.94* | -0.75 | 0.38 |
| Overall | -2.26 | -3.25 | -3.56 | -4.44* | -1.61 | 0.49 |
| WI (L/d) | 63.8 | 88.4 | 85.9 | 88.8 | 69.9 | 7.65 |
| WI/DMI (L/kg) | 4.9 | 7.5 | 7.0 | 6.4 | 6.5 | 0.70 |

[1]Diet included 0, 18 and 24%FFS groups.

[2]Cow included M = multiparous cow and P = primiparous cow.

[3]FMPP = during the 1st month postpartum

[4]SMPP = during the 2nd month postpartum

[5]Overall = average values of the whole period of experiment

[6]Cow and diet x cow interaction differed significantly ($p<0.05$)

*Least squares means in the same row differed significantly ($p<0.05$) between types of cows.

**Least squares means in the same row differed significantly ($p<0.01$) between types of cows.

## Effects of FFS supplementation on milk production of crossbred Friesian cows during the first 2 month postpartum

Average milk yield of cows in each group of diet and two types of cows during the first 8 week postpartum are shown in Figure 8 and 9, respectively. Average milk yield, milk yield per DMI (milk yield/DMI) and 4% fat corrected milk (4%FCM) of crossbred Friesian cows fed different diets during all periods are shown in Table 4. During all periods no diet x cow interaction were observed ($p>0.05$) for average milk yield, milk yield/DMI. During the FMPP average milk yield did not differ ($p>0.05$) among dietary groups but multiparous cows had greater milk yield than ($p<0.01$) primiparous cows. During the SMPP cows fed 24%FFS had greater ($p<0.05$) milk yield than cows fed 0 and 18%FFS. Multiparous cows also had greater ($p<0.05$) average milk yield than primiparous cows. For overall period, 24%FFS diet significantly increased ($p<0.05$) average milk yield when compared with 0 and 18%FFS groups (19.4 versus 16.3, 17.1 kg/d, respectively). The differences were also observed between multiparous and primiparous cows (18.9 versus 16.3 kg/d; $p<0.01$). In addition during both FMPP and SMPP, 24%FFS diet significantly increased ($p<0.05$) average milk yield/DMI when compared with 0%FFS diet but did not differ ($p>0.05$) between multiparous and primiparous cows. For overall period 24%FFS diet significantly increased ($p<0.05$) average milk yield when compared with 0 and 18%FFS groups (1.83 versus 1.47, 1.64, respectively). FFS at 18% and 24% of diet increased feed efficiency by 11.6% and 24.5% respectively. During the FMPP diet x cow interaction was observed ($p<0.05$) for average 4%FCM. Thus in this period treatment combinations (dietary treatment) for average 4%FCM is summarized in Table 5. This period multiparous cows in all diets could produce significantly higher ($p<0.05$) average 4%FCM than primiparous cows (17.0, 17.9 and 17.0 kg/d versus 12.8, 13.0 and 15.6 kg/d, respectively). However, during the SMPP and overall no diet x cow interactions were observed for average 4%FCM. During both periods 24%FFS diet produced significantly greater ($p<0.05$) average 4%FCM than 0 and 18%FFS diets. Multiparous cows produced significantly higher ($p<0.01$) amount of 4%FCM than primiparous cows during the SMPP and overall period (19.1 versus 15.6 and 18.2 versus 14.7 kg/d, respectively).

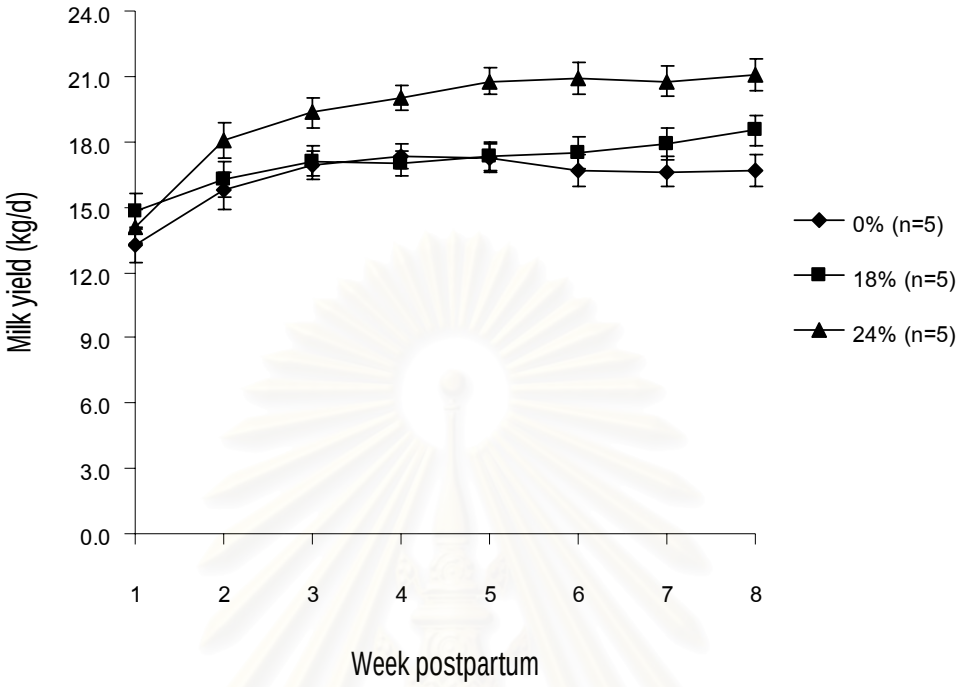


Figure 8. Average milk yield for cows fed each diet (0, 18 and 24%FFS) during the first 8 week postpartum. Error bars indicated pooled standard errors.

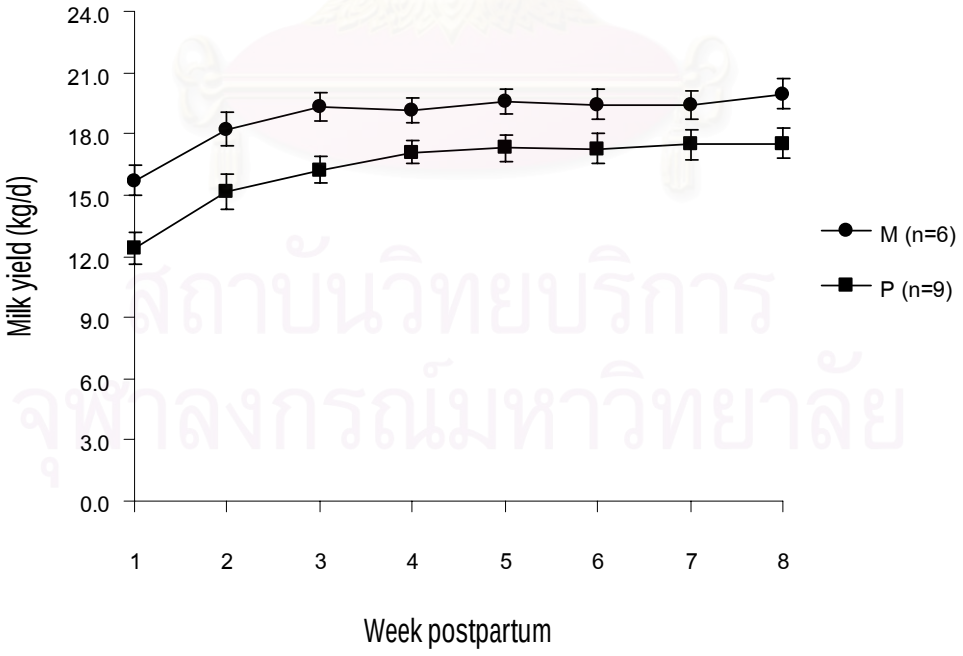


Figure 9. Average milk yield for cow (M = multiparous cow and P = primiparous cow) during the first 8 week postpartum. Error bars indicated pooled standard errors.

Table 4. Effects of FFS supplementation on average milk yield, milk yield/DMI and 4%FCM of crossbred Friesian cows during the first 2 month postpartum

| | Diet[1] | | | Cow[2] | | Pooled |
|---|---|---|---|---|---|---|
| Items | 0% | 18% | 24% | M | P | SE |
| Number of cows | 5 | 5 | 5 | 6 | 9 | - |
| Average milk yield (kg/d) | | | | | | |
| FMPP[3] | 15.9 | 16.3 | 17.9 | 18.1** | 15.2 | 0.65 |
| SMPP[4] | 16.8[b] | 17.8[b] | 20.9[a] | 19.6* | 17.4 | 0.68 |
| Overall[5] | 16.3[b] | 17.1[b] | 19.4[a] | 18.9** | 16.3 | 0.63 |
| Average milk yield/DMI | | | | | | |
| FMPP | 1.61[b] | 1.72[ab] | 1.92[a] | 1.81 | 1.70 | 0.06 |
| SMPP | 1.34[b] | 1.56[ab] | 1.75[a] | 1.50 | 1.60 | 0.06 |
| Overall | 1.47[b] | 1.64[b] | 1.83[a] | 1.65 | 1.65 | 0.05 |
| Average 4%FCM (kg/d) | | | | | | |
| FMPP[6] | 14.9 | 15.5 | 16.3 | 17.2 | 13.8 | 0.57 |
| SMPP | 15.9[b] | 16.3[b] | 19.0[a] | 19.1** | 15.6 | 0.65 |
| Overall | 15.4[b] | 15.9[b] | 17.6[a] | 18.2** | 14.7 | 0.58 |

[1]Diet included 0, 18 and 24%FFS groups.

[2]Cow included M = multiparous cow and P = primiparous cow

[3]FMPP = during the 1st month postpartum

[4]SMPP = during the 2nd month postpartum

[5]Overall = average values of the whole period of experiment

[6]Diet, cow and diet x cow interaction differed significantly ($p<0.05$)

[abc]Least squares means in the same row with different superscripts differed significantly ($p<0.05$) within diet.

*Least squares means in the same row differed significantly ($p<0.05$) between types of cows.

**Least squares means in the same row differed significantly ($p<0.01$) between types of cows.

Table 5. Effects of FFS supplementation on EB and average 4%FCM of crossbred Friesian cows during the FMPP

| | Dietary treatment[1] | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0% | | 18% | | 24% | | Pooled |
| items | M | P | M | P | M | P | SE |
| Number of cows | 2 | 3 | 2 | 3 | 2 | 3 | |
| Average EB (Mcal/d) | | | | | | | |
| FMPP[2] | -3.67$^{bc}$ | -2.01$^{c}$ | -8.24$^{a}$ | -1.65$^{c}$ | -5.89$^{ab}$ | -3.76$^{bc}$ | 0.67 |
| Average 4%FCM (kg/d) | | | | | | | |
| FMPP | 17.0$^{a}$ | 12.8$^{b}$ | 17.9$^{a}$ | 13.0$^{b}$ | 17.0$^{a}$ | 15.6$^{b}$ | 0.57 |

[1]Dietary treatment included 1. multiparous cow fed 0%FFS; 2. primiparous cow fed 0%FFS; 3. multiparous cow fed 18%FFS; 4. primiparous cow fed 18%FFS; 5. multiparous cow fed 24%FFS; 6. primiparous cow fed 24%FFS.

[2]FMPP = during the 1st month postpartum

[abc]Least squares means in the same row with different superscripts differed significantly ($p<0.05$).

## Effects of FFS supplementation on body condition score of crossbred Friesian cows during the first 2 month postpartum

Least squares means BCS of cows in each group of diet from calving to the first 8 week postpartum are shown in Figure 10. No diet x cow interaction was observed. At calving BCS of cows in 0, 18 and 24%FFS groups were 3.2, 3.0 and 3.1 but there were no difference ($p>0.05$). At the end of the experimental period, BCS in 0, 18 and 24%FFS groups were 2.8, 2.7 and 2.7, respectively in which no difference was found. In addition, from calving to 8 week postpartum, each diet could not influence ($p>0.05$) BCS.

BCS of multiparous and primiparous cows from calving to the first 8 week postpartum are shown in Figure 11. At calving and the end of the experimental period, BCS of multiparous and primiparous cows were 3.1 and 2.8, respectively. From calving

to 8 week postpartum there were no difference ($p>0.05$) of BCS between two groups of cows.

## Effects of FFS supplementation on body weight of crossbred Friesian cows during the first 2 month postpartum

Least squares means BW of cows in each group of diet from calving to the first 8 week postpartum are shown in Figure 12. No diet x cow interaction was detected ($p>0.05$). At calving BW in 0, 18 and 24%FFS groups were 445, 469 and 479 kg, respectively. At the end of experimental period BW in 0, 18 and 24%FFS groups were 431, 456 and 459 kg, respectively. However, no difference of BW was found in both periods. During the first 8 week postpartum, each diet did not influence ($p>0.05$) BW of cow.

Additionally BW in two types of cows from calving to the first 8 week postpartum is shown in Figure 13. At calving the difference was observed ($p<0.01$) when compared between multiparous and primiparous cows (526 versus 402 kg). At the end of experimental period, multiparous cows had more BW ($p<0.01$) than primiparous cows (500 versus 396 kg). During the first 8 week postpartum BW were significantly different ($p<0.01$) between two types of cows.

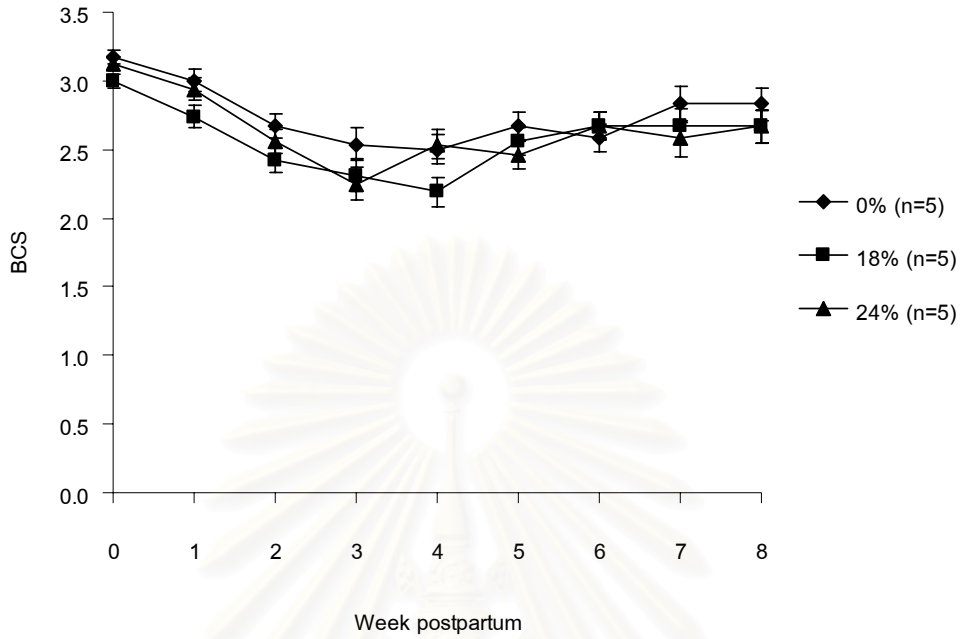


Figure 10. Least squares means BCS for cows fed each diet (0, 18 and 24%FFS) from calving to the first 8 week postpartum. Error bars indicated pooled standard errors.

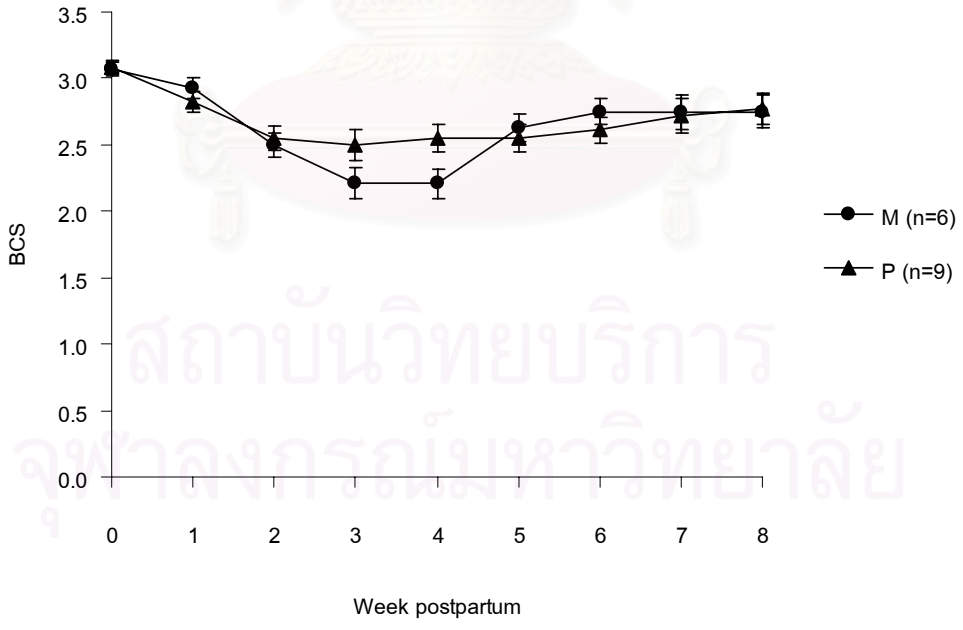


Figure 11. Least squares means BCS for cow (M = multiparous cow and P = primiparous cow) from calving to the first 8 week postpartum. Error bars indicated pooled standard errors.

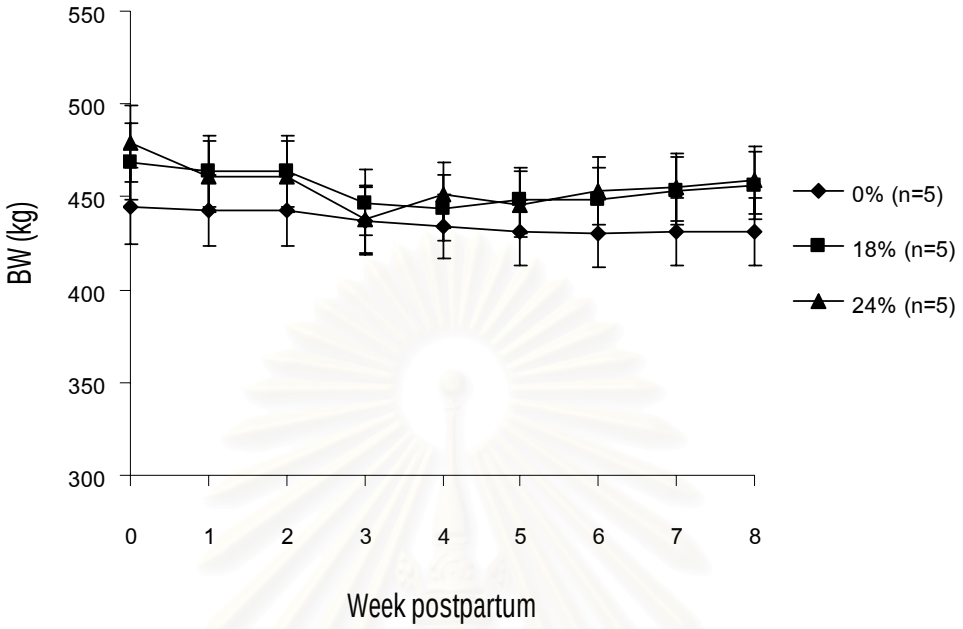


Figure 12. Least squares means BW for cows fed each diet (0, 18 and 24%FFS) from calving to the first 8 week postpartum. Error bars indicated pooled standard errors.

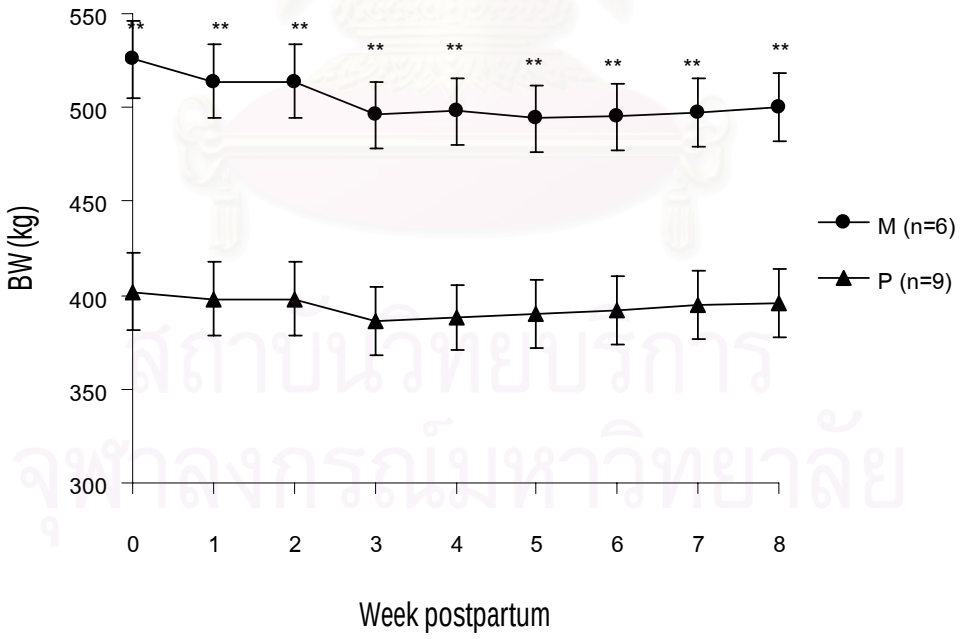


Figure 13. Least squares means BW for cow (M = multiparous cow and P = primiparous cow) from calving to the first 8 week postpartum. Asterisks indicated a difference ($p<0.01$) between two types of cow. Error bars indicated pooled standard errors.

## Effects of FFS supplementation on body condition score and body weight loss of crossbred Friesian cows during the first 2 month postpartum

Average BCS loss and BW loss of crossbred Friesian cows during experimental period are shown in Table 6. No diet x cow interaction was observed ($p>0.05$) for average BCS loss and BW loss. During all experiment period diet and types of cows did not affect average BCS loss. However during the FMPP cows that fed 24%FFS diet, tended to loose ($p<0.06$) more BW than cows fed 0 and 18%FFS diets (-25.7 kg versus -5.6, -14.5 kg, respectively). Also multiparous cows lost ($p<0.06$) more BW than primiparous cows (-21.2 kg versus -9.3 kg). During the SMPP cows fed 18%FFS diet had greater average BW loss than cows fed 0 and 24%FFS diets (-6.3 kg versus -4.9, -4.8 kg, respectively) but there were no difference ($p>0.05$). In addition for overall period cows fed 24%FFS diet had higher average BW loss than cows fed 0 and 18%FFS diets (-15.2 kg versus -4.9, -10.4 kg, respectively) but there were no difference ($p>0.05$). In both period types of cows did not influenced ($p>0.05$) average BW loss. During the SMPP and overall, average BW loss for multiparous and primiparous cows were -9.5 versus -1.2 and -15.1 versus -5.2 kg, respectively.

Table 6. Effects of FFS supplementation on average BCS and BW loss of crossbred Friesian cows during the first 2 month postpartum

| | Diet[1] | | | Cow[2] | | Pooled |
|---|---|---|---|---|---|---|
| Items | 0% | 18% | 24% | M | P | SE |
| Number of cows | 5 | 5 | 5 | 6 | 9 | - |
| Average BCS loss | | | | | | |
| FMPP[3] | -0.5 | -0.6 | -0.6 | -0.6 | -0.5 | 0.06 |
| SMPP[4] | -0.4 | -0.3 | -0.5 | -0.4 | -0.4 | 0.08 |
| Overall[5] | -0.5 | -0.5 | -0.6 | -0.5 | -0.5 | 0.06 |
| Average BW loss (kg) | | | | | | |
| FMPP | -5.6 | -14.5 | -25.7 | -21.2 | -9.3 | 3.30 |
| SMPP | -4.9 | -6.3 | -4.8 | -9.5 | -1.2 | 4.68 |
| overall | -4.9 | -10.4 | -15.2 | -15.1 | -5.2 | 3.19 |

[1]Diet included 0, 18 and 24%FFS groups.

[2]Cow included M = multiparous cow and P = primiparous cow.

[3]FMPP = during the 1st month postpartum

[4]SMPP = during the 2nd month postpartum

[5]Overall = average values of the whole period of experiment

## Effects of FFS supplementation on percentages of milk composition, pH and VFA concentration of rumen fluid of crossbred Friesian cows during the experimental period

Least squares means of milk composition percentage, pH and VFA concentration of rumen fluid are shown in Table 7. No diet x cow interaction was observed for average percentages of milk composition, pH and VFA concentration. Average percentages of milk protein, fat, lactose, solid not fat and total solid were not significantly different ($p>0.05$) in cows fed 0, 18 and 24%FFS diet. Types of cow had no effect ($p>0.05$) on milk compositions.

The concentrations of individual VFA included C2, C3, C4 and valerate (C5). Least squares means of ruminal fluid pH from cows fed 0, 18 and 24%FFS diets were 6.6, 6.6 and 6.4, respectively whereas multiparous and primiparous cows were 6.6 and 6.5. Diet and cow did not affect ($p>0.05$) pH of ruminal fluid. The percentage of C2 in 0, 18 and 24%FFS diets were 63.5, 64.6 and 63.6, respectively, whereas in multiparous and primiparous cows were 64.2 and 63.7. Diet and types of cows did not affect ($p>0.05$) the percentage of C2. Similar results were also found for C3, C4, C5 and C2:C3 ratio.

Table 7. Effects of FFS supplementation on average percentages of milk composition, pH and VFA concentration of rumen fluid of crossbred Friesian cows during the experimental period

| | Diet[1] | | | Cow[2] | | Pooled |
|---|---|---|---|---|---|---|
| Items | 0% | 18% | 24% | M | P | SE |
| Number of cows | 5 | 5 | 5 | 6 | 9 | - |
| Average milk composition (%) | | | | | | |
| Protein | 3.30 | 3.17 | 3.32 | 3.24 | 3.25 | 0.06 |
| Fat | 3.64 | 3.41 | 3.40 | 3.56 | 3.38 | 0.11 |
| Lactose | 4.88 | 4.91 | 5.06 | 4.93 | 4.94 | 0.07 |
| Solid not fat | 8.88 | 8.73 | 9.06 | 8.84 | 8.87 | 0.12 |
| Total solid | 12.47 | 12.11 | 12.40 | 12.37 | 12.18 | 0.23 |
| pH | 6.6 | 6.6 | 6.4 | 6.6 | 6.5 | 0.07 |
| VFA concentration (mol/100 mol) | | | | | | |
| C2 | 63.5 | 64.6 | 63.6 | 64.2 | 63.7 | 1.23 |
| C3 | 20.3 | 20.8 | 21.6 | 19.8 | 22.0 | 0.56 |
| C4 | 10.9 | 12.6 | 12.1 | 12.3 | 11.4 | 0.67 |
| C5 | 1.2 | 1.3 | 1.2 | 1.1 | 1.3 | 0.05 |
| Ratio C2/C3 | 3.2 | 3.2 | 3.0 | 3.3 | 2.9 | 0.10 |

[1]Diet included 0, 18 and 24%FFS groups.

[2]Cow included M = multiparous cow and P = primiparous cow.

## Effects of FFS supplementation on concentration of plasma glucose of crossbred Frisian cows from a week prepartum to the first 8 week postpartum

From a week prepartum to the 8$^{th}$ week postpartum the concentration of plasma glucose in each diet group are shown in Figure 14. No diet x cow interaction was observed for concentration of plasma glucose weekly and average concentration of plasma glucose during experimental period which was not affected ($p>0.05$) by diet and types of cow. In 0, 18 and 24%FFS groups were 55.57, 59.26 and 56.54 mg/dl, respectively. A week prepartum, the concentration of plasma glucose in 0, 18 and 24%FFS groups were 54.7, 56.6 and 55.0 mg/dl, respectively. During experimental period diet did not affect ($p>0.05$) the concentration of plasma glucose.

The concentration of plasma glucose in multiparous and primiparous cows from a week prepartum to the 8$^{th}$ wk postpartum is shown in Figure 15. A week prepartum, the concentration of plasma glucose in multiparous and primiparous cows were 55.5 and 55.4 mg/dl. However, types of cows did not affect ($p>0.05$) the concentration of plasma glucose. During the first 8 week postpartum, average concentration of plasma glucose in multiparous and primiparous cows were 56.27 and 57.98 mg/dl.

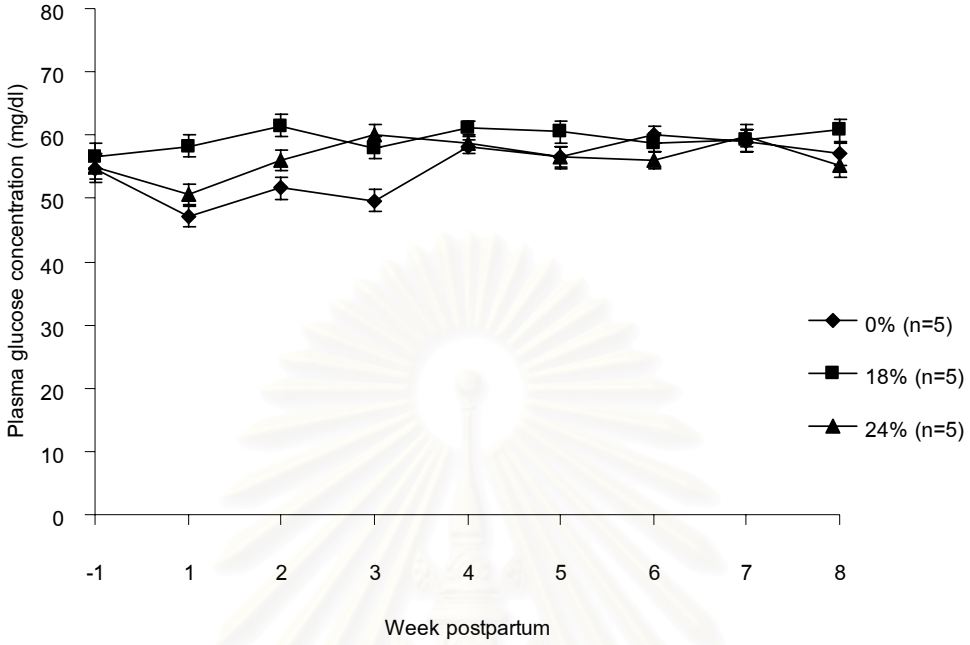


Figure 14. Least square means concentrations of plasma glucose for cows fed each diet (0, 18 and 24%FFS) from a week prepartum to the first 8 week postpartum. Error bars indicated pooled standard errors.

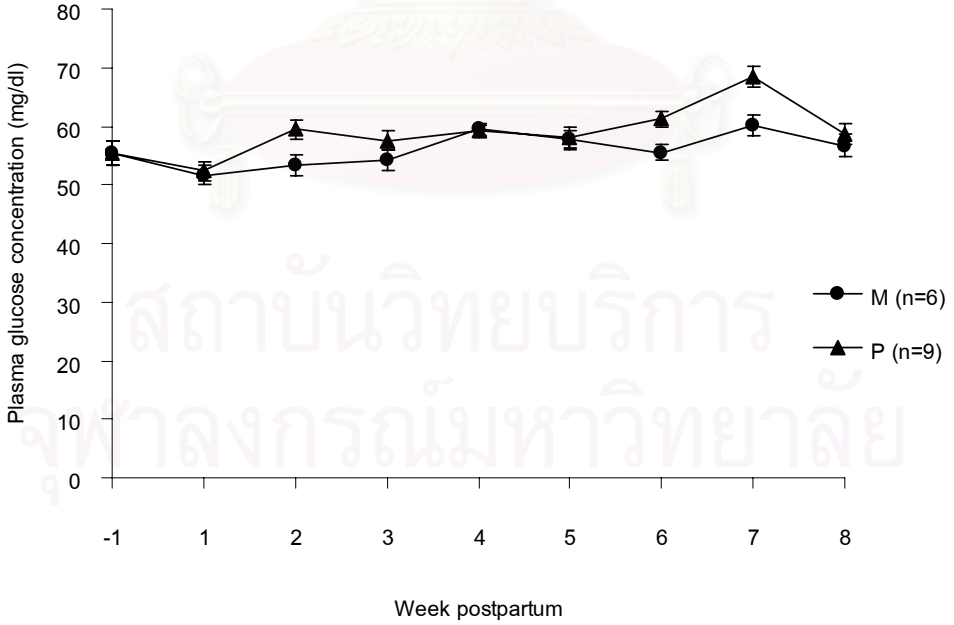


Figure 15. Least square means concentrations of plasma glucose for cow (M = multiparous cow and P = primiparous cow) from a week prepartum to the first 8 week postpartum. Error bars indicated pooled standard errors.

## Effects of FFS supplementation on concentration of plasma NEFA of crossbred Frisian cows from a week prepartum to the first 8 week postpartum

The level of plasma NEFA; from cows fed 0, 18 and 24% FFS diet; during a week prepartum to the first 8 week postpartum, are shown in Figure 16. No diet x cow interaction was observed for concentration of NEFA and average concentration of plasma NEFA during experimental period which were not affected ($p>0.05$) by diet and types of cows. This period average plasma NEFA levels of cows were fed 0, 18 and 24%FFS diets were 201, 209 and 225 μmol/l, respectively. Pooled SE was 12.17. A week prepartum, the concentration of plasma NEFA from cows received 0, 18 and 24%FFS diets were 230, 232 and 233 μmol/l, respectively. In addition the 1$^{st}$ week postpartum level of plasma NEFA in all cows started dramatically to increase. This week NEFA from cows received 24% FFS diet tended to be increased more than 0 and 18%FFS (422 versus 333 and 367 μmol/l, respectively). However, no significant difference was found ($p>0.05$). Then the 2$^{nd}$ week postpartum the plasma NEFA declined to the same level before parturition. No effect of diet on plasma NEFA level was found.

The concentrations of plasma NEFA in multiparous and primiparous cows were shown in Figure 17. A week prepartum, the concentrations of plasma NEFA in multiparous and primiparous cows were 211 and 252 μmol/l. The 1$^{st}$ week postpartum level of plasma NEFA in multiparous cows tended to have higher concentration of plasma NEFA than primiparous cows (448 versus 300 μmol/l). During the first 8 week postpartum, average concentrations of plasma NEFA in multiparous and primiparous cows were 222 and 200 μmol/l.

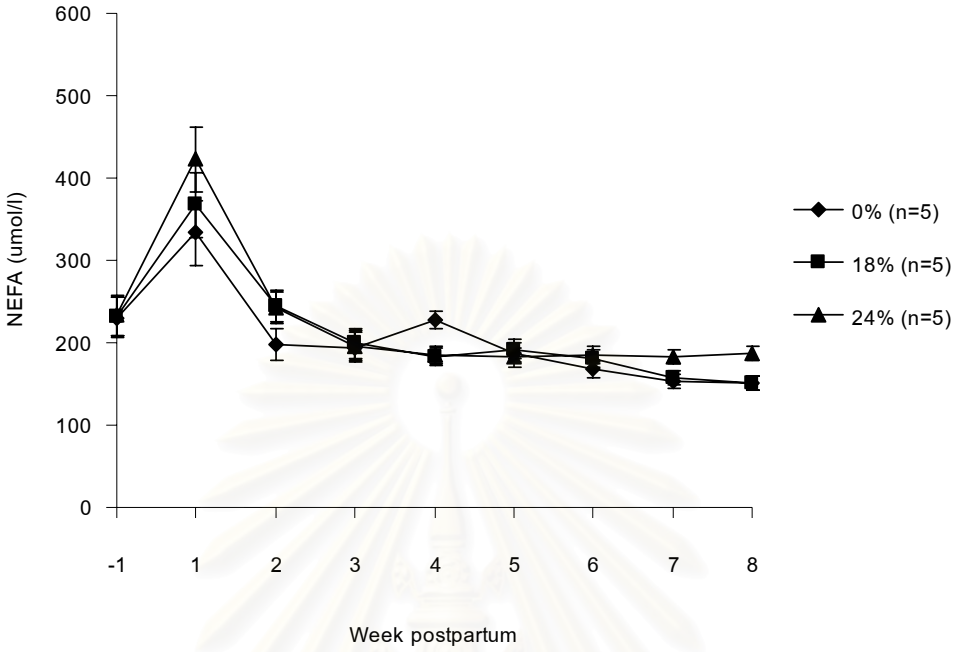


Figure 16. Least squares means concentrations of plasma NEFA for cows fed each diet (0, 18 and 24%FFS) from a week prepartum to the first 8 week postpartum. Error bars indicated pooled standard errors.

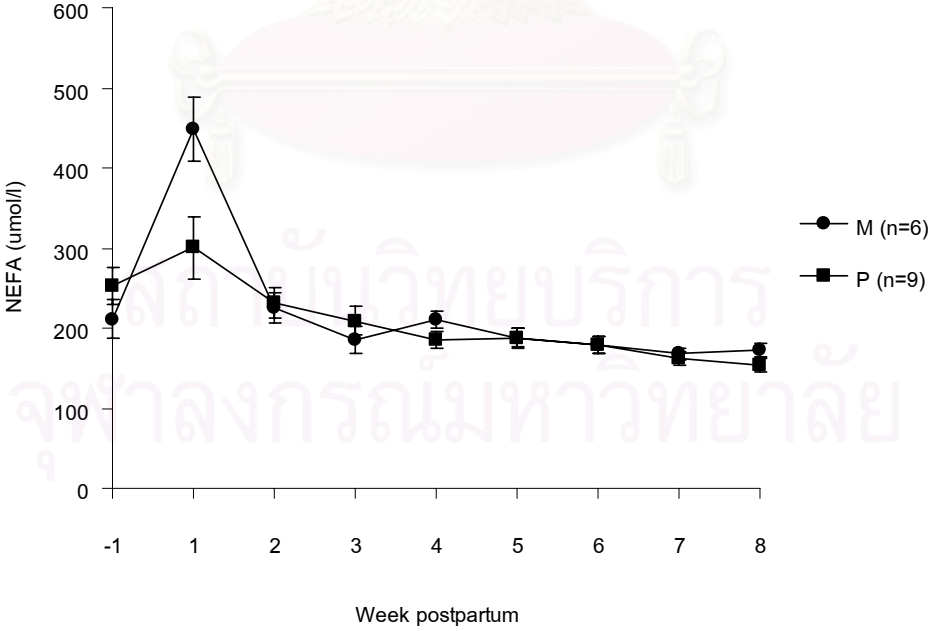


Figure 17. Least squares means concentrations of plasma NEFA for cow (M = multiparous cow and P = primiparous cow) from a week prepartum to the first 8 week postpartum. Error bars indicated pooled standard errors.

## Effects of FFS supplementation on ovulation rate, concentration of plasma P4 and follicular development of crossbred Friesian cows during the experimental period

No diet x cow interaction was observed for concentration of plasma P4, size of the largest ovulatory follicle and number of follicles. The ovulation rate, concentration of plasma P4 and follicular development are shown in Table 8. Cows received 18%FFS diet has the greater ovulation rate than cows received 0 and 24%FFS diets (100.0% versus 80.0 and 60.0%, respectively). The ovulation rate of multiparous cows was greater than primiparous cows (83.3 versus 77.8%). The concentration of plasma P4 in 0% FFS and 18%FFS groups were greater ($p<0.01$) than 24%FFS group (10.5, 11.1 versus 6.1 ng/ml, respectively). Although primiparous cows had higher concentration of plasma P4 than multiparous cows (9.1 versus 10.2 ng/ml) but there were no difference ($p>0.05$). The size of the largest ovulatory follicle in 0, 18 and 24%FFS groups were 12.7, 12.0 and 13.3 mm, respectively whereas multiparous and primiparous cows were 12.5 and 12.8 mm. However, diet and cow did not influence ($p>0.05$) the size of the largest ovulatory follicle. Also number of follicles in each cow including small, medium and large size, from 1 d before ovulation until the $10^{th}$ d of estrus cycle were not affected ($p>0.05$) by diets and types of cows.

Table 8. Effects of FFS supplementation on ovulation rate, concentration of plasma P4 and follicular development of crossbred Friesian cows during the experimental period

| | Diet[1] | | | Cow[2] | | Pooled |
|---|---|---|---|---|---|---|
| Items | 0% | 18% | 24% | M | P | SE |
| Number of cows | 4 | 5 | 3 | 5 | 7 | - |
| 0vulation rate (%) | 80.0 | 100.0 | 60.0 | 83.3 | 77.8 | - |
| | (4/5) | (5/5) | (3/5) | (5/6) | (7/9) | |
| Plasma P4[3] (ng/ml) | 10.5[a] | 11.1[a] | 6.1[b] | 9.1 | 10.2 | 0.73 |
| Size of the largest ovulatory follicle (mm) | 12.7 | 12.0 | 13.3 | 12.5 | 12.8 | 0.79 |
| Number of follicles[4] | | | | | | |
| Small size (3.0-5.9 mm) | 1.68 | 1.14 | 0.91 | 1.40 | 1.07 | 0.18 |
| Medium size (6.0-9.9 mm) | 0.47 | 0.48 | 0.44 | 0.33 | 0.58 | 0.11 |
| Large size (>10 mm) | 0.09 | 0.08 | 0.14 | 0.13 | 0.08 | 0.02 |

[1]Diet included 0, 18 and 24%FFS groups.

[2]Cow included M = multiparous cow and P = primiparous cow.

[3]Average value from 7th to 10th d after ovulation

[4]Average number of follicles from 1 d before ovulation until the 10th d of estrus cycle

[abc]Least squares means in the same row with different superscripts differed significantly ($p<0.01$) within diet.

# CHAPTER V

# Discussion

## Effects of FFS supplementation on feed intake, energy balance and water intake

There were several studies (Ruegsegger and Schultz, 1985; Faldet and Satter, 1991; Shauff et al., 1992; Abu-Ghazaleh et al., 2002[a]) showing that there were not significant difference ($p>0.05$) in DMI when FFS was replaced soybean meal in diet fed to cows. In addition to the study of Knapp et al. (1991) which reported that cows fed 0, 12, 18 and 24%FFS of diet, consumed the similar amount of DM. Similarly, average DMI during all period in this study were not significant difference ($p>0.05$) among dietary groups. However, multiparous cows had greater ($p<0.05$) average DMI than primiparous cows. During all periods average DMI (%BW) were not affected ($p>0.05$) by diets and cows. Similarly to previous research, which reported that DMI (%BW) was not affected by supplementation of FFS when compared with soybean meal (Faldet and Satter, 1991). Jenkins (1993) and NRC (2001) suggested that total dietary fat should not exceed 6 to 7% of dietary DM. FFS in the present study were processed through an extruder to rupture the seeds (Dhiman et al., 1999) and referred to as “protected fats” or “bypass fat” (Gransworthy, 2002). This type of oil seed was relatively inert in the rumen but available postruminally (Coppock and Wilks, 1991). Therefore high amount of fat in FFS supplementation diet in this study could not depress DMI and fiber digestion.

The objective of dietary supplemental fat in the early postpartum period is to lessen the negative EB of the herd. However production of milk or FCM often is increased when fat is supplemented in diet (Staples et al., 1998). Schmidely et al. (2005) reported FCM increased in the goats fed 10 and 20%FFS diets (3.3 and 5.2%EE, respectively) more than ($p<0.05$) those fed 0%FFS diet (1.4%EE) without any change in energy intake. Thus the goats fed FFS at 10 and 20% levels of diets had significantly lower ($p<0.05$) EB than those fed 0%FFS diet. This result is in agreement with the present study. Cows fed FFS diets had lower EB than control diet. More pronounce was found in multiparous cows than primiparous cows. The present results

showed that cows in all dietary groups entered to some degree of NEB. During the FMPP multiparous cows fed 18%FFS diet had more some degree of NEB than ($p<0.05$) multiparous cows fed 0%FFS diet. During the SMPP and overall period cows fed 24%FFS diet tended to have more negative EB than cows fed 0%FFS but there was no difference ($p>0.05$). Increment of milk yield and 4%FCM in cows fed 18 and 24% FFS diet were the response of greater NEB found in cows fed FFS supplementation diets.

Dairy cattle consume large amount of water (NRC, 2001) that is necessary for maintaining body fluids and proper ion balance; for digestion, absorption and metabolized nutrient; for elimination waste material and excess heat from the body; for providing a fluid environment for the fetus; and for transportation nutrients to and from body tissues (Waldner and Looper, 2002). No study had been observed on WI in dairy cattle fed FFS diet. In this study cows fed 18 and 24% FFS diets tended to consume higher water than cows received 0%FFS diet however no difference was found on WI and WI/DMI. Types of cows had also no influence on WI and WI/DMI. From this study it is likely that fat supplementation from FFS could increase water consumption during early lactation period in crossbred Friesian cattle.

## Effects of FFS supplementation on milk production

Previous studies reported that milk yield increased when supplemented FFS in diet (Dhiman et al., 1999; Abu-Ghazaleh et al., 2002[a]). Knapp et al. (1991) studied EE in 0, 12, 18 and 24%FFS of diets were 3.0, 5.1, 6.4 and 7.0% respectively. Milk yield increased ($p<0.05$) when levels of FFS in diets increased (34.9, 37.5, 38.5 and 38.8 kg/day, respectively). In the present study average milk yield during the SMPP and overall period increased in cows fed 24%FFS when compared with cows fed 0 and 18%FFS (20.9 versus 16.8, 17.8 and 19.4 versus 16.3, 17.1). Knapp et al. (1991) suggested that feeding 12%FFS consisted of 5.1%EE could improve milk yield by 7.4%. Comparison to the present study 18 and 24% FFS diets consisted of 4.5 and 5.9%EE improved milk yield by 4.9% and 19% respectively.

It was found in this study that FFS could enhance ($p<0.05$) feed efficiency. FFS at 18% and 24% increased feed efficiency by 11.6% and 24.5% respectively. The

present result agreed with Dhiman et al. (1999) reported in cows fed 12%FFS (4.9%EE) increased more feed efficiency than cows fed 0%FFS (2.7%EE) by 15.1%. Faldet and Satter (1991) reported that cows fed 13%FFS (5.6%EE) produced by 13.8% more 3.5%FCM than cows fed 0%FFS (3.3%EE). Similar to the present study, during SMPP and overall periods 24%FFS diet produced significantly ($p<0.05$) more average 4%FCM than 0%FFS diets. In both periods FFS at 24% increased average 4%FCM by 19.5% and 14.3%, respectively. Milk yield often increases after FFS supplementation. Because of FA from FFS are incorporated directly into milk fat by the mammary (Chilliard et al., 2003). Then FA mobilization from adipose tissue and the requirement of NADPH for mammary fatty acid synthesis decrease (Grummer and Carroll, 1991).Thus glucose is spared to require for lactose synthesis, since lactose is the major osmotic component in milk (Garnsworthy, 2002). The osmotic relationship of lactose and milk secretion makes its concentration stable despite differences in diet composition (Chouinard et al., 1997).

Type of cows had an influence on 4% FCM production. Multiparous cows fed 18 and 24% FFS diets produced more 4% FCM than primiparous cows by 37.7% and 9.0%, respectively.

Therefore positive response of milk yield, feed efficiency and 4%FCM to feeding FFS in this study could be attributed to many factors such as experimental design, primiparous versus multiparous cows, stage of lactation, level of production, protein content of diets fed, forage fed, and type and extent of heat treatment (Faldet and Satter, 1991).

## Effects of FFS supplementation on body condition score and body weight

Abu-Ghazaleh et al. (2002[a]) reported that feeding 13.3%FFS (5.5%EE of diet) increased ($p<0.05$) milk yield when compared with feeding 0%FFS (3.0%EE of diet) in Holstein cow. Diet treatments did not influence ($p>0.05$) BCS (2.69 versus 2.73, respectively). Similarly AbuGhazaleh et al. (2002[b]) reported that BCS was not different ($p>0.05$) between cow fed 10.6%FFS (4.9%EE of diet) and cow fed 0%FFS (3.1%EE of diet). These results were similar to the present study. From calving to 8$^{th}$ week

postpartum, diet and types of cow could not influence BCS and average BCS loss in all dietary groups. After calving cows fed 24, 18 and 0%FFS diet started to recover their BCS at the 4th, 5th and 5th week postpartum, respectively. These results indicated that these cows in all dietary groups entered to some degree of NEB.

Previous studies reported that FFS supplementation in diet did not influence ($P>0.05$) BW when compared with soybean meal based diet (Faldet and Satter, 1991; Schauff et al., 1992; Dhiman et al., 1999). In the present study from calving to 8th week postpartum, FFS diet could not influence BW but types of cow could affect BW ($p<0.05$). In addition to study of Dhiman et al. (1999) who reported that 12%FFS supplementation in diet tended to loose more BW than ($p=0.06$) 0%FFS diet. Due to cows needed high energy to support high milk yield. This result is in agreement with the present study. Cows fed 24%FFS diet, tended to loose ($p<0.06$) more BW than cows fed 0 and 18%FFS diets during the FMPP. The loss of BW during this period due to the high energy demand to support lactation and the compensation for the deficiency feed intake to demand (Rukkwamsuk et al., 2001).

## Effects of FFS supplementation on average percentages of milk composition, pH and VFA concentration of rumen fluid

Milk composition including percentage of protein, fat, lactose, solid not fat and total solid were not affected ($p>0.05$) by diet and types of cow. Previous studies reported that percentages of milk protein (Rauegsegger and Schultz, 1985; Dhiman et al., 1999), milk fat (Faldet and Satter, 1991; Schauff et al., 1992), milk lactose (Chouinard et al., 1997; Dhiman et al., 1999), milk SNF and TS (Schuaff et al., 1992; Dhiman et al., 1999) were not altered ($p>0.05$) when supplemented FFS in the diet. From all results indicated that FFS supplementation diet did not affect the percentage of individual milk composition especially protein and fat. Due to fat and protein was encapsulated within the seed, bypassed to lower digestive tract so rumen fermentation was not interfered.

As previously discussed, feeding free polyunsaturated oils can affect rumen fermentation adversely and reduce fiber digestion. However the present study, FFS supplementation diet and type of cow did not affect ($p>0.05$) pH, individual VFA concentration of rumen fluid and ratio C2:C3. Previous study reported that pH and individual VFA (C2, C3 and C4) concentration were unaffected by increasing of FFS to the diet (Knapp et al., 1991). Increasing levels of FFS in diet provided direct evidence that increasing EE levels of diet in appropriate form without exceed 6 to 7% of dietary DM (NRC, 2001), did not affect to rumen fermentation and depression of fiber digestion.

## Effects of FFS supplementation on concentration of plasma glucose and NEFA

Low blood glucose seems to be a critical factor in the etiology of ketosis and perhaps of fatty liver (Grummer and Carroll, 1991). However, blood glucose concentrations generally did not change under conditions of fat supplementation (Staples, et al., 1998). Previous study reported that concentration of plasma glucose did not differ ($p>0.05$) when 12.9%FFS (5.3%EE of diet) compared 0%FFS (3.4%EE of diet) in diet fed to cows (Ruegsegger and Schultz, 1985). Similarly, Beam and Butler (1997) fed different fat level (0, 7.6 or 15.3%tallow; 3.3, 5.2 or 7.1%EE, respectively) in diet of multiparous Holstein cow, no effect ($p>0.05$) on plasma glucose was found at the beginning of parturition. This result agreed with the present study. FFS supplementation diets did not affect ($p>0.05$) the concentration of plasma glucose during the experimental period apart from an increment of milk yield. Thus FFS supplementation had the possibility to prevent low plasma glucose that may cause ketosis and fatty liver during early lactation in high producing cows.

In addition to the mobilization of body fat in dairy cows during early lactation, results in elevated blood NEFA levels to compensate for energy requirement (Van knegsel et al., 2005). Sklan et al. (1994) reported that cow fed 2.5%calcium soaps of FA (4.9%EE) tended to increase concentration of plasma NEFA but this did not differ ($p>0.05$) when compared to cow fed 0%calcium soaps of FA (2.8%EE). In the study of Beam and Butler (1997) reported that feeding different fat level (0, 7.6 or 15.3%tallow; 3.3, 5.2 or 7.1%EE, respectively) in diet to multiparous Holstein cow showed no alteration of the plasma NEFA level. This is in agreement with the present study, diet

and types of cow did not affect ($p>0.05$) the concentration of plasma NEFA. The present result showed that concentration of plasma NEFA in cows of all dietary groups increased sharply immediately at the 1st week postpartum and progressive declined during the 2nd week through to the 8th week postpartum. Thus it indicated that cows of all dietary groups entered a period of high energy requirement and mobilized their adipose tissue reserve. Cows would enter some degree of NEB during 1st week postpartum.

## Effects of FFS supplementation on ovulation rate, concentration of plasma P4 and follicular development

The reproductive performance of lactating dairy cows was improved by inclusion of fat supplementation in the diet (Staples et al., 1998). However, several studies reported that fat supplementation in diet decreased plasma P4 concentration. Carroll et al. (1990) reported that during the 8th d of the first estrous cycle (30 d postpartum) multiparous Holstein cows fed 5%prilled long chain FA (DM basis) had lower ($p<0.05$) mean plasma P4 level than multiparous Holstein cows fed 0%prilled long chain FA. Similarly, cows consumed an isoenergetic containing control diet (2.7%EE) or dietary fat supplemented with linseed (5.0%EE) or FFS (5.0%EE). Both dietary fats diet had lower ($p<0.001$) plasma P4 than control diet during the 3rd through 8th d after ovulation (Robinson et al., 2002). In contrast to the study during the first estrous cycle no differences in serum P4 concentration in multiparous Brahman cows was found when received either 8.3%rice bran (5.2%EE of diet) or 0%rice bran (3.7%EE of diet). Due to these effects of dietary fat occurred later for the postpartum period or because of rice bran's hypocholesterolemic properties (De Fries et al., 1998). In this present study cows in 18%FFS group had greater ovulation rate than 0 and 24%FFS groups. In addition increased concentrations of plasma P4 have been associated with greater ovulation. Although dietary fat are thought to have cholesterol esters and store precursor for steroidogenesis. However, lesser ovulation rate in association with lesser level of P4 was found in cows received 24% FFS diet. It may be partly due to the lower level of cholesterol supply as a result from the expense of higher milk yield in cows fed 24%FFS diet. Staples et al. (1998) reported that cows produced high milk yield has been linked to lowered fertility in lactating dairy cows. In addition NEB is related to

reduction serum P4 concentrations and lower fertility in dairy cows (Butler, 2003). EB status and BW loss of cows in the present study indicated that cow feeding 24%FFS entered some degree of NEB to response on high milk yield. The predominant FA in cows supplemented with FFS including PUFA would increase the oxidation of FA rather than storage as TG (Squires, 2003). Thus lacked of a precursor for increment of plasma P4 concentration might suspect to occur in cows fed with 24%FFS diet.

Not only fat supplementation improved reproductive performance of lactating dairy cows but also affected the development of ovarian follicle during the postpartum (Staples et al., 1998). In contrast to previous study (Robinson et al., 2002) who reported that cows fed an isoenergetic containing control diet (2.7%EE of diet) or dietary fat supplemented with linseed (5.0%EE of diet) or FFS (5.0%EE of diet) showed similar size of the largest ovulatory follicle. Similarly, the present study found that diets and types of cow did not affect size of the largest ovulatory follicle. In addition to the follicular development in multiparous Brahman cows received either increasing level of fat in diet or no inclusion of fat (0 versus 8.3%rice bran; 3.7 versus 5.2%EE of diets) did not differ ($P>0.05$) during the $35^{th}$ d postpartum (Webb et al., 2001). Similar to the present study diet and cow did not affect ($p>0.05$) number of follicles which included small, medium and large size. It is probably from the present result that cows in all dietary groups entered some degree of NEB. NEB primarily appears to interfere with the ability of the hypothalamo-hypophyseal axis to develop the pulsatile LH pattern for fostering ovarian follicular development and ovulation (Butler and Smith, 1989). Consequently, FFS supplementation in this study did not affect follicular development.

In conclusion, fat in form of oilseed from FFS had some effects on milk yield, milk yield/DMI, 4%FCM, and EB. No effect on DMI, DMI (%BW), milk composition, pH and VFA concentration of ruminal fluid, BCS and BW, plasma glucose and NEFA concentrations. FFS supplementation at 24% level reduced ovulation rate and concentration of plasma P4, but follicular development in crossbred Friesian cows during first 2 months postpartum was not interfered by FFS supplementation diets.

# REFERENCES

Abu-Ghazaleh, A. A., Schingoethe, D. J. and Hippen, A. R. 2002[a]. Feeding fish meal and extruded soybeans enhances the conjugated linoleic acid (CLA) content of milk. J. Dairy Sci. 85: 624–631.

Abu-Ghazaleh, A. A., Schingoethe, D. J., Hippen, A. R., Kalscheur, K. F. and Whitlock, L. A. 2002[b]. Fatty acid profiles of milk and rumen digesta from cows fed fish oil, extruded soybeans or their blend. J. Dairy Sci. 85: 2266-2276.

Accorsi, P. A., Govoni, N., Gaiani, R., Pezzi, C., Seren, E. and Tamanini, C. 2005. Leptin, GH, PRL, insulin and metabolic parameters throughout the dry period and lactation in dairy cows. Repro. Dom. Anim. 40: 217–223.

Agazzi, A., Bayourthe, C., Nicot, M.C., Meynadier, T., Moncoulon, R. and Enjalbert, F. 2004. In situ ruminal biohydrogenation of fatty acids from extruded soybean: effects of dietary adaptation and of mixing with lecithin or wheat straw. Anim. Feed Sci. Technol. 117: 165-175.

AOAC. 1990. Official method of analysis. 15th ed. Washington, DC: Association of Official Analytical Chemists.

Bauchart, D. 1993. Lipid absorption and transport in ruminants. J. Dairy Sci. 76: 3864-3881.

Bauman, D. E., Perfield II, J. W., de Veth, M. J. and Lock, A. L. 2003. New perspectives on lipid digestion and metabolism in ruminants. Proc. Cornell Nutr. Conf. 175-189.

Beam, S. W. and Butler, W. R. 1997. Energy balance and ovarian follicle development prior to the first ovulation postpartum in dairy cows receiving three levels of dietary fat. Biol. Reprod. 56: 133–142.

Beam, S. W. and Butler, W. R. 1999. Effects of energy balance on follicular development and first ovulation in postpartum dairy cows. J. Reprod. Fertil. Suppl. 54 suppl. 1: 411–424.

Boland, M. P., Lonerganand, P. and O'Callaghanz, O. 2001. Effect of nutrition on endocrine parameters, ovarian physiology, and oocyte and embryo development. Theriogenology. 55: 1323-1340.

Butler, W. R. 2000. Nutritional interactions with reproductive performance in dairy cattle. Anim. Reprod. Sci. 60-61: 449-57.

Butler, W. R. 2003. Energy balance relationships with follicular development, ovulation and fertility in postpartum dairy cows. Livest. Prod. Sci. 83: 211–218.

Butler, W. R. and Smith, R. D. 1989. Interrelationships between energy balance and postpartum reproductive function in dairy cattle. J. Dairy Sci. 72: 767–783.

Carroll, D. J. Jerred, M. J. Grummer, R. R. Comb, D. K. Pierson, R. A. and Hauser, E. R. 1990. Effects of fat supplementation and immature alfalfa to concentrate ratio on plasma progesterone, energy balance and reproductive traits of dairy cattle. J. Dairy Sci. 73: 2855-2863.

Chilliard, Y., Ferlay, A., Roul, J. and Lamberet, G. 2003. A review of nutritional and Physiological factors affecting goat milk lipid synthesis and lipolysis. J. Dairy Sci. 86: 1751-1770.

Chouinard, P. Y., Levesque, J. Girard, V. and Brisson, G. J. 1997. Dietary soybeans extruded at different temperatures: Milk composition and in situ fatty acid reactions. J. Dairy Sci. 80: 2913–2924.

Coppock, C. E. and Wilks, D. L. 1991. Supplemental fat high energy rations for lactating cows: effects on intake, digestion, milk yield, and composition. J. Anim. Sci. 69: 3826-3837.

Clark, C. E. F., Fulkersona, W. J., Nandraa, K. S., Barchiab, I. and Macmillanc, K. L. 2005. The use of indicators to assess the degree of mobilization of body reserves in dairy cows in early lactation on a pasture-based diet. Livest. Prod. Sci. 65: 91-105.

De Fries, C. A., Neuendorff, D. A. and Randel, R. d. 1998. Fat supplementation influences postpartum reproductive performance in Brahman cows. J. Anim. Sci. 76: 864-870.

De Vries, M. J. and Veerkamp, R. F. 2000. Energy balance of dairy cattle in relation to milk production variables and fertility. J. Dairy. Sci. 83. 62-69.

Dhiman, T. R., Helmink, E. D., Mcmahon, D. J., Fife, R. L. and Pariza, M. W. 1999. Conjugated linoleic acid content of milk and cheese from cows fed extruded oilseed. J. Dairy Sci. 82: 412-419.

Diskin, M. G., Mackey, D. R., Roche, J. F. and Sreenan J. M. 2003. Effects of nutrition and metabolic status on circulating hormone and ovarian follicle development in cattle. Anim. Reprod Sci. 78: 345-370.

Doreau, M. and Chilliard, Y. 1997. Digestion and metabolism of dietary fat in farm

animals. Br J Nutr. 78 Suppl. 1: S15-S35.

Drackley, J. K. 2007. “Overview of fat digestion and metabolism in dairy cows.” [On line]. Available:http://www.thedairysite.com/articles/793/overview/of*fat-digestion-and-metabolis-in-dairy-cows.

Edmonson, A. J., Lean, I. J., Weaver, L. D., Farve, T. and Webster, G. 1989. A body condition scoring chart for Holstein dairy cows. J. Dairy Sci. 72: 68–78.

Elliott, J. P., Drackley, J. K., Fahey, G. C. and Shanks, R. D. 1995. Utilization of supplemental fat by dairy cows fed diets varying in content of nonstructural carbohydrates. J. Dairy Sci. 78: 1512–1525.

Erwin, E. S. 1961. Volatile fatty acid analysis of blood and rumen fluid by chromatography. J. Dairy Sci. 44: 1768-1771.

Faldet, M. A. and Satter, L. D. 1991. Feeding heat–treated full fat soybeans to cows in early lactation. J. Dairy Sci. 74: 3047-3054.

Faldet, M. A., Son, Y. S. and Satter, L. D. 1992. Chemical, in vitro, and in vivo evaluation of soybeans heat-treated by various processing methods. J. Dairy Sci. 75: 789-795.

Functon, R. N. 2004. Fat supplementation and reproduction in beef females. J. Anim. Sci. 82 suppl. E: E154–E161.

Garnsworthy, P. C. 2002. Fat in dairy cow diets. In: Recent developments in ruminant nutrition 4. Wiseman, J. (ed.) Hampshire: Nottingham University Press. 399-416.

Garnsworthy, P. C. and Webb, R. 2002. The influence of nutrition of fertility in dairy cows. In: Recent developments in ruminant nutrition 4. Wiseman, J. (ed.) Hampshire: Nottingham University Press. 499-516.

Glew, R. H. 1997. Lipid metabolism II: pathway of metabolism of special lipids. In: Text book of biochemistry with clinical correlations. 4th ed. Devlin, T. M. (ed.) New York: A John Wiley & Songs. 395-443.

Grinther, O. J., Kastelic, J. P. and Knof, L. 1989. Composition and characteristics of follicular waves during the bovine estrous cycle. Anim. Reprod. Sci. 20: 3187-3200.

Grummer, R. R. 2007. Strategies to improve fertility of high yielding dairy farms: management of the dry period. Theriogenology. 68 suppl. 1: S281–S288.

Grummer, R. R. and Caroll, D. J. 1991. Effects of dietary fat on metabolic disorders a reproductive performance of dairy cattle. J. Anim. Sci. 69: 3838–3852.

Hawkins, D. E., Niswender, K. D., Oss, G. M., Moeller, C. L., Odde, K. G., Sawyer, H. R. and Niswender, G. D. 1995. An increase in serum lipids increases luteal lipid content and alters the disappearance rate of progesterone in cows. J. Anim. Sci. 73: 541-545.

Hayirli, A. R., Grummer. R. R., Nordheim, E. V. and Crump, P. M. 2002. Animal and dietary factors affecting feed intake during the prefresh transition period in Holsteins. J. Dairy Sci. 85: 3430–43.

Ingvarsten, K. L. and Andersen, J. B. 2000. Integration of metabolism and intake regulation: a review focusing on periparturient animals. J. Dairy Sci. 83: 1573-1597.

Ishler, V. and Varga, G. 2000. “Soybeans and soybean by products for dairy cattle.” [On line]. Available:http://www.das.psu.edu/dairynutrition/document/soybean.pdf.

Jenkins, T. C. 1993. Lipid metabolism in the rumen. J. Dairy Sci. 76: 3851-3863.

Jouaney, J. P. 2006. Optimizing rumen functions in the close-up transition period and early lactation to drive dry matter intake and energy balance in cows. Anim. Reprod. Sci. 96: 250–264.

Kahn, W., Kenney, R. and Volkmann, D. 1994. Veterinary reproduction ultrasonography. Ultrasonography in the cow. Germany: Times mirror international publishers limited. 83-184.

Knapp, D. M., Grummer, R. R. and Dentine M. R. 1991. The response of lactating dairy cows to increasing levels of whole roasted soybean. J. Dairy Sci. 74: 2563-2572.

Komaragiri, M. V. S., Casper, D. P. and Erdman, R. A. 1998. Factor affecting body tissue mobilization in early lactation dairy cows 2. Effect of dietary fat on mobilization of body fat of protein. J. Dairy Sci. 81: 169-175.

Konigsson, K., Savoini, G., Govoni, N., Invernizzi, G., Prandi, A., Kindahl, H. and Veronesi, M. A. 2008. Energy balance, leptin, NEFA and IGF-I plasma concentrations and resumption of post partum ovarian activity in Swedish red and white breed cows. Acta Vet Scand. 50: 1-3.

Lubojacka, V., Pechova, A., Dvorak, R, Drastich, P., Kummer, V. and Poul, J. 2005. Liver steatosis following supplementation with fat in dairy cow diets. Acta. Vet. Bro. 74: 217-224.

Lucy, M. C. 2000. Regulation of ovarian follicular growth by somatotropin and insulin like growth factors in cattle. J. Dairy Sci. 83: 1635–47.

Lucy, M. C. 2001. ADSA Foundation scholar award reproductive loss in high-producing dairy cattle: Where will it end? J. Dairy Sci. 84: 1277–1293.

Lucy, M. C., Savio, J. D., Badinga, L., De La Sota, R. L. and Thatchers, W. W. 1992. Factors that affect ovarian follicular dynamics in cattle. J. Anim. Sci. 70: 3615-3626

Lucy, M. C., Staples, C.R., Michel, F. M. and Thatcher, W. W. 1991. Energy balance and size and number of ovarian follicles detected by ultrasonography in early postpartum dairy cows. J. Dairy Sci. 74: 473–82.

Matthews, H. R. Freedland, R. A. and Miesfeld, R. L. 1997. Glycogen metabolism, pentose-phosphate pathway, and metabolism of other carbohydrates. In: Biochemistry: a short course. New York: A John Wiley & Songs. 163-177.

McGarry, J. D. 1997. Lipid metabolism I: utilization and storage of energy in lipid form. In: Text book of biochemistry with clinical correlations. 4th ed. Devlin, T. M. (ed.) New York: A John Wiley & Songs. 361-393.

Montiel, F. and Ahuja, C. 2005. Body condition and suckling as factors influencing the duration of postpartum anestrus in cattle: a review. Anim. Reprod. Sci. 85: 1–26.

Murphy, M. R. 1992. Nutritional factors affecting animal water and waste quality. J. Dairy Sci. 75: 326-333.

National Research Council. 1989. Nutrition requirements of dairy cattle. 6th ed. Washington DC: National Academy of Sciences. 157.

National Research Council. 2001. Nutrient requirements of dairy cattle. 7th ed. Washington DC: National Academy of Sciences. 363.

Noseir, W. M. 2003. “Ovarian follicular activity and normal profile during estrous cycle in cows: the development of 2 versus 3 waves.” [On line]. Available:http://www.RBEj.com/content/1/1/50.

Opsomer, G., Grohn, Y. T., Hertl, J., Coryn, M., Deluyker, H. and De Kruif, A. 2000. Risk factors for post partum ovarian dysfunction in high producing dairy cows in Belgium: a field study. Theriogenology. 53: 841–857.

Palmquist, D. L. and Jenkins, T. C. 1980. Fat in lactation rations: a review. J. Dairy Sci. 63: 1-14.

Parr, R. A. 1992. Nutrition-progesterone interactions during early pregnancy in sheep. Reprod. Fertil. Dev. 4: 297-300.

Ponter, A. A., Parsy, A. E., Saade, M., Mialot, J.P., Ficheux, C., Ponter, C. D. and

Grimard, B. 2006. Effect of supplement rich in linolenic acid added to the diet of postpartum dairy cows on ovarian follicle growth and milk and plasma fatty acid composition. Reprod. Nutr. Dev. 46: 19-29.

Reist, M., Erdin, D., Euw, D., Tschuemperlin, K., Leuenberger, H., Delavaud, C., Chilliard, Y., Hammon, H. M., Morel, C., Kuenzi, N. and Blum, J. W. 2003. Concentrate feeding strategy in lactating dairy cows: metabolic and endocrine changes with emphasis on leptin. J. Dairy Sci. 86: 1690–1706.

Robinson, R. S., Pushpakumara, P. G. A., Cheng, Z. Peters, A. R., Abayasekara, D. R. E. and Wathes, D. C. 2002. Effects of dietary polyunsaturated fatty acids on ovarian and uterine function in lactating dairy cows. Reprod. 124: 119-131.

Roche, J. F. 2006. The effect of nutritional management of the dairy cow on reproductive efficiency. Anim. Reprod. Sci. 96: 282–296.

Ruegsegger, G. J. and Schultz, L. H. 1985. Response of high producing dairy cows in early lactation to the feeding of heat treated whole soybeans. J. Dairy Sci. 68: 3272–3279.

Rukkwamsuk, T., Rungruang, S., Choothesa, A. and Wensing T. 2008. Performance of periparturient dairy cows fed alfalfa hay in total mixed ration: a field trial in Thailand. Kasetsart J. (Nat. Sci.). 42: 11 – 18.

Rukkwamsuk, T., Wensing, T. and Geelen, M. J. H. 2001. Changes in fat component in milk of dairy cows during the postparturient period. J. Kasetsart Vet. 11: 1-9.

Sakaguchi, M., Sasamoto, Y., Suzuki, T., Takahashi, Y. and Yamada, Y. 2004. Postpartum ovarian follicular dynamics and estrous activity in lactating dairy cows. J. Dairy Sci. 87: 2114 – 2121.

Schei, I., Volden, H. and Bævre, L. 2005. Effects of energy balance and metabolizable protein level on tissue mobilization and milk performance of dairy cows in early lactation. Lives. Prod. Sci. 95: 35 – 47.

Schauff, D. J., Elliott, J. P., Clark, J. H. and Drackley, J. K. 1992. Effects of feeding lactating dairy cows diets containing whole soybeans and tallow. J. Dairy Sci. 75: 1923 – 1935.

Schmidely, P., Morand-Fehr, P. and Sauvant, D. 2005. Influence of extruded soybeans with or without Bicarbonate on milk performance and fatty acid composition of goat milk. J. Dairy. Sci. 88: 757-765.

Shrestha, H. K., Nakao, T., Higaki, T., Suzuki, T. and Akita, M. 2004. Resumption of

postpartum ovarian cyclicity in high producing Holstein cows. Theriogenology. 61: 637–49.

Shrestha, H. K., Nakao, T., Suzuki, T., Akita, M. and Higaki, T. 2005. Relationships between body condition score, body weight, and some nutritional parameters in plasma and resumption of ovarian cyclicity postpartum during pre-service period in high-producing dairy cows in a subtropical region in Japan. Theriogenology. 64: 855–866.

Sklan, D., Kaim, M., Moallem, U. and Folman, Y. 1994. Effect of dietary calcium soaps on milk yield, body weight, reproductive hormones and fertility in first parity and older cows. J. Dairy. Sci. 77: 1652-1660.

Spicer, L. J., Tucker, W. B. and Adams, G. D. 1990. Insulin-like growth factor-1 in dairy cows; relationships among energy balance, body condition, ovarian activity, and estrus behavior. J Dairy Sci. 73: 929–37.

Spicer, L. J., Francisco, C. C., Jones, D. and Chambarian, C. S. 2004. “Changes in ovarian follicular growth during early lactation in Holstein cows.” [On line]. Available:http://www.ansi.okstate.edu/research/2004rr/15/15.htm.

Spicer, L. J., Vernon, R. K., Tucker, W. B. Wettemann, R. P., Hogue, J. F. and Adams, G. D. 1993. Effects of inert fat on energy balance, plasma concentrations of hormones, and reproduction in dairy cows. J. Dairy. Sci. 76: 2664-2673.

Squires, E. J. 2003. Endocrine manipulation of reproduction. In: Applied Animal Endocrinology. Cambridge: CABI Publishing. 154-191.

Staples, C.R., Burke, J. M. and Thatcher, W. W. 1998. Influence of supplemental fats on reproductive tissues and performance of lactating cows. J. Dairy Sci. 81: 856-871.

Stich, V. and Berlan, M. 2004. Physiological regulation of NEFA availability: lipolysis pathway. Proc. Nutr. Soc. 63: 369-374.

Thomas, M. G., Bao, B. and Williams, G. L. 1997. Dietary fats varying in their fatty acid composition differentially influence follicular growth in cows fed isoenergetic diets. J. Anim. Sci. 75: 2512-2519.

Van Knegsel, A. T. M., Brand, H. V. D., Dijkstra, J., Tamminga, S. and Kemp, B. 2005. Effect of dietary energy source on energy balance, production, metabolic disorders and reproduction in lactating dairy cattle. Reprod. Nutr. Dev. 45: 665–688.

Van Soet, P. J., Roberton, J. B. and Lewis, B. A. 1991. Methods for dietary fiber,

neutral detergent fiber, and nonstrach polysaccharides in relation to animal nutrition. J. Dairy Sci. 75: 3583-3597.

Vazquez-anon, M., Bertics, S., Luck, M., Grummer R. R. and Pinheiro, J. 1994. Peripartum liver triglyceride and plasma metabolisms in dairy cows. J. Dairy Sci. 77: 1521-1528.

Waldner, D. N. and Looper, M. L. 2002. “Water for dairy cattle.” [On line]. Available:http://www.osuextra.com.

Waltner, S. S., McNamara, J. P. and Hillers, J. K., 1993. Relationships of body condition score to production variables in high producing Holstein dairy cattle. J. Dairy Sci. 76: 3410–3419.

Wang, A. S., Jan, D. F., Chen, K. J., Yang, D. W. and Fan, Y. K. 2004. Dietary supplementation of fat increased milk percentage without affecting ruminal characteristics in Holstein cows in a warm tropical environment. Asian–Aust. J. Anim. Sci. 17: 213–220.

Webb, S. M., Lewis, A. W., Neuendorff, D. A. and Randel, R. D. 2001. Effects of rice bran, lasalosid, and sex of calf on postpartum reproduction in Brahman cows. J. Anim. Sci. 79: 2968-2974.

Wildman, E. E., Jones, G. M., Wagner, P. E. Bowman, R. L., Troutt, H. F. and Lesch, T. N. 1982. A dairy cow body condition scoring system and its relationship to selected production characteristics. J. Dairy Sci. 65: 495-501.

Wiltbank, M., Lopez, H., Sartori, R., Sangsritavong, S. and Gumen, A. 2006. Changes in reproductive physiology of lactating dairy cows due to elevated steroid metabolism. Theriogenology. 65: 17–29.

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