

EFFECTS OF MANAGEMENT PROGRAM IN BEEF CATTLE ON CONCEPTION  
RATE AND GROWTH PERFORMANCE OF CALVES



CHITANON SUEAPHENG

A Thesis Submitted to University of Phayao  
in Partial Fulfillment of Requirements  
for the Master of Science in Animal Science

June 2021

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Thesis

Title

Effects of Management Program in Beef Cattle on Conception Rate and Growth  
Performance of Calves

Submitted by Chitanon Sueapheng

Approved in partial fulfillment of the requirements for the  
Master of Science Degree in Animal Science  
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**Title:** EFFECTS OF MANAGEMENT PROGRAM IN BEEF CATTLE ON CONCEPTION RATE AND GROWTH PERFORMANCE OF CALVES

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**Keywords:** Concentrate feed Creep feeding Khao Lamphun Cattle hematology Growth performance Body proportions

### ABSTRACT

The objective of this research was to study the effects of supplementation of concentrate on reproductive systems in postpartum cows and calf. In this study, experiment 1, 72 White Lamphun cows were randomly assigned into 2 groups, 36 each. 1) cows without concentrate diet, 2) cows were supplemented with concentrate diet. The body score of the cows were increased in the group that supplemented with concentrate feed ( $3.39 \pm 0.21$ ) compared to control group ( $3.04 \pm 0.14$ ,  $P < 0.001$ ). While 30 and 60 days postpartum, the cows supplemented with concentrate feed had the rate return to estrus 63.89% (23/36) and 97.22% (35/36), respectively ( $P = 0.193$ ) tended to higher than without supplementation of concentrate feed (50%, 18/36 and 91.67% ,33/36), respectively ( $P = 0.686$ ). Accordingly, the mean level of progesterone hormone was significantly higher than without supplementation of concentrate feed ( $15.66 \pm 7.17$  vs  $10.37 \pm 3.55$ ) ( $P < 0.001$ ). In conclusion, the cows that supplemented with concentrate feed had higher body score, pregnancy rate, hormone levels in the reproductive system than control group.

Experiment 2 aimed to determine the effect of creep feeding on growth performance, haematological and serum biochemical profile of khao Lamphun calf. Number 72 khao Lamphun calf. Throughout 90–day data of collect data between no supplementation of concentrate ( $n=36$ ) and supplementation creep feeding ( $n=36$ ) on birth weight, and weaning weight was collected. The calves' birth weights supplementation creep feeding ( $18.06 \pm 0.53$ ) no supplementation of concentrate ( $17.89 \pm 0.57$ ) did not Statistically different. Final liveweight of supplementation creep feeding was  $101.92 \pm 13.00$  kg was greater than no supplementation of concentrate was  $89.17 \pm 10.43$  kg ( $P < 0.0001$ ). and Average Daily Gain (ADG) of supplementation creep feeding was  $0.42 \pm 0.06$  g/d was greater than no supplementation of concentrate was  $0.36 \pm 0.05$  g/d ( $P < 0.0001$ ). Body proportions at birth weight of no supplementation of concentrate heart girth (GIR), hip height (HH) and Body length (BL) were  $61.08 \pm 2.17$ ,  $63.14 \pm 3.30$  and  $55.47 \pm 1.76$  respectively and supplementation creep feeding was  $60.89 \pm 1.70$ ,  $62.69 \pm 2.55$  and  $56.17 \pm 1.99$  cm respectively did not Statistically different. Weaning weight of supplementation creep feeding was GIR ( $110.33 \pm 6.82$  cm), HH ( $101.89 \pm 4.90$  cm) and BL ( $93.00 \pm 6.90$  cm) was greater than no supplementation of concentrate was  $104.64 \pm 7.58$ ,  $98.86 \pm 5.90$  and  $89.64 \pm 5.76$  cm respectively Statistically different. as well as haematological and serum biochemical values. Results showed that creep feeding significantly influenced overall body weight gain and some blood and serum biochemical variables. Generally, blood and serum biochemical values obtained were within normal ranges showing that there were no

adverse effects of creep feeding or source of protein on the animals. It was therefore, concluded that the practice of creep feeding of calves should be adopted to enhance their growth rate in the study environment.



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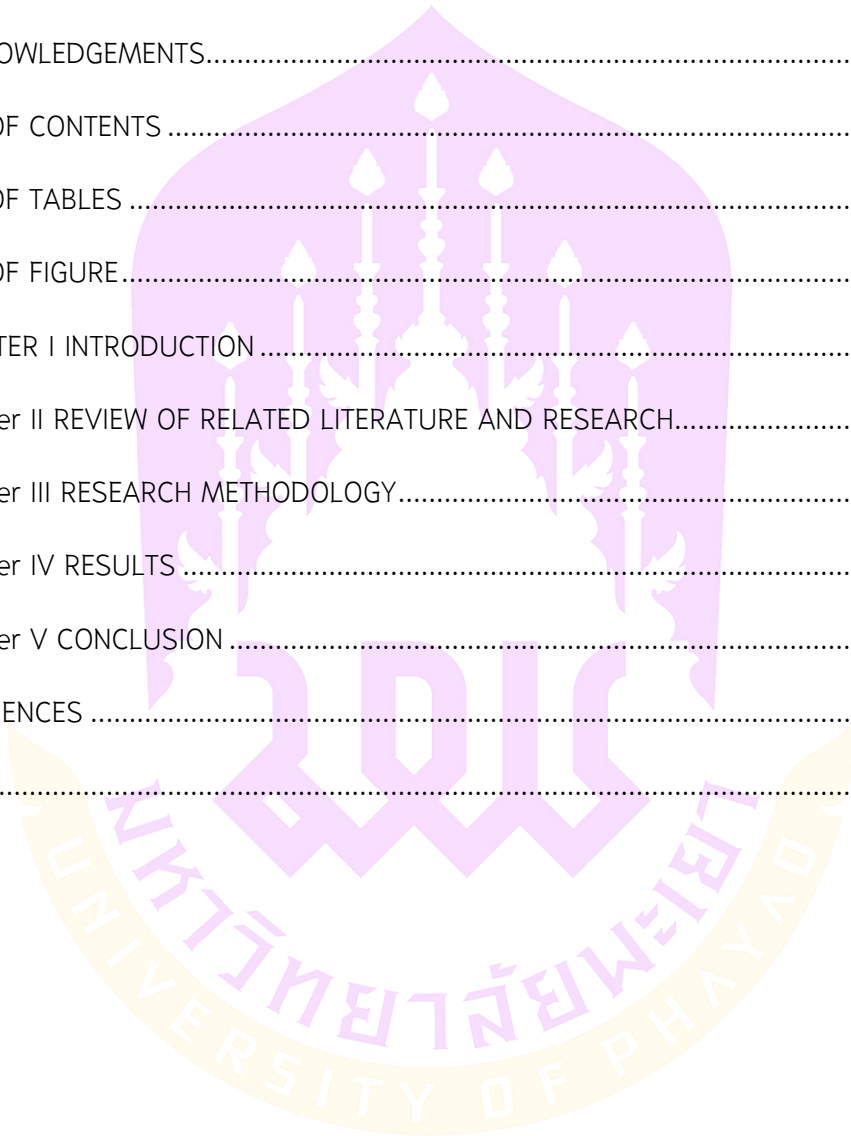
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## CHAPTER I

### INTRODUCTION

In Thailand, most of the beef cattle are raised in the northeast, about 2,655,382 heads in the north, 925,951 heads, 1,055,365 heads in the central Region and 808,653 heads in the south, most commonly in the northeast and central regions (Development DOL, 2015). The demand for high quality beef cattle has increased in Thailand, but the population beef cattle is not sufficient to cover demands for domestic consumption (DLD, 2004; Waritthitham et al. 2010). Therefore, increase number of cattle production systems is expected to be necessary in Thailand. Globally, developments in animal breeding, nutrition and health will continue to increase animal productivity.

The first group is Thai native cattle which make up 61% of the population, although they have been decreasing in number by 0.7% annually. Native cattle in Thailand have characteristics of *Bos indicus* (Zebu), including the distinctive dorsal hump. There are four native breeds officially recognized by the Department of Livestock Development: Kho–Khao Lamphun (Northern Thailand), Kho–Isaan (Northeastern Thailand), Kho–Lan (Central Thailand), and Kho–Chon (Southern Thailand). These breeds may have originated from different regions of Thailand (Wangkumhang et al., 2015). Nevertheless, changes in the agricultural system and increased demand of meat consumption have subsequently resulted in decreased number of the Khao Lamphun cattle (Dumrongsri et al. 2014). Because of the population decreases in the number of White Lamphun cattle, the possibility of conserving typical breed in tropical country for future needs was considered. This breed is often well adapted to tropical environments and climates, it also able to use poor quality feeds and to cope parasitic diseases (Charoensook et al. 2013). However, it slow growth rate and small size. It has been reported that birth weight was 18 kg, weaning weight at the age of 200 days, average 122 kg, fully grown weight, male 350–450 kg, female 300–350 kg (Department of Livestock Development, 2018).

Therefore, in order to further improve cattle performance, supplementation as creep feeding in postpartum cow and calve is special interest. Shike et al. (2007), Parish and Rhinehart (2009) and Vinales et al. (2013). Shike et al. (2007) reported 21% higher

body weight gain (BWG) in creep fed calves compared to non-creep fed animals. In order to attain the genetic potential for growth, young animals must be fed rations that are adequate and balanced in addition to the dam's milk. For calves, preweaning performances are very important determinants of overall performance at weaning and post weaning (Myer et al. 1999; Arthington et al. 2008). Pre-weaning performance enables early assessment of the economic value of the animal and the dam or sire (or both), and the assignment of roles subsequently. Calf performance may be considered a trait of the cow in successive lactations (Vinoles et al. 2013) and prediction of the future most probable producing ability (MPPA) of a cow with respect to preweaning growth traits of her calves is of immense advantage. Cow productivity is a trait of the cow which depends on the weaning rate and weaning weight of a cow's calves over a number of lactations (Vinoles et al. 2013).

Since, Lower BCS at calving resulted in a lesser proportion of first-calving cows returning to oestrus early in the breeding period, increasing ICI (Dziuk & Bellows 1983). Supplementation in cattle after birth with energy feed ingredients and protein affects the condition of the body score and improve the reproductive system to have the opportunity to return faster to the next pregnancy. Research to improve the feed efficiency of cows. Shakil et al. (2019) studied on of supplementation in heifer weighing  $150 \pm 5$  kg was carried out, within 15 days encounter heifer cow gained 5.27 kg which higher than controls with only 4.53 kg gain. Ciccioni et al. (2003) found out that with 11% protein concentrate supplementation a Hected high fertility postpartum cows and from body weigh 406 kg increased in the final weight to 440 kg body weight Therefore, when the postpartum cows have a higher performance, they can increase the production efficiency of the calves.

However, the importance of haematological indices in animal husbandry is well acknowledged. Metabolic disturbances usually by inappropriate feeding without overt clinical manifestation could lower performance and productivity (Radostits et al. 2000; Mamun et al. 2013). Such altered metabolic status could be reflected in altered haematological indices. The significantly different haematological indices between treatments in the present study could relate to the different feeding materials and feeding regimes. Mamun et al. (2013) had stated that factors such as nutrition and stress affect blood values of animals.

The objectives of the study are that supplying limited-creep feeding supplementation to Khao Lamphun calves could improve growth performance, blood hematological in Khao Lamphun calves and affect reproductive systems, progesterone hormone and estradiol hormone in the Khao Lamphun postpartum cows.

### **Objectives**

1. To determine the effect of concentrate feed supplementation on body condition score the reproductive systems, progesterone hormone and estradiol hormone in the Khao Lamphun cows.
2. To determine the effect of creep feeding supplementation on growth performance and hematology in Khao Lamphun calves.

### **Hypotheses**

1. Concentrate feed supplementation could affect reproductive systems, progesterone hormone and estradiol hormone in the Khao Lamphun postpartum cows.
2. Concentrate feed supplementation could improve growth performance and blood hematological in Khao Lamphun calves.

### **Scope of the Research**

1. This research focuses on the effected of concentrate supplementation on the reproductive of postpartum cows
2. This research focuses on the effects of creep feeding growth performance and hematology in Khao Lamphun calves.

### **Expected Benefits**

1. Concentrate supplementation resulted in the postpartum cows on the reproductive systems, progesterone hormone and estradiol hormone in the Khao Lamphun cows.
2. Concentrate supplementation resulted in creep feeding higher growth performance and hematology in Khao Lamphun calves.

**Definition**

Concentrate feed, Creep feeding, Khao Lamphun Cattle, hematology, Growth performance, Body proportions



## Chapter II

### REVIEW OF RELATED LITERATURE AND RESEARCH

#### 1. Beef production system

In a transitional country such as Thailand, the livestock sector is undergoing highly dynamic changes. This has resulted from rapidly increasing demand for meat and meat products. The changes in the demand for livestock products have been driven by income growth, urbanization and educational background. Affect the Increase of slaughtering cattle. But forecasting indicated the decrease of slaughtering cattle from 1,252,000 cattle in 2014 to be 626,408 cattle in 2018, and it would be similar if the number of cows in the cow-calf production is kept at least 80% every year. The Office of Agricultural Economics annually conducts surveys of beef production system in 2015 through 2019, Thai beef production increased at a rate of 7.15 percent per year. In 2019, there were 1.178 million cattle productions. This was up from 1.126 million units in 2018. 4.62 percent. Since the government encourages farmers to feed beef cattle as a primary occupation, and the natural rise of cattle breeders, there is also good breeding of beef cattle. This makes the annual rate better. In 2015– 2019, demand for Thai cattle consumption increased slightly at a rate of only 0.08% per annum, with 1.262 million cattle consumption expected, or 212.02 thousand tons of cattle, a slight increase from 0.08% in 2018. as a result of increased demand for the domestic market.



**Table 1** Production, Exports, Imports and consumption beef and products of Thailand in 2015 – 2020

item	2558	2559	2560	2561	2562	Rate increase (Percentage)
Production <sup>1/</sup> (million)	0.914	0.938	0.980	1.126	1.178	7.15
(1000 tonnes of carcass weight)	153.48	157.58	161.73	189.17	197.90	7.15
Export <sup>2/</sup> (1000 tonnes)	2.462	0.172	0.106	0.096	0.075	-53.07
Import <sup>2/</sup> (1000 tonnes)	10.314	10.477	12.18	13.07	13.68	8.18
Consumer <sup>1/</sup> (Million heads)	1.258	1.259	1.260	1.261	1.262	0.08
(1000 tonnes of carcass weight)	211.344	211.512	211.680	211.848	212.02	0.08

## 2.Method of raising a cow to produce a calf

Cow-calf production is the first stage of the beef production process. An average of about 2.2 years elapses between the breeding of a beef cow or heifer to the time their offspring are ready for slaughter. Heifer calves may be retained for herd expansion or replacements, sold to other producers as replacements, or sold along with the steers to feedlot operators to grow out for slaughter. In raising cattle to produce calf. The resulting cattle must be derived from a good breed of cattle, or artificial insemination and breeding, so that male cattle can be sold at the cattle market and can be used as a good quality cow. Similarly, female cattle are raised to be calf crops. However, in the care of the cows, the cattle roughages and concentrates are important to livelihoods. In Thailand, grass and by-products are used from the waste of farming to be used for greater food value (Livestock Department, 2009). The raising of beef cattle in Thailand was started by selection and by the crossbred steers or bulls from the farms or cattle markets. They were raised in barns and received ad libitum good quality forage, supplemented by concentrate feed until reaching their target weight.

The beef cattle which comprises of Native and Native Crossbred cattle are capable of utilizing agriculture by-products or low quality feed to convert to meat. They can graze on uncultivated land and consume all crop residues.

### **3. Khao Lamphun cattle**

Thai native cattle are classified into four breeds (Kow-Lamphun, Kho-Esarn, Kho-Lan and Kho-Chon cattle) showed in different region of Thailand. They are small body size, heat tolerant, insect and disease resistant, good grazers and great reproductive performance, however, the native cattle breed raised in the north-eastern part of the Thailand (Kho-Esarn), also used for beef production, through a crossbreeding system, which cross with exotic breeds as two-way crossbred (Thai native x Brahman, Angus or Holstein Friesian), and three-way crossbred or synthetic line for improve performance and produced the high-quality meat. However, Thai consumers are willing to pay the fattening beef or crossbred beef meat rather than native beef cattle, even though the price of their meat is higher. Hence, the evaluations of genetic identification of cattle breeds are raise in Thailand that purpose for produced high quality meat is necessary. For protect consumers from fraudulent quality claims. There is becoming more challenging, which can be used for breeding strategies and further traceability system for beef cattle breeds in Thailand.

Sacred oxen in Hindi refer to angels who serve as a vehicle for Shiva, one of the supreme Hindu Gods, representing labor and strength. In addition, they are the pet under the care of the other gods, Krishna and Phra Phon Thep, representing abundance and fertility. As a result, the oxen in the Royal Ploughing Ceremony are very significant since they are the symbol of strength and abundance and they also the indication of prediction for abundance of the national water and rice crops at the beginning of the planting season. (Nature and environment, 2014)

### **4. General information of Khao Lamphun cattle**

Thai native cattle (*Bos indicus*) are classified by the information of ecotypes as follows: The Northern ecotype (Khao Lamphun), the North-eastern ecotype, the Central ecotype, and the Southern ecotype (Charoensook et al. 2013). External characteristics of the

Khao Lamphun cattle include pink-skinned, white hair, white and long horns as well as back legs and hooves (Jaisin et al. 2014). The major group of Khao Lamphun cattle is raised in the upper north of Thailand, particularly Chiang Mai, Lamphun, and Lampang provinces. The Khao Lamphun cattle are tolerant to a poor quality of feedstuffs. They are well adapted to internal and external parasites and are also resistant to diseases such as Anaplasmosis (Charoensook et al. 2013). In 2013, the number of Khao Lamphun cattle was estimated to be approximately 5,000 head, with most being raised in backyard farms of small farmers. Importantly, the Khao Lamphun cattle are classified as an endangered-maintained breed because the total number of breeding females is less than 1,000 head (Charoensook et al. 2013). Khao Lamphun cattle is native cattle in Thailand. In 1993, he said, the characteristics of the Khao Lamphun cattle in various ways were as follows:

1. The head is small, the eye bone is not very blurry. The head bone between him lifts up in the middle and has tresses up but not pronounced. He's small and medium. The flesh is amber or orange, small ears, pink leather, delicate hairs on the back of the ears, and in the ear cavity there is little hair.
2. Medium eyes, not very big. Black eyes are not well, pinkish-yellow eyes, long white eyelashes, long pink-yellow eyes.
3. Short nose, pink or ampan.
4. Short chin When the mouth is tight, the chin is firmly attached to the upper lip.
5. Short neck, slack neck, moderate sex, female may be found.
6. The back is smooth, the buttocks are slightly raised, the scapula is noticeably elevated.
7. The tail is medium-sized, slender from the beginning to the tip, with white-yellow tail hairs, medium-tail tassels. Long, wavy hairs are almost to the ground. Good insect repellent.
8. The abdomen is not very wide or deep, suitable for the body, pink leather, smooth, short and short.
9. Mature male weight 350-450 kg, female 300-350 kg



*Figure 1 Khao Lamphun bull: select sacred oxen for the royal ploughing ceremony*

### **5. Growth performance of The Khao Lamphun**

Results from the production of growth capacity of the Lamphun according to the report, The experiment was conducted with a protein concentrate of 14–15 percent at a rate of 1.5 percent of the body weight and the full amount of fresh grass was found to be between 600–700 grams/day. This was in line with the 1991 trial of the Khao Lamphun, with concentrate 15.6 percent crude protein at 1.0 and 1.5 percent to . body weight the cattle were given ruzi grass and rice straw asroughage. It was found that the average growth rate were 543.90 and 699.36 g/day. But in cattle that grazing in hamil guinea–Centro pasture plots with 2,3 and 4–heads/ha the, a growth rate was only 267 gram/day, The weight of weaning at 200 days was 137.12 kg, the weight at 400 kilograms was 164.70 kg and 600 kilograms a day was 198.43 kg. (Phayao Livestock Research and Breeding Center, Phayao, 1999) as shown in Table 2.

*Table 2 Average of some characteristics of Khao Lamphun cattle in Phayao Livestock Research and Breeding Center, Phayao during the year 1999–2003*

Economic characteristics	Average
Age at estrus (month)	17.90
First weight estrus (kg.)	204.62
Calving interval (day)	458.93
Calving rate per year (%)	79.00
Dead rate pre-weaning (%)	1.27
Birth weight (kg.)	21.11
Weaning weight at 200 day (kg.)	137.12
Body weight at 400 day (kg.)	164.70
Body weight at 600 day (kg.)	198.43
Hip height at birth (cm.)	70.68
Body length at birth (cm.)	55.47
Heart girth at birth (cm.)	63.00
Body weight of breeder (kg.)	429.40
Body weight of cow(kg.)	273.35

**Source:** Phayao Livestock Research and Breeding Center, Phayao (1999)

## **6.Problems of upstream cattle farming**

Nowadays, people in Thailand cattle consumption is increasing, but there is a lack of networking between the production and marketing of cattle, since the upstream cattle are connected to cattle in the middle of the water, the cattle and cattle farmers, and downstream is a cattle cooperative with a cattle processing plant that can be consumed into cattle. This group is a potential addition to the quality cattle market that can help create value-added and develop the market of beef cattle. Therefore, the management of the basic cattle system in Thailand needs to begin to develop from the care of cattle breeders from the breeding integrity of the body and the reproductive system. Care of the cows must be taking into account the period before pregnancy until the period of birth.

## 7. Management of cows

Raising a breeder to be healthy is the main factor and is important for the mating, which is consistently given to calf every year. And it should be managed to meet the quality of the feed that the body needs at various stages. (Department of Livestock Development, 2015) It has managed to produce good cattle. as follows:

Good health for breeding or high mixing rates, including regular lying to the cubs every year. It is associated with diet that is used to feed the cattle in each range with different needs. The management of the parenting of the cattle can be classified from a different phase according to the dietary requirements of the cattle for 3 phases. as follows:

Phase 1: from the cows at tomother breeder giave birth to a calf a ged child 3 to 4 months (120 days). After birth, the mating period until pregnancy. It is necessary to obtain adequate diets to meet the needs of the body from calving. The 2004 male peak reported that the stage of calving to mating, if the cows had an increased in weight, would be able to increase the rate of sticking to the mother and reduce the distance to the baby.

Phase 2: The stage where the cows are four to six months pregnant is the stage when the cattle are born near the weaning period. The pregnant cow in this period needs diet to nourish the body and has less need for diet than at any other stage.

Phase 3, the pregnant cow, 3 months before giving birth.

## 8.Important factors of breeder management

Breeders, whether it's beef cattle, care must be managed from good reproduction to higher rates of mixing or easy-to-stick. This is to give the birth weight to weaning good performance in the born child. Good yield is the goal of farmers who want to breed cattle that meet their desired characteristics, together with the cattle breeders who are completely in line with the reporting of Peerasak (2007) good reproductive performance will result in a good combination of cows. There are also factors of age and season that directly affect the breeder and the cattle.

Peerasak (2007) reported that, the breeding of the cows, at secondcalf are more likely to be more complete than first calving or older cattle. The mating timethe coldest part of the year. The age of the seasonal calf also affects the birth weight and weaning weight of the calf. as follows:

Charan (1969) stated that the age of the calf of cattle with different influences on the weight of the cattle ( $P < 0.01$ ), the cattle born will have a weaning weight when the cattle age from 2 to 5 years will be up in the 5–7 years, with the mother raising the baby to a stable weaning weight, and then the breast will yield less after the 7th year.

Sombat (1987) reported that the growth rate of fresh grass-fed cattle has significantly higher growth rates than cattle that fed rice straw as roughages. ( $P < 0.01$ ) Similarly, "The growth of the Brahman crossbred × Charolais of Kasetsart University, it was found that the newborn weight, weaning weight, and growth rate before weaning each year differ due to climate change. The climate influences the quantity and quality of the feed crops received by cattle, thus affecting the birth weight, the weaning weight and growth rate before weaning of cattle each year.

### **9. Reproduction of cattle Khao Lamphun**

The production of beef for sale to consumers is the basis of the success of cattle production. Therefore, farmers need knowledge and anatomical expertise of male and female cattle, which consists of hormones and hormonal functions, is essential for the management of various aspects of the reproductive system, such as estrus and estrus cycle.

The estrous cycle of Thai native cattle that resided in northeast Thailand is ranged 18 to 21 days (Chasombat et al. 2014). According to dairy ultrasonographic studies the number of follicular waves ranges between one and four waves per cycle, but the predominant pattern for Thai native cattle is of four waves (47.6 and 82.8% for heifers and cows, respectively; Sakhong et al. 2011). On the other hand, Chasombat et al. (2014) informs that follicular dynamics in Thai native heifers are classified by two (70%) or three (30%) follicular waves.

### **10 Estrus and endocrinology of the estrous cycle of cattle**

Reproductive performance of the cow and heifer is one of the most important factors that influence ranch profitability. Understanding the biological mechanisms associated with getting a cow or heifer bred can be a significant management tool for increasing realized income.

Estrous cycles give females repeated opportunities to become pregnant throughout their productive lifetime. The cycle is regulated by the hypothalamic–pituitary–gonadal axis, which produces hormones that dictate reproductive events. The reproductive axis is composed of the hypothalamus, pituitary, and the ovary (Figure 2)

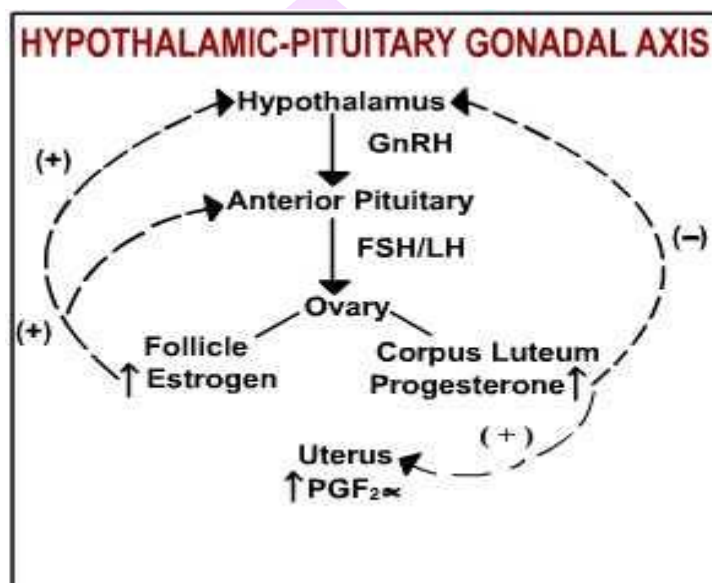


Figure 2 The reproductive axis is composed of the hypothalamus, pituitary, and the ovary

Source: <https://beef.unl.edu/learning/estrous.shtml>

The hypothalamus is a specialized portion of the ventral brain. Its primary function is to produce gonadotropin–releasing hormone (GnRH) in response to circulating estrogen, or to cease GnRH production in response to progesterone. The pituitary is composed of the anterior and posterior lobes. In this module, we will discuss the function of the anterior lobe as it relates to reproduction in beef females. The anterior pituitary is located directly beneath the hypothalamus in a small depression of the sphenoid bone. It produces the gonadotropins follicle–stimulating hormone (FSH) and luteinizing hormone (LH) in response to GnRH and estrogen. FSH and LH production is inhibited by progesterone. The third portion of the reproductive axis consists of the ovaries, located in the pelvic cavity of the cow. Follicles are structures on the ovarian surface that contain ova (egg) and produce estrogen. Follicles range in size and maturity at different stages of the cycle, but usually only one is selected to ovulate. A corpus luteum (CL) is a structure that forms from the previous cycle's ovulation



point. The CL is responsible for progesterone production. Both estrogen and progesterone are produced following FSH and LH stimulation of the ovary. The uterus is also found in the pelvic cavity. It likewise contributes to reproductive control, as it produces prostaglandin F<sub>2</sub>α (PGF<sub>2</sub>α).

The sequence of hormonal release essentially begins with the synthesis and release of GnRH from the hypothalamus. This polypeptide hormone is transported to the anterior pituitary through a highly specialized capillary network called the hypothalamo–hypophyseal portal system. GnRH functions to stimulate the anterior pituitary to produce and release FSH and LH. FSH and LH are transported through systemic blood circulation to the ovaries, where they initiate a series of morphological changes that lead to ovulation and pregnancy if fertilization occurs.

The primary hormones produced by the ovary are estrogen and progesterone. These hormones are transported by the blood stream to "target" tissues to cause a reaction. Estrogen is produced by the follicle, which is located on the ovary. As the follicle grows, more estrogen is produced. As increasing amounts of estrogen are released into the blood stream and travel to the anterior pituitary, it acts in a positive feedback fashion, stimulating pulsatile LH release. It also affects the nervous system of the cow, causing restlessness, phonation, mounting, and most importantly, the willingness to be mounted by other animals. Estrogen causes the uterus to contract, allowing sperm to be transported through the female reproductive tract more efficiently after insemination. Other effects of high estrogen concentrations in the blood include increased blood flow to the genital organs and the production of mucus by glands in the cervix and vagina. These characteristics are all signs of estrus, or sexual receptivity.

Progesterone produced by the CL prevents cyclicity by acting on the anterior pituitary in a negative feedback fashion; therefore, decreasing the release of FSH and LH. It prepares the uterus for reception of fertilized ova and subsequent pregnancy. It also helps the cow maintain pregnancy by suppressing uterine contractions and promoting development of the uterine lining.

A fifth hormone important in female reproduction is prostaglandin F<sub>2</sub>α(PGF<sub>2</sub>α). This hormone is secreted by the endometrium of the uterus and also affects structures on the

ovary, helping to initiate ovulation by causing the demise of the CL, which results in withdrawal of progesterone's negative feedback mechanism. High circulating concentrations of progesterone stimulate the production and release of PGF<sub>2a</sub> from the uterus.

### **11. Physiology of the estrous cycle**

The estrous cycle of the cow is generally about 21 days long, but it can range from 17 to 24 days in duration. Each cycle consists of a long luteal phase (days 1–17) where the cycle is under the influence of progesterone and a shorter follicular phase (days 18–21) where the cycle is under the influence of estrogen. The cycle begins with standing heat, or estrus. This time of peak estrogen secretion can last from 6 to 24 hours, with ovulation occurring 24 to 32 hours after the beginning of estrus.

Ovulation marks the beginning of the luteal phase, and is the culmination of a process called oogenesis, in which germ cells mature under the proper conditions. Germ cells are contained in thousands of tiny structures called follicles that contain receptors for FSH, which in turn stimulates the growth and maturation of responsive follicles. Most follicles develop in patterns referred to as follicular waves. The first one or two waves consist of a group of follicles being recruited and grown to 3–5 mm in diameter. These follicles then grow to 6–9 mm in diameter in response to FSH, and one of the follicles may even reach 12–15 mm. However, these follicles generally don't become ovulatory due to the inhibitory action of progesterone on the anterior pituitary's production of FSH. Instead, these first waves of follicles undergo atresia (regression or death) after being broken down and invaded by connective tissue. It isn't until the second or third follicular wave that one follicle is selected to become the ovulatory, or Graafian, follicle. This follicle matures to a diameter of 16–18 mm and consists of an ovum or egg surrounded by many layers of cells, around which forms a central cavity filled with fluid and encompassed by several thin cell layers. illustrates the morphological changes that occur on the ovary throughout the cow and heifer's estrous cycle. Once ovulation has occurred and the egg is released, the cells on the ovary that made up the ovulatory follicle differentiate to form luteal cells. This occurs under the influence of LH, and results in a structure called a corpus luteum (CL). The CL is responsible for progesterone production. Peak of progesterone production occurs about day 12 when the CL

is fully mature. As previously noted, progesterone inhibits LH and FSH release by the anterior pituitary and prevents ovulation by inhibiting follicular development and maturation.

After about 14 days of progesterone influence, the uterus begins to release pulses of prostaglandin (PGF<sub>2</sub>a) into its venous drainage to the ovaries. Prostaglandin lyses the luteal tissue of the CL and causes its regression, resulting in a rapid decline of circulating progesterone and removing progesterone's negative feedback on the anterior pituitary. By about day 17, the luteal phase of the estrous cycle comes to an end.

The follicular phase begins with the removal of the blocking action of progesterone, which allows for greater amplitude and frequency of GnRH pulses. Greater GnRH results in more FSH and LH production, which in turn supports follicular development of the ovulatory follicle in the follicular wave. This Graafian follicle produces increasing amounts of estrogen, which causes positive feedback to the anterior pituitary. Once estrogen levels reach a threshold level, a surge of LH (at least 10 times greater than tonic levels) results in ovulation.

## 12. Controlling the estrous cycle

The problem with the problem is that it is estrus, so there is a way of studying the problem of estrus. And the problem of silent estrus is how to use the estrus induction program. It stimulates the breeder to have a growth of egg and ovarian, along with ovulation, which can be achieved by injecting hormones into the cattle body (Bo et al. 2010) Induction programs can help solve the problem of estrus and estrus. The benefit of induction is that those who perform artificial insemination can plan for the same time. in a given period (De souze et al. 2013). Various hormones that can be used in estrus induction. There are many types of applications: Progesterone, Gonadotrophin-releasing hormone GnRH and Prostaglandin (PGF<sub>2</sub>α). The form of induction is used both the injection method and the method of silicone rods made by vaginal insertion (Wilson et al. 2010).

## 13. Estrus synchronization

Ball et al. (2004) explained that the induction of estrus in cattle is managed to keep the cows at the same time in many cases. At the same time, using synthetic hormones that are presented in nature, taking into account the estrus cycle of the cows, the various

hormones in which the estrus of cow in each cycle depends on the management of hormones that have changed like in the normal animal estrus cycle. In hormonal function in the cycle, estrus occurs. This is caused by the function of the pituitary gland GnRH is a secretion FSH and LH. If the case LH Higher levels of hormonal secretion will result in subsequent ovulation (Mihm et al. 2002). Estrus synchronization of cows can be practiced as follows:

1. Supplementation in feed with substances Melengestrol Acetate (MGA) It is a product that is used to mix in the diet of cattle to affect the reproductive system.
2. The skin is also embedded in the ear area Norgestomet is a product of P4 by burying the rods in the ear area. Similarly, you can use of Norgestomet For Estrus synchronization Compared to the use of CIDR It was found that the estrus rate of cattle is equal. is worth the 100% (Gabriel et al. 2004)
3. Vaginal sponge pattern, how long it takes 9–19 It is used in combination with other hormones, such as intramuscular injections, when the device is removed or after 48 hours of disassembly. (Wildeus, 2000)
4. Progesterone tube to the vagina It is a device that releases progesterone into the vagina of the breeder. (controlled internal drug release, CIDR) It looks t-shaped. The part of the body is a solid silicone rod, which is coated with. At the end, there is a rope for the convenience of removing the device from the vagina of progesterone the cattle, which, in accordance with the program of estrus in the cattle, can quickly increase the concentration of progesterone in the cattle bloodstream. (Rathbone et al. 2002)

#### **14. Management of cattle in the near-term birth and the postpartum period.**

Cows is cared for and well managed, has a healthy body. Breastfeeding for healthy mothers is an important factor in allowing cattle to continue to produce in the long run. Therefore, the problem of metabolic syndrome in the breast feeding phase is complicated due to a condition that can lead to subsequent abnormalities in the breast feeding process. Accumulation of fatty liver Acidosis in rumen stomach (rumen acidosis) and low blood calcium (hypocalcemia). These problems affect the production efficiency of the cattle, especially breastfeeding. The problem is how to manage diets for the cow of the maternity co-parent. (Hutjens, 1996)

## 15. Comparison of AD<sub>3</sub>E injections in cows

Some vitamins have been recognized as having unique influence on immunity during vaccination, affecting both humoral and cell mediated response (Reddy et al., 1987). This immune- stimulatory effect reported is proven in vitamin A, E and D in livestock (Reddy et al. 1985). Several researched showed that vaccination efficacy can be more improved by supplementations especially vitamins which have effect on immune system such as vitamin A, E and D (Priyantha et al. 2012).

Some studies refer to that the deficiency in the vitamins& minerals may increase the incidence of retained fetal membrane (RFM) in cows (Akar and Yaldiz. 2005), injection of AD<sub>3</sub>E weekly during the last month of gestation before calving improved the reproductive parameters with normal placental dropping, rapid uterine involution and high incidence of pregnancy as compared with control group (Abdulhameed et al. 2009). Also another study concluded that the supplementing periparturient lactating buffalo with protected fat and injecting vitamin AD<sub>3</sub>E mixture increase milk production efficiency throughout the final 100 days of lactation (Hafez, 2012).

Fat soluble vitamins (i.e. A and E) are potently antioxidants. Animals cannot produce these vitamins in their bodies; hence an exogenous regular supply is needed to cover the physiological requirements and to sustain high production performance. During the per parturient period (transitional period) the concentrations of these vitamins reduce dramatically in the peripheral blood (Goff, Stabel, 1990 and Weiss et al. 1994). Thus, animals are venerable to different metabolic disorders, contagious diseases and a reduction in milk production and quality during this period (Block, 2010).

Vitamin E is a lipid soluble antioxidant present in cellular membranes that protect the cell from free radical by preventing lipid peroxidation (Chew et al. 1995; Tengerdy et al. 1983). It was proven by the different studies that the effect of enhancing antibody response in calves is by oral supplementation (Chew et al. 1995). Dairy cows injected with 1000 mg dl- alpha- tocopheryl acetate prepartum reported greater bactericidal activity at calving although; phagocytosis was not affected (Hogan et al. 1993). The positive benefit has been also proven in experimental animal by supplementary vitamin E that age dependent deterioration of the immune system can be altered (Oskar et al., 2001). The

same effect was also reported with vaccine in human antibodies titer against hepatitis B vaccine and this significantly increased in subjects receiving supplemental vitamin E (Oskar et al. 2001). Furthermore, it was demonstrated that alpha tocopherol intake was negatively correlated with rates of clinical mastitis (Afzal et al. 1984).

Vitamin A was also considered as a potent antioxidant that received a significant effect on immunity, though disease etiology (Chew et al. 1995; Smith et al., 2005) was found as a modulating agent on cellular and noncellular host defense system in animals (Chew et al. 1995). Beta carotene is caused by an induced lymphocyte proliferation and blastogenesis in cattles and pigs. It was also perceived causing increased helper/inducer T lymphocyte, peripheral monocytes, interleukin 2 receptors, transferring receptors, natural killer cells, cytotoxicity and tumor necrosis factors (Chew et al. 1995; Merker, 1985). A similar result was noticed in Holstein cows by higher phagocytic activity of netrophils, higher bactericidal activity during peripartum and lower intra-mammary infection during the lactation. However, the study further concluded that lower concentration of Vitamin A associated with sub-optimal host defense mechanism lowered the somatic cell counts in milk (Chew et al. 1995). On the other hand, carotinoids may modulate immune function by deactivating reactive chemical species such as free radicals, singlet oxygen and photochemical sensitizers (Chew et al. 1995). The main function of Vitamin D is to regulate calcium homeostasis, bone function and resumption (Catorna et al. 2004). Meanwhile, it has been demonstrated that the effect of immune response is basically on peripheral mononuclear cells which acts as immune regulators in animal physiology (Catorna et al. 2004). Significant effect was examined on helper T cell which activates stronger cell mediated immunity against an infectious disease in human (Cartona et al., 2004). Therefore, vitamin supplementation AD<sub>3</sub>E have vaccination efficiency in animals.

Hasan (2016) Studied and trial the effects of vitamin injections AD<sub>3</sub>E In the pregnancy and from the blood test, it was found that using AD<sub>3</sub>E during the first 3 months of pregnancy in the cattle, cow it affects the growth of embryo and caured easy calving easy calving. Similarly, it can help solve the problem of the cows diet. It increases the efficiency of the diet, which has a positive effect on the mother's body for the good of the feed.

Sarker et al. (2015) Studied the effects of injections GnRH, AD<sub>3</sub>E and minerals of around estrus in a heifer. The experiments were divided into 4 groups of 15 characters each. 1) injection of GnRH 500  $\mu$ g 2) injection of AD<sub>3</sub>E 10 mg; 3 times a day 3) injection of minerals 15 mg 20 days 4) Control group, observe the behavior for 30 days. All 4 groups of experiments will perform abdominal examination from an ultrasound machine during 28–35 days after AI. Cows in experiment in 1, 2 and 3 groups had a higher average estrus cycle than control group (in Table 3). Therefore, hormonal and vitamin used can help the cow to have an estrous cycle and getting higher pregnant.

*Table 3* Effects of GnRH exposure, vitamin AD<sub>3</sub>E injections, and mineral on entering the estrous cycle in heifer

Treatment Type	Number of heifers treated	Number of heifers responded	Proportion of heifers responded (%)	Number of heifers conceived	Proportion of heifers conceived (%)
Received treatment	45	21	46.67 <sup>a</sup>	16	35.56 <sup>a</sup>
Received notreatment	15	1	6.67 <sup>ab</sup>	0	0.00 <sup>b</sup>

Source: Sarker et al. (2015)

Sarker et al. (2015) studied on the results of GnRH hormone injections, AD<sub>3</sub>E vitamin injections and minerals in cattle, the reported showed the response to estrus cycles and pregnancy rates are after injection of GnRH affects the proportion of cattle entering the average high estrus cycle average is 60.00%, AD<sub>3</sub>E average is 46.67% and mineral the lowest average is 33.33%, respectively (P<0.05). Similarly, cattle in the group that received the hormone GnRH had a percentage of pregnancy at 46.67% which higher than the group that received the minerals, 26.67% (P<0.05) (Table 4).

Table 4 GnRH, vitamin AD<sub>3</sub>E injections, and mineralization in heifers.

Drugs used for treatment	Number of heifers treated	Number of heifers responded	Proportion of heifers responded (%)	Number of heifers conceived	Proportion of heifers conceived (%)
GnRH inj	15	9	60.00 <sup>a</sup>	7	46.67 <sup>a</sup>
AD <sub>3</sub> E inj	15	7	46.67 <sup>ab</sup>	5	33.33 <sup>ab</sup>
Vitamin mineral solution	15	5	33.33 <sup>b</sup>	4	26.67 <sup>b</sup>

Source: Sarker et al. (2015)

## 16. The cow-to-calf connection for the postnatal development of calves after birth

The transfer of nutrients from mother to fetus ceases and the neonate must orally ingest nutrients and digest them. Colostrum is typically the first food ingested by the calf. Thus, colostrum intake exposes the gastrointestinal (GI) tract of neonatal calves to nutrients (e.g., fat, lactose) and various bioactive substances such as immunoglobulins, IGF, insulin, or cholesterol (Koldovský, 1989). Bioactive substances found in mammary secretions elicit their biological effects directly on the wall of the calf's GI tract, are first transported across intestine into the calf's circulation, or both. These substances are either locally synthesized in maternal mammary tissues, are transferred from blood circulation by various mechanisms, or both (Baumrucker and Albrecht, 2014; Baumrucker and Bruckmaier, 2014). The transfer of some bioactive substances present in colostrum is mediated by cell surface receptors and receptor-like transporters located within the calf GI tract (Baumrucker and Albrecht, 2014; Ontsouka and Albrecht, 2014).

## 17. Management of the Calf at Birth

As calving time approaches, the cow due to calve needs to be watched closely for any complications. Cows and heifers should calve in a clean, dry, grassy lot or a clean, well-bedded pen. Pens should be square and should provide 150 to 200 square feet of space;



they should have good lighting and ventilation but be free from drafts. Beef cows can calve outside if a windbreak is available.

The newborn calf should begin to breathe shortly after the umbilical cord breaks. Mucus around the nostrils should be removed. Do not pound on the calf's chest or lift it by the rear legs since this can do more harm than good. Shortly after birth, the navel cord should be dipped (not sprayed) with a 7% tincture of iodine solution. (Do not use teat dip or weaker iodine solutions.)

The cow should be allowed to lick the calf after delivery. In cold weather or if the cow does not lick the calf, the calf should be dried with clean cloths. This practice not only dries the calf but stimulates the calf's blood circulation. Generally, dairy calves are removed from their dam shortly after the dam has licked the calf clean (within one hour). (Donna et al, 2006)

### **18. Early Colostrum Intake**

Colostrum is secreted by the mammary gland shortly before and after calving. True colostrum is obtained only from the first milking. After the first milking and for the next two and a half days, the cow's milk is called transition milk. Colostrum provides a calf with its primary source of nutrients. As shown in Table 5, true colostrum contains twice as much dry matter and total solids, two to three times as many minerals, and five times as much protein as whole milk. Colostrum also contains various hormones and growth factors that are necessary for growth and development of the digestive tract. Colostrum is lower in lactose, thus decreasing the incidence of diarrhea. Milk obtained after the first milking is inferior in quality to the first milking and should not be fed to the newborn calf as colostrum milk.

Calves are born with little defense or immunity against disease. They acquire resistance to disease from their dam through timely and adequate intakes of high-quality colostrum, their mother's first milk. Calves that do not receive adequate amounts of quality colostrum early in life are more susceptible to diseases. Holstein calves should be hand-fed 5 to 6 pints or 3 quarts of good quality colostrum within an hour of birth and again within 12 hours or the next regular feeding. (Donna et al, 2006)

## 19. Feed of the calf after birth

The newborn calf is not protected, the report said. This is transmitted through breast milk Virote (2003), also known as lymphatic milk, with a protein of 14.0% and a TDN of more than 75%, in line with Beanden et al. (2004) that compared normal lymphatic milk and milk, it was found that lymphatic milk contained 14% protein and normal milk contained only 3.1% protein, as shown in Table 5. To encourage more calf eating supplements as comply with Chalang (1998) "it is necessary for the cow to be concentrated, especially the cow's need, and reduces the lactation of the cattle. A concentr diet ate for calf should be high in protein at 18–22% and with energy 2.75–2.99 McalME/kg/DM (76–82% TDN)

Table 5 a comparison of the chemical Colostrum of lymphatic milk and normal milk.

Constituent	Cow	
	Colostrum	Milk
Total solids (%)	23.9	12.9
Fat (%)	6.7	4.0
Protein (%)	14.0	3.1
Lactose (%)	2.7	5.0
Ash (%)	1.11	0.74

Source: Beanden et al. (2004)

## 20. Creep feeding

Creep feeding a tight grip on feed costs is a priority for every beef producer. Creep feeding calves can be a good return on investments in certain situations. Maintaining the calf's efficiency at an early age is becoming much more critical with modern market requirements. The gross income of the cow/calf enterprises is partially dependent on the weaning weight of the calves. Outside of changing weaning date, there are management strategies that can increase calf weaning weight. However, chasing increased output or calf weaning weight can have some negative downstream implications to profitability of the cowherd. Strategies to increase calf weaning weight could include increased selection for maternal milk production, increased genetic selection for growth, increase in forage quality

consumed by the calf, and creep feeding the calf to increase nutrient intake. Creep feeding of beef calves usually is reserved for specific market and management situations such as high calf prices, low feed prices, dry lot operations, fall born calves, and purebred bull calves. Creep feeding suckling calves can increase market weight of the calf at weaning. Creep feeding must take into account the economics of the cost of gain, potential market endpoint, and the influence of sale price of the calves. For instance, the cost of the gain from creep feeding has to be less than the value of the gain to be a profitable nutritional strategy. Because there is data that suggests non-creep fed calves catch up with their creep fed mates post-weaning, the highest return is realized if calves are sold at weaning. Under severe drought conditions, creep feeding can be used to sustain minimal calf growth. A risk of creep feeding is getting calves too fat, resulting in price discounts. In addition, long-term data shows that creep feeding heifers will lower their long-term productivity by decreasing lifetime milk production.

Growth has a crucial impact on the economic value of animals. Growth rate determines the ultimate weight and size of cattle at maturity as well as carcass yield on slaughter. Growth is an increase in size and functional capabilities of tissues and organs of the body. In cattle breeding programmes, growth traits like body weight, weight gain and feed efficiency are considered as selection criteria. Linear body parameters such as body length, height at withers, heart gait, etc are also important growth indices and are positively associated with body weight (Otoikhian et al. 2008; Ozkaya and Bozkurt, 2008; 2009). They are used to predict body weight in animals including cattle (Ozkaya and Bozkurt, 2008; 2009). Body condition score on the other hand is useful in assessing energy reserves, marbling, and carcass quality. It could also be used to assess growth performance (Assan, 2013). In order to attain the genetic potential for growth, young animals must be fed rations that are adequate and balanced in addition to the dam's milk. For calves, preweaning performances are very important determinants of overall performance at weaning and post weaning (Myer et al. 1999; Arthington et al. 2008). Pre-weaning performance enables early assessment of the economic value of the animal and the dam or sire (or both), and the assignment of roles subsequently. Calf performance may be considered a trait of the cow in successive lactations (Vinoles et al. 2013) and prediction of the future most probable

producing ability (MPPA) of a cow with respect to preweaning growth traits of her calves is of immense advantage. Cow productivity is a trait of the cow which depends on the weaning rate and weaning weight of a cow's calves over a number of lactations (Vinoles et al. 2013). Provision of adequate and good quality feed for cattle feeding is a major constraint to cattle production in Nigeria and sub-Saharan Africa (Sowande et al. 2008). Nomadism and Pastoralism are production systems in response to variations in geographical distribution of forage resources and seasonal variation in availability of grazing and water resources for animal (cattle) production. The shortage of grasses for feeding of cattle becomes even more acute during the dry season when grasses are both in short supply and low in quality (Ensminger, 1991; Sowande et al. 2008). Pastures with low nutrient quality, which is most common in late summer, or low nutrient quantity, which is common in winter or during drought, cannot meet calves' nutrient needs for maximum growth (Parish and Rhinehart, 2009). Scarcity of feed resources affects both cow and calf performances: low milk production, shorter lactation period, and poor growth and development of the calf. Generally milk produced by cows is insufficient to meet the nutritional needs of the calf and support maximum growth and development (Shike et al. 2007). Parish and Rhinehart (2009) stated that milk production in beef cows peaks at about 2 months after calving and decrease subsequently and that milk from the lactating cow offers only about half the nutrient that a 3- to 4-months old calf needs for maximum growth. These indices indicate that as cows' milk yield decreases, calf nutritional demand increases. In order to bridge this gap, calves are given access to supplemental feeding (creep feeding). Weaning is a stressful event in the life of the calf (Lynch et al. 2012). The stress of weaning manifests in altered behavior (Price et al. 2003; Enriquez et al. 2010), secretion of hormonal mediators of stress (Blanco et al., 2009), and altered immune functions (Arthington et al. 2008; Enriquez et al. 2011) evident post weaning. Even though proper nutrition generally cannot prevent stress or infection, it can assist in preparing the animal for a period of stress, can decrease the adverse effect of stress and can enhance recovery from stressful periods. From the foregoing, methods of increasing the availability of nutrients for the cow-calf pair would be of benefit for increasing the productivity of the beef herd (Vinoles et al. 2013). The effect of pre-weaning concentrate feeding on physiological and immunological responses of calves has

also been studied (Lynch et al. 2012). Generally, proper nutrition to the calf will help prevent the immunosuppression caused by stress and thus enhance the health and performance of the animal. Creep feeding is advocated as a means of reducing weaning stress in calves through the familiarization to a palatable feed, such as concentrates. It has been reported to decrease morbidity in feedlots (Myers et al. 1999).

## **21. Compare the growth performance in the pre-weaning period**

According to the study, the effect of thickening supplements on pre-weaned cattle to the growth rate at the first stage of weaning EW or early weaned and TW or traditionally weaned. They are divided into 4 groups, 1) group in the early stages of weaning that has been fed concentrate receive EWS (90 day ;n = 7), 2) group in the early stages of weaning that do not receive concentrate fed receive EWNS ( day ;n = 7), 3) The group of cattle in the weaning phase that receives the concentrate for TWS (150 day ;n = 6), 4) group in the weaning phase that does not receive TWNS concentrate food (150 day ;n = 7) The results showed that the growth rate of EW and TW groups during the 0 to 90 days of weaning did not differ statistically (0.967 kg and 1.002 kg, respectively). When comparing the growth rate of pre-weaned cattle that have been fed and not fed during the first 2 months, there is no difference. However, after that, it was found that the group who received the diet had a higher daily growth rate than those who did not receive the supplement (1.087 kg and 0.907 kg, respectively). In conclusion, dietary supplements that calf need It can lead to a way to increase the nutrition in the pre-weaned period, it can help increase the daily growth rate of calves. (Blanco et al., 2008) However, feeding the newborn calf at  $32.20 \pm 2.29$  kg, the growth rate is  $519.05 \pm 49.79$  g/day and has a newborn weight of  $32 \pm 2.29$  kg, not different from flavomycin supplements at 50, 100 and 200 mg/kg. Consistent with the study It was found that supplements concentrate proteins at 16%, 18% and 20% it was levels, found that all 3 experimental groups had a growth rate. body length, the weaning age, the amount of concentrate, the roughage, as well as the total cost of feed. were not statistically different ( $P > 0.05$ ) and it was found that 18% concentrate had the highest effect on pre-weaned calf. Therefore, the use of diet efficiency with growth at the first-stage weaning phase can be selected for at 16% Protein.

Bojarpour et al. (2010) compared the growth and ingestion rate of weaned dairy calves at different ages (at 8 weeks and 13 weeks) appeared after weaning for a week (week 9), weaned calf at 8 weeks, with a low daily growth rate. However, during the 10–13 weeks, the two groups had a growth rate per day, varying statistically insignificantly. The dry matter intake of the two groups of animals during weeks 8–9 varies statistically, but during the 10–13 weeks the weaned calf at 8 weeks have higher ingestion of dry matter, as the cattle are fed a concentrate diet for dairy calves, which is a stimulant.

Jasper et al. (2002) Reported in the pre-weaning period, fully breasted dairy-treated with a better weight gain than the milk-limited dairy cow at the level of 10 percent of body weight twice a day. After weaning (37–42 days) and after weaning the two cattle, the group had no different growth and no effect on eating diet after weaning (day 63), as Pisut et al. (1993) with a study of weaning cattle at different ages using Holstein Friesian calve: the possibility of producing quality cattle 37 males, weaning at the age of 45, 60, 90 and 120 days, and then fattening to gain at 250 kg body weight. It was found that the growth of cattle did not differ throughout the trial. However, the amount of milk powder and the feed consumed varies according to the different weaning period. But after 120 days, all cattle consumed concentrate diet and roughage in different quantities, and the group weaned at 60 days with the lowest production costs.

## **22. Effect of supplementation feed to hematological of cows**

The importance of haematological indices in animal husbandry is well acknowledged. Metabolic disturbances usually by inappropriate feeding without overt clinical manifestation could cause reduced performance and productivity (Radostits et al. 2000; Mamun et al. 2013). Such altered metabolic status could be reflected in altered haematological indices. The significantly different haematological indices between treatments in the present study could relate to the different feeding materials and feeding regimes. Mamun et al. (2013) had stated that factors such as nutrition and stress affect blood values of animals. The significantly higher Hemoglobin, and Red Blood Cell in the control animals could indicate higher oxygen capacity of the blood as a result of greater demand of oxygen by the tissues while the higher neutrophil and neutrophil:lymphocyte ratio indicate greater

stress profile of non-creep fed calves compared to creep fed animals. The haematological values reported in the present study were however consistent with some published blood parameters of apparently healthy calves.

Nutrition among other factors influences serum total protein, urea, globulin and bilirubin concentrations (Klinkon and Jezek, 2012). Serum protein and urea concentration is hence influenced by the amount of protein in diets. Klinkon and Jezek (2012) stated that increased urea concentration in serum of calves is indicative of increased protein catabolism. On the other hand, creatinine is synthesized during endogenous metabolism in muscles and do not depend on nutrition (Klinkon and Jezek, 2012). The similarity in serum creatinine levels of experimental units is hence not surprising. Aspartate transaminase (AST (SGOT)) activity is increased above normal range in pathological situations that cause cell necrosis such as damage to liver cells (Klinkon and Jezek, 2012).

Hematological analysis is helpful for the diagnosis of diseases of the blood system, as well as the diagnosis of many organs and systemic diseases. The diagnosis of disease can sometimes be based solely on the number of whole blood cells (CBC), but blood flow charts can provide useful information for the prognostic diagnosis, monitoring, and prognosis of an individual's future disease progression. Specific characteristics of bovine blood cells and diseases related to abnormal bovine hematological characteristics.

In all vertebrates, blood contains (1) almost colorless plasma (2) several types of white blood cells or white blood cells (3) red blood cells or red blood cells stained with hemoglobin, and (4) smaller cells called platelets or platelets. Plasma carries dissolved food, waste, internal secretions and some gases. (Tibbo, 2004)

### **23. Bovine hematological reference ranges**

The most appropriate reference range is generated from a group of healthy animals with environmental and physiological characteristics as similar to the patient as possible. As in all species, a certain amount of physiological variability is observed in hematologic profiles of cattle. Variables that contribute to the thresholds and width of reference intervals include age, sex, stress, diet, body condition, reproductive status, recent activity, hydration, ambient temperature, and altitude (Krimer et al, 2011, Woodet al, 2010) Reference intervals for

bovine hematologic parameters from 3 sources are summarized in Table 6. Many commonly used hematology reference intervals originate from research undertaken in the 1960s. A 2010 study by George et al (2010) compared reference intervals of healthy North American cows from 1957 to 2006 to account for changes in the CBC count during the past 50 years. The main findings included a significant increase in reference ranges for neutrophil counts over the study period, whereas reference intervals for lymphocyte, monocyte, and eosinophil counts as well as hemoglobin concentration had decreased. Genetic selection and decreased prevalence of bovine virus diarrhea were suggested as possible reasons for higher neutrophil counts (George et al, 2010). Animals persistently infected with Bovine viral diarrhea virus (BVDV) exhibit significant neutropenia (Piccinini et al 2006), thus the absence of persistently infected animals in the study might have contributed to increased neutrophil numbers. The reduced eosinophil count was assumed to be caused by decreased exposure to parasites due to modern husbandry and parasite control programs. The authors recommended that laboratories should establish their own reference ranges to reflect current cattle populations (George et al, 2010).

*Table 6 Bovine hematology reference intervals according to 3 sources. \**

Parameter	Unit	Wood and Quiroz-Rocha (2010)	Kraft and Dürr (2005)	George et al. (2010)
Erythrocytes	10 <sup>6</sup> /μl	4.9–7.5	5–10	5.1–7.6
Hematocrit	%	21–30	28–38	22–33
Hemoglobin	g/dl	8.4–12.0	9–14	8.5–12.2
Mean corpuscular volume	fl	36–50	46–65	38–50
Mean corpuscular hemoglobin	pg	14–19	11–17	14–18
Mean corpuscular hemoglobin concentration	g/dl	38–43	31–34	36–39
Red cell distribution width	%	16–20	NA	15.5–19.7
Reticulocytes	10 <sup>3</sup> /μl	0	NA	NA



Leukocytes	103/ $\mu$	5.1–13.3	5–10	4.9–12.0
Segmented neutrophils	103/ $\mu$	1.7–6.0	1.0–3.5	1.8–6.3
Band neutrophils	103/ $\mu$	0–0.2	0.0–0.2	Rare
Lymphocytes	103/ $\mu$	1.8–8.1	2.5–5.5	1.6–5.6
Monocytes	103/ $\mu$	0.1–0.7	0–0.3	0–0.8
Eosinophils	103/ $\mu$	0.1–1.2	0.3–1.5	0–0.9
Basophils	103/ $\mu$	0–0.2	0–0.1	0–0.3
Platelets	103/ $\mu$	160–650	300–800	193–637
Mean platelet volume	fl	4.6–7.4	NA	4.5–7.5

\* NA = not available

† Reference intervals for the ADVIA 120 hematology analyzer from 99 clinically healthy cows, 50% in first lactation, all milking for 30–150 days, from 10 farms in Ontario, Canada.

‡ Mainly based on several German original research papers.

§ Reference intervals for the ADVIA 120 hematology analyzer from 58 healthy adult dairy Holstein cows in mid-lactation (at least 18 weeks into lactation) and producing 50–70 pounds of milk per day from 4 herds in California.

## 24. Red blood cell parameters and their interpretation

Red blood cells (RBC) are formed in the bone marrow, especially in the top of the femur and in vertebrates (Anderson, 2003). The main function of red blood cells is the transport of oxygen bound to hemoglobin. The life span of bovine red blood cells is relatively long, 130–160 days (Wood and Quiroz-Rocha, 2010).

Erythrocytes have an average diameter of 5–6  $\mu$ m in cattle, which is small compared to other species. The key function of erythrocytes is the transport of oxygen, which is bound to hemoglobin. Erythropoiesis, which takes approximately 5 days, is stimulated by erythropoietin and occurs in the bone marrow parenchyma. Bovine erythrocytes have a relatively long-life span of 130–160 days. (Brockus 2011, Wood and Quiroz-Rocha 2010). An RBC count typically includes the total number of RBCs, hematocrit (HCT), hemoglobin (HGB), erythrocyte indices, and occasionally the red cell distribution width (RDW). Erythrocyte indices include mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) (Brockus 2011).

In general, beef cattle breeds have higher RBC counts than dairy cattle, bulls have greater RBC counts than cows, and nonlactating cows have higher RBC counts than lactating cows (Wood and Quiroz-Rocha 2010). In calves, HGB, MCH, and MCHC decrease during the first month and then start to increase during the first 3 months of life (Mohri et al. 2007). In young calves, RBC counts might be higher, and MCV and MCHC might be lower than in adults (Brun-Hansen 2006, Jones and Allison 2007). Common indications for RBC analysis are clinical anemia or hemorrhage. In absolute anemia, RBCs, HGB, and/or HCT are decreased. Relative anemia is caused by an increase of plasma volume (e.g., during pregnancy or after fluid therapy). Anemia can be categorized into regenerative and nonregenerative anemia according to the bone marrow response, and can also be classified with regard to the cell size (normocytic, macrocytic, and microcytic, indicating normal, increased, and decreased MCV, respectively) and hemoglobin concentration (normochromic, hypochromic, and hyperchromic, indicating normal, decreased, and increased HGB, respectively) (Brockus, 2011; Baily, 1978).

Causes for regenerative anemia are hemorrhage or hemolysis. With acute blood loss, the RBC parameters are initially within the reference ranges because cells and plasma are lost in the same proportion. Diminished RBC count and HGB can be found only after several hours, when fluid in the blood vessels is replaced and dilutes the blood. In cattle, regeneration of erythrocytes begins after approximately 2 days, and takes weeks to be fully accomplished. In chronic hemorrhage, RBC count, HCT, and HGB are decreased while reticulocytes as well as MCV are increased. In ruminants, only a moderate rise in reticulocytes is observed in responding anemia. If regenerative capacity is depleted, chronic bleeding anemia can become nonregenerative. Brockus (2011) Causes for hemorrhage include trauma, abomasal ulcers, hemorrhagic enteritis, vena cava syndrome, blood sucking parasites (e.g., *Haemonchus* spp., lice, or ticks), hemostasis defects, and vessel erosion or rupture. Hemolytic anemia is caused by blood parasites, toxins, electrolyte imbalances, hyposmolality, (Brockus 2011; Gründer 2006; Jones and Allison 2007) or autoimmune reactions.<sup>40</sup> In cattle, common causes for hemolysis include unsuitable food and toxic plants, such as cabbage (*Brassica* spp.), onions (*Allium cepa*), rye grass (*Lolium*

spp.), or red maple (*Acer rubrum*) (Brockus, 2011; Baily, 1978; Brockus, 2011; Gründer, 2006; Hutchison, 1977; Wood and Quiroz–Rocha, 2010) Microorganisms infecting ruminant RBCs include rickettsia (*Anaplasma marginale*) (Allison and Meinkoth, 2010), protozoa (*Babesia* spp., *Theileria* spp., *Trypanosoma* spp., and *Sarcocystis* spp.) (Allison and Meinkoth, 2010; Brockus 2011; Jones and Allison 2007) or bacteria (*Mycoplasma wenyonii*, *Leptospira* spp., and *Clostridium* spp.) (Allison and Meinkoth, 2010; Jones and Allison, 2007). Further factors are copper deficiency or chronic copper intoxication (Jones and Allison, 2007; Wood and Quiroz–Rocha 2010), hypophosphatemia (postparturient hemoglobinuria) (Brockus, 2011; Gründer, 2006) and water intoxication (Gründer, 2006; Kraft and Dürr, 2005).

## **25. White blood cell parameters and their interpretation**

White blood cells (WBC) play an important role in immune defense and include other subpopulations such as neutrophils, eosinophils and basophils, monocytes and lymphocytes. White blood cells are produced and matured in the bone marrow, whereas in the case of lymphocytes, white blood cells are produced in lymphoid tissue (Webb and Latimer, 2011).

White blood cells (WBCs) or leukocytes play an essential role in immune defense, and include different subpopulations: neutrophil, eosinophil, and basophil granulocytes, monocytes, and lymphocytes. Leukocytes are produced and mature in the bone marrow, and, in the case of lymphocytes, in the lymphoid tissues. The number of leukocytes in the blood constitutes only a small percentage of the total population and undergoes wide fluctuation. In the vasculature, a marginal pool and a circulatory pool of neutrophils are differentiated. The marginal neutrophils are attached to the endothelial cells, but detach and join the circulatory pool if blood pressure rises and the blood flow velocity increases. Therefore, every change in blood pressure can result in a change in the amount of leukocytes present in the blood (Kraft and Dürr, 2005).

A complete WBC count is composed of the total number of leukocytes, the relative differential blood count, and the absolute differential blood count. Usually, the different subpopulations, as well as band and segmented neutrophils can be distinguished. (Kraft and Dürr, 2005; Wood and Quiroz–Rocha, 2010).

In cattle, the total number of WBCs decreases with age (Gründer, 2006). Lymphocytes are the dominant subpopulation, but the lymphocyte proportion varies with age. The newborn calf has more granulocytes than lymphocytes (Jones and Allison, 2007; Wood and Quiroz-Rocha, 2010). During the first month of life, calves exhibit a decrease in the overall number of WBCs, neutrophils, and lymphocytes, followed by an increase thereafter.<sup>31</sup> Within approximately 3 months, the lymphocyte percentage increases up to 80% of the total circulating WBC population. In the adult, the lymphocyte concentration decreases progressively but remains the dominant cell type. The neutrophil-to-lymphocyte ratio in adult cattle is approximately 1:2, which is low compared to other domestic animals. (Jones and Allison, 2007; Wood and Quiroz-Rocha, 2010). Eosinophils are below adult reference ranges after birth and increase with age (Kraft and Dürr, 2005; Wood and Quiroz-Rocha, 2010).

Compared with other species, cattle have a small bone marrow reserve for granulocytes. This results initially in a neutropenic rather than a neutrophilic reaction in an early inflammatory process. Neutrophilia and a left shift might be observed only after the speed of granulopoiesis is increased. After 3–5 days, immature and mature neutrophils might rebound (Gründer, 2006; Jones and Allison, (2007; Wood and Quiroz-Rocha, 2010). Cattle neutrophil counts rarely exceed 30,000 cells/ $\mu$ l.<sup>46,48</sup> Neutrophil numbers decrease in the postpartum period,<sup>18</sup> and lactating cows have lower WBC counts than nonlactating cows. Wood and Quiroz-Rocha, 2010

Indications for a leukogram include diagnostics, general health assessments, monitoring of a disease, or monitoring of therapeutic actions. However, it is seldom possible to come to a definite diagnosis based solely on a WBC count (Kraft and Dürr, 2005). Sequential leukograms can help establish a prognosis. The return of the leukogram within normal limits together with clinical improvement can be interpreted as a favorable sign, whereas a rapid fall in leukocytes without clinical improvement is regarded as a grave prognostic sign. Guarded or poor prognoses should be formulated with persisting neutropenia, a degenerative left shift, or severe persistent leukocytosis (Gründer, 2006; Jones and Allison, 2007).

The mechanisms underlying leukocytosis include increased release from the bone marrow, decreased emigration into the tissues, and a shift of cells from the marginal into the circulatory pool.<sup>48</sup> Physiologic leukocytosis is seen in association with stress, excitement, fear, exercise, or parturition. A stress leukogram is characterized by neutrophilia, lymphocytopenia, eosinopenia, and occasionally monocytosis. It is triggered by endogenous or exogenous corticosteroid exposure (Jones and Allison, 2007; Kraft and Dürr, 2005). A short-term physiological leukocytosis is also observed after epinephrine release. Jones and Allison (2007) Causes for pathological leukocytosis include infectious diseases, endogenous or exogenous intoxication, endocrine conditions, central nervous disorders, anaphylactic shock, leukemia, and bovine leukocyte adhesion deficiency (BLAD). Kraft and Dürr (2005)

Leukopenia is caused by decreased production, increased tissue demand, and consumption combined with marginalization. In general, it is observed in connection with viral infections, circulatory shock, peracute inflammation, cytotoxic substances, as well as hematopoietic stem cell disorders and bone marrow atrophy Kraft and Dürr (2005). In cattle, leukopenia often occurs with metabolic disorders, liver disease, and infectious diseases (e.g., mucosal disease, paratuberculosis, or salmonellosis). Panleukopenia, a depression of all WBC subpopulations, is observed in viral disease (e.g., mucosal disease, infectious bovine rhinotracheitis), rickettsiosis, bacterial septicemia, and purulent splenitis Gründer (2006).

The most common causes for neutrophilia are chronic inflammation and stress. Chronic inflammation has been reported among other infections of the udder, urogenital tract, gastrointestinal tract, liver, respiratory tract, heart, and central nervous system. In bovines, neutrophilia is also commonly observed with acute purulent processes, such as endometritis or metritis, retained placenta, acute bacterial mastitis, and foreign body peritonitis Gründer (2006). Inflammatory neutrophilia is seen in viral, bacterial, protozoal, parasitic, and fungal infections. Additionally, neutrophilia is observed with noninfective inflammation (traumatic injuries, necrosis, infarction, burns, thrombosis, etc.), neoplasia, intoxication, endocrine disorders, hemorrhage, and hemolysis Kraft and Dürr (2005). In cattle, stress-induced neutrophilia is also associated with abomasal displacement, ketosis, indigestion, and dystocia Yıldız et al (2011). Severe leukocytosis, exceeding 40,000 cells/ $\mu$ l and sometimes even 100,000 cells/ $\mu$ l, based on a marked increase in neutrophils may be

a sign of BLAD in Holstein Friesians. BLAD is usually diagnosed in calves, and most affected calves die within 1 year of age.

Neutropenia occurs in ruminants during the first 1 or 2 days of severe, acute inflammation, including sepsis, mastitis, peritonitis, metritis, enteritis, and pneumonia. It can be caused by viral (BVDV, Bluetongue virus, Border disease virus), Tornquist and Rigas (2010). rickettsial (*Anaplasma phagocytophilum*,<sup>43,46</sup> *Ehrlichia ruminantium*), protozoal (*Theileria* sp.), Tornquist and Rigas (2010) and fungal infections, as well as bone marrow disease, toxins, neoplasia, or idiosyncratic drug reactions Kraft and Dürr (2005).

Conditions commonly associated with eosinophilia include type I (immediate) hypersensitivity reactions and parasitic infection (Jones and Allison,2007; Kraft and Dürr ,2005; Tornquist and Rigas, 2010). Additional causes are neoplasia, infections, and drug reactions. Eosinopenia might also occur in the early phase of infectious diseases, uremia, and acute hemolysis (Kraft and Dürr, 2005). Extreme eosinopenia has been associated with *Theileria* infections (Tornquist and Rigas, 2010).

Basophilia has been linked to hyperlipidemia and occasionally to allergies, ulcerations, Kraft and Dürr (2005) and parasitic infections (ticks) (Tornquist and Rigas (2010). Basopenia is not commonly reported in the literature because basophil reference intervals often include zero (Tornquist and Rigas (2010).

Lymphocytosis can occur in the healing phase of infectious diseases, during chronic antigenic stimulation due to infectious agents, neoplasia, and hypoadrenocorticism (Kraft and Dürr, 2005). Reactive lymphocytosis is observed during chronic purulent diseases, such as hepatitis, peritonitis, pericarditis, nephritis, mastitis, or bronchopneumonia. Gründer (2006), Bovine leukemia virus (BLV) is the causative agent of enzootic bovine leukosis. Infection with BLV results in a persistent lymphocytosis driven by an increase in B cells with abnormal lymphocyte morphology in up to 30% of affected cattle. Only a small percentage of animals with lymphocytosis develop bovine lymphoma. In some cases, WBC counts may exceed 100,000 cells/ $\mu$ l (Bienzle, 2011); Jones and Allison, 2007).

Reasons for lymphocytopenia include acute stress, viral or bacterial infection, immune suppression, chronic renal insufficiency, and application of corticosteroids (Jones and Allison,2007; Kraft and Dürr, 2005). It is also induced by loss of lymph and disruption of

lymph node architecture (e.g., in paratuberculosis, inflammation, or neoplasia) (Webb and Latimer, 2011).

Monocyte numbers are variable in cattle and are thus not a sensible indicator for a specific disease (Jones and Allison, 2007). Monocytosis has been observed during acute stress and in the healing phase of acute as well as chronic infections. It is further caused by hemolysis, hemorrhage, exudative inflammation, necrosis, ulceration, and corticosteroid therapy (Kraft and Dürr, 2005; Webb and Latimer, 2011). Monocytosis was also found to be a successful marker of bacteremia and bacterial endocarditis Webb and Latimer (2011) as well as puerperal infections.<sup>19</sup> Monocytopenia may be associated with endotoxemia (Tornquist and Rigas, 2010; viremia, and inflammation; Jones and Allison, 2007). but has so far not been proven to have much clinical relevance (Webb and Latimer, 2011).

With automated cell counters, falsely low WBC counts might occur due to clumping of leukocytes, and falsely high WBC counts might be caused by nucleated red blood cells, insufficient lysis of erythrocytes, excessive Heinz bodies, or clumping of platelets (Webb and Latimer, 2011).

## **26. Platelet parameters and their interpretation**

Platelets are anuclear cytoplasmic fragments of megakaryocytes, and play an essential role in hemostasis (Boudreaux et al., 2011). With an average mean platelet volume (MPV) of 4.0–4.8 femtoliters, bovine platelets are small compared to those of other species. Up to 30–40% of platelets are sequestered in the spleen and enter circulation in response to epinephrine release. Thrombocytes are also stored in the liver and bone marrow (Boudreaux et al., 2011) Bovine platelets survive up to 10 days in peripheral blood. Wood and Quiroz–Rocha (2010) In intact blood vessels, platelets circulate predominantly in the marginal pool. Kraft and Dürr (2005) The total number of platelets is influenced by the amount of production, consumption, sequestration, and loss (Boudreaux et al 2011).

Platelet numbers increase significantly during the first 2 weeks of age and more slowly thereafter during the first 3 months. Platelet counts in calves might be within Mohri et al (2007) or above adult reference intervals (Brun–Hansen, 2006). The following

parameters can be estimated: total number of platelets, MPV, thrombocrit or plateletcrit, and platelet distribution width (Boudreaux et al., 2011).

severe hemorrhage or increased bleeding tendency. This includes clinical signs such as petechia, ecchymosis, hematuria, epistaxis, melena, hematemesis, and hyphema (Thomas, 2010).

Thrombocytosis occurs physiologically as a consequence of epinephrine-induced splenic contraction. Essential or primary thrombocytosis is an uncommon myeloproliferative condition. Reactive or secondary thrombocytosis is triggered by cytokine release and is observed in connection with stress, chronic blood loss, inflammation, neoplasia, or iron deficiency. Enhanced thrombopoiesis is also seen with inherited megakaryocyte disorders (Boudreaux et al., 2011). An increased platelet count might be associated with an increased risk for thrombosis (Boudreaux et al., 2011; Kraft and Dürr, 2005).

Thrombocytopenia is found in excessive consumption (e.g., with blood loss, disseminated intravascular coagulation, or thrombocytopenic thrombotic purpura), decreased platelet production (e.g., in myelophthisis or bone marrow hypoplasia linked to toxins), destruction (e.g., due to infections, toxins, drugs, neoplasia, or immune-mediated), or distribution disorders (e.g., splenomegaly) (Boudreaux et al., 2011; Kraft and Dürr, 2005; Thomas, 2010). Examples for infectious diseases causing thrombocytopenia include salmonellosis, leptospirosis, babesiosis, theileriosis, (Thomas, 2010) anaplasmosis, and BVDV infection (Boudreaux et al., 2011; Thomas, 2010). Examples for toxins leading to platelet destruction are bracken fern (*Pteridium aquilinum*), mycotoxins (trichothecene), black fly (*Simuliidae* spp.) toxin, and coumarin rodenticides (Gründer, 2006; Thomas, 2010). Thrombocytopenia associated with leukopenia and bone marrow depletion is observed in bovine neonatal pancytopenia, a hemorrhagic disease of newborn calves observed since 2006, which is thought to be linked to an inactivated vaccine against BVDV in dams.

Enlarged platelets are associated with accelerated production as well as myeloproliferative disorders and hyperthyroidism. Decreased MPV or platelet fragments are observed in iron-deficiency anemia, bone marrow failure, and immunemediated thrombocytopenia (Boudreaux et al., 2011).



Automated cell counters should be calibrated adequately to account for the small size of bovine platelets. Falsely elevated platelet counts might be flagged if fragmented RBCs or WBCs are erroneously counted as platelets by an automated analyzer. Platelet clumping might be a reason for indication of thrombocytopenia in automated cell counters. Platelet clumping can result from exposure to EDTA exceeding 4–6 hr or from use of heparin as an anticoagulant (Jones and Allison, 2007; Thomas, 2010). Storage of blood in EDTA tubes over 4 hr at room temperature or refrigerated might cause platelet swelling, resulting in falsely increased MPV. Storage over 24 hr can result in fragmentation of platelets (Boudreaux et al., 2011).

## **27. Effect of supplementation feed to progesterone hormone**

Postpartum nutrition plays a significant role in the onset of ovarian cyclicity, the expression of normal estrous cycles, and conception rates (Robinson et al., 2006). However, the importance of protein nutrition on the reproductive performance of the modern high-yielding cow is ambiguous. There are numerous studies reporting direct negative effects (Larson et al. 1997), indirect or associative effects (Barton et al., 1996), or no effects (Kenny et al. 2001) of elevated dietary protein content on reproductive performance. For example, work by Jordan and Swanson (1979) showed that an increase in dietary CP content increased the interval from calving to first ovulation and reduced the pregnancy rate. However, other studies have failed to support this observation (Laven and Drew, 1999). Westwood et al. (1998) also reported confounding evidence on the effect of dietary protein concentration on conception rates. However, Westwood et al. (1998) concluded that a stronger relationship existed between dietary CP concentration and conception rates in older animals compared with primiparous animals. Work by Butler (2001) illustrated that elevated blood urea concentration coupled with suboptimal early luteal progesterone concentrations are detrimental to embryo survival. Interestingly, Orihuela (2000) indicated that a severe protein deficiency will interfere with reproductive processes, as will an excessively high protein concentration in the diet. The detrimental effects of protein deficiency were previously

emphasized by Westwood et al. (1998) who stated that low-CP diets result in suboptimal microbial protein synthesis in the rumen and are associated with reproductive failure.

## **28. Effect of supplementation feed to estrogen hormone**

Energy and protein intakes are considered to be the most important nutritional factors. Inadequate amount of energy delays sexual maturity in heifers (Graves and McLean, 2003). It is also reported that if energy deficient rations are fed to heifers that have begun normal estrous cycles, they may stop cycling (McDonald et al., 1988).

Fat supplementation has positive influence on reproductive status of dairy cows by altering the size of the dominant follicle, hastening the interval to first postpartum ovulation, increasing progesterone levels during the luteal phase of the estrous cycle, modulating uterine prostaglandin synthesis, and improving oocyte and embryo quality (Staples et al. 1998; Santos et al., 2008). Linoleic (C18:2n-6) and linolenic (C18:3n-3) acids are classified as essential fatty acids and must be supplied in the diet because they could not be synthesized by mammalian cells (Santos et al. 2008). PUFAs of the n-6 and n-3 families seem to have the most remarkable effects on reproductive responses of cattle (Santos et al. 2008). Previous studies of PUFAs from sunflower seed, soybean oil and flaxseed showed positive influence on reproductive performance (Ryan et al. 1992; Petit et al., 2004), due to fatty acids which are the precursor for synthesis of reproductive hormones such as estradiol and progesterone levels via cholesterol and prostaglandins via arachidonic acid (Staples et al. 1998; Robinson et al. 2002; Zachut et al. 2010).

## Chapter III

### RESEARCH METHODOLOGY

3.1. Experiment 1 Effects of Concentrate Feed Supplementation on the Reproductive Systems, Progesterone and Estradiol Levels in the Khao Lamphun Cows

#### 3.1.1 Ethical Principles and Guidelines for the Use of Animals

The study was conducted in accordance with the standards of laboratory animals for scientific purposes, which are supervised by the Animal Conduct Control Committee for Scientific Work, Phayao University certificate UP AE61-01-01-002.

#### 3.1.2 Experimental Design and Animal managements

A total of 72 Khao Lamphun 2 months postpartum cows from the Phayao Livestock Research and Breeding Center, Phayao were randomly divided according to Paired Observation into 2 treatment groups, 1) Control group – No concentrate feed on postpartum cows (n= 36 heads) and 2) Treatment group – Supplemented with concentrate feed on postpartum cows (n= 36 heads). Using a bull in the herd for natural mating in each group. The experimental animals were raised in an open pasture Group 1. fed only with Ruzi grass or rice straw *ad lib.* after returning back to the stall and Group 2. fed with Ruzi grass or rice straw *ad lib.* as basal diets and supplemented with concentrate feed (12%CP), supplements are fed at 0.5% of body weight per day. (Table 7). The experimental period last for 90 days. (Figure 2)

#### 3.1.3 Samples collection and Chemical analysis

The feed samples were collected for proximate analysis of Dry Matter (DM), Crude Protein (CP), Crude Fiber (CF), Ether extract (EE) and calculate the value of Nitrogen Free Extract (NFE) according to the method of AOAC (1990) and the Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) were analysed according to the method of Van Soest et al. (1991)

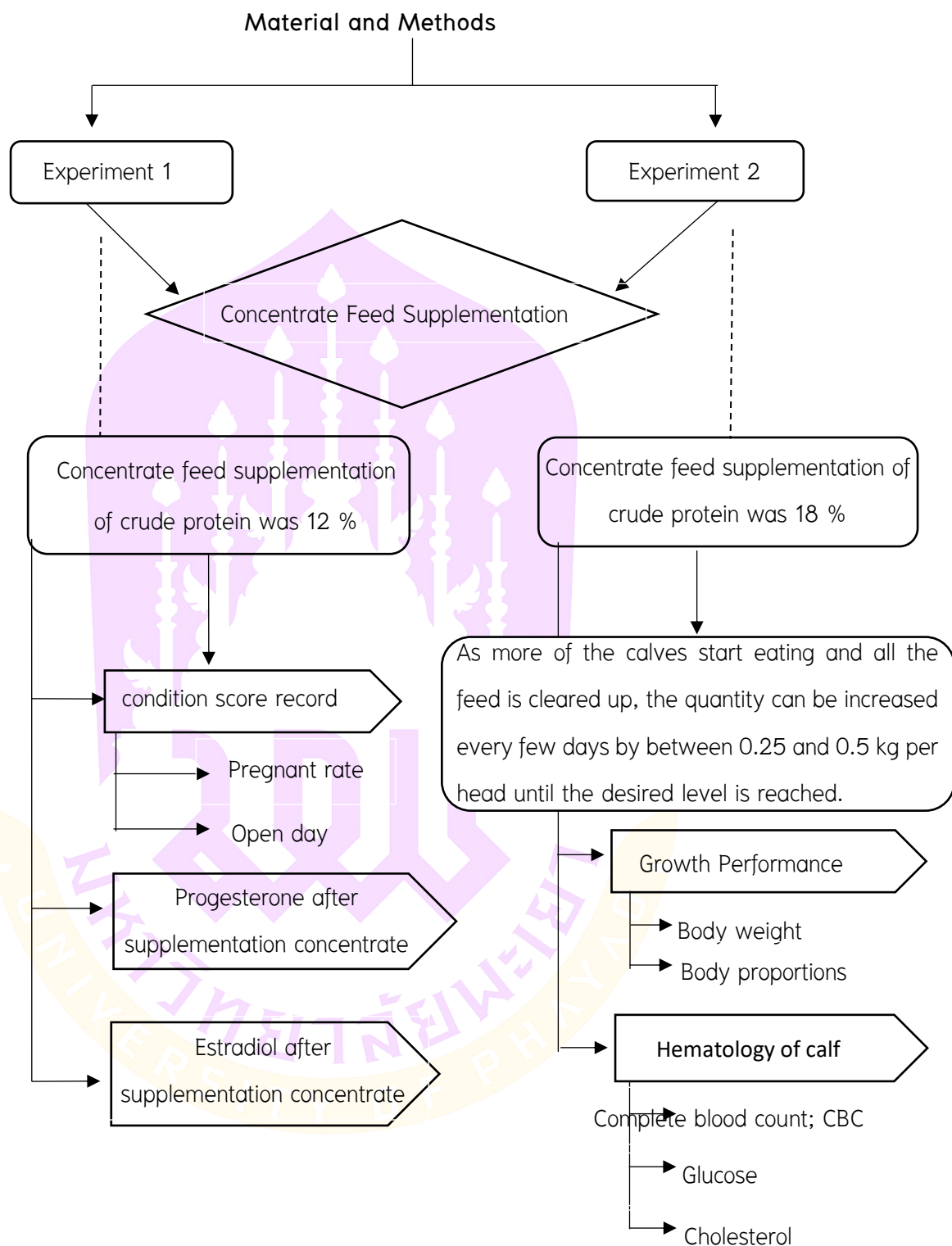


Figure 3 Plan of studies on control and treatment groups in cow-calf raising systems in concentrate feed supplementation.

Table 7 Chemical composition of concentrate feed for Khao Lamphun cow.

Item	Roughage		Concentrate
	Ruzi grass	Rice straw	CP 12%
DM	90.40	92.64	93.33
Ash	8.60	13.40	4.39
CP	10.20	2.52	12.25
CF	23.40	29.63	7.77
EE	5.90	2.14	2.86
NDF	63.20	70.24	42.45
ADF	29.20	51.16	6.48
NFE	52.00	44.96	65.81

<sup>1</sup> DM = dry matter, Ash =, CP = crude protein, EE = ether extract, CF = crude fiber, NFE = nitrogen free extract.

### 3.1.4 Study Area

The experiment was conducted at Phayao Research and Breeding Center. The terrain and climatic characteristics are latitude at 19° 9' north and longitude at 99° 55' °C east at a moderate sea level of 440 meters, the average tropical savannah temperature of 25°C and an average relative humidity of 73%. (Department of Land Development, 2007)

### 3.1.5 Data collection

Body condition score (BCS)

This was determined monthly by physical examination of each calf and scoring was based on the Scottish body condition scoring system (Agra Point International, 2003). The system consists of five grades determined by assessment of the degree of fat covering in the loin area between the hook (hip) bone and the last rib. Depending on the fat covering a score (subjective) of between 1 (very thin) and 5 (very fat) was assigned to each cow in the experiment. Thus, an animal with very prominent (visible) spine, individual ribs and tail head (no fat cover) received a score of 1. A score of 2 indicated some fat covering of individual ribs and tail head but these structures were still felt by mare touch. A score of 3 was given

to an animal whose short ribs were felt by firm pressure and whose tail head had fat cover that was easily felt. An animal whose short ribs could not be detected even with firm pressure and with obvious fat deposit around tail head received a score of 4 while a score of 5 was assigned to an animal whose characteristic bone structure was not noticeable, and tail head a loose fold of flesh. Animals that fell in between these classes received intermediate scores such as 1.5, 2.5, 3.5, etc. Daily changes in body condition score was calculated as the difference between two consecutive scores divided by the number of days in the interval.

### **Pregnancy rates**

Pregnancy rate is a common trait utilized when evaluating fertility and pregnancy rates and these were analyzed for during the study period of 90 d. Pregnancy diagnosis using in palpation, Palpation may be done with either hand. One hand may be used to grasp the cow's tail to use as leverage to push the other hand into the rectum. The covered, lubricated hand should be shaped into a wedge by bringing the fingers close together. The wedge-shape of the hand helps in the initial thrust into the rectum. As the hand goes through the cow's rectum, the hand should be formed into a cone to push aside fecal material and straighten the folds of the rectum. The cow will naturally strain against the palpator's hand. The palpator should allow the muscle contractions to subside, and then continue pushing the hand through the rectum.

### **Hematology and blood biochemical profiles**

Blood sampling: On the last day of each month, blood samples (approximately 10 mL) were collected from the jugular vein of each cow before the morning feeding in tubes containing sodium heparin as an anticoagulant. Plasma samples were separated by centrifuging at 1,000×g for 20 min, and supernatant obtained was stored at –20°C for further analysis. Progesterone hormone were determined using applicable kits (Jiuqiang Biological Technology Co., Ltd, Beijing, China) and an automated analyser (HITACHI 7020 Automated Biochemical Analyzer; HITACHI, Tokyo, Japan).

### 3.1.6 Statistical analyses

Data collected were subjected to Multivariate Analysis of Variance (ANOVA) by using the R (64 bit) computer software to compare treatment effects and pregnancy rates using the Chi-squared test and to compare progesterone hormone by using the one-sample t-test. Effects were accepted as significant at the 95% probability level. Dalgaard (2002)

## 3.2. Experiment 2: Effects of creep feeding on growth performance and hematology in Khao Lamphun calves

In total, 72 Khao Lamphun calf from the Phayao Livestock Research and Breeding Center, Phayao were used as experimental animals. The animals were randomly divided into 2 groups : 1) Control group –no creep feed supplementation for calves (n=36) and 2) Treatment group – creep feed supplementation for calves (n= 36). The animals were raised in an open pasture on and fed on a diet containing either Ruzi grass or rice straw with supplement in cow to Crude Protein concentrate were 18 % (Table 1) for 90 day.

The experiment was designed according to a paired observation. Production performance was recorded based on body weight gain (BWG), Heart girth (HG), Hip height (HH) and Body length (BL).

### 3.2.1 Feed Management

The experimental animals were divided into 2 treatment groups. Natural service breeding was used as feed sources in the control group. The diet for creep feed was a mixture of cassava chips, corn germ meal, rice bran, soybean meal, molasses, urea, DCP 18 and premix (Table 8). Hand plucked pasture samples (n=3 samples, Samples were sent in triplicate to a commercial laboratory for chemical composition analysis ( Dairy One Laboratory, Ithaca, NY) . Samples were analyzed by wet chemistry procedures for concentrations of CP (method 984.13; AOAC, 2006) and NDF (Van Soest et al. 1991; method by using an Ankom 200 fiber analyzer, Ankom Technology Corp.).

The creep feed group was fed with of the with supplement in cow to crude protein concentrate were 18 % (Table 8). Creep supplements are usually fed at 1.5% of calf body weight per day. with about 0.25 kg per head being fed daily for the first few days. As more

of the calves start eating and all the feed is cleared up, the quantity can be increased every few days by between 0.25 and 0.5 kg per head until the desired level is reached.

Proximate analysis of Dry Matter (DM), Crude Protein (CP), Crude Fiber (CF), Ether extract (EE) according to the method AOAC (1990). Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) Analysis according to the method of (Van Soest et al. 1991) and calculate the value. Nitrogen Free Extract (NFE).



Figure 4 A permanent creep is useful where a concentrated ration is fed to calves.

### 3.2.2 Chemical composition of the experimental diet

The chemical composition of the experimental diet consists of dry matter (DM) 87.27%, ash 5.35%, crude protein (CP) 18.23%, crude fiber (CF) 5.50%, ether extract (EE) 1.90%, neutral detergent fiber (NDF) 40.76%, acid detergent fiber (ADF) 8.12% and nitrogen free extract (NFE) 56.31%. (Table 8)



*Table 8 Percentage of feedstuffs in the creep feed ration*

Ingredient	Concentrate CP 18%
Cassava chips (kg.)	29.00
Corn germ meal (kg.)	24.70
Rice bran (kg.)	26.00
Soybean seeds (kg.)	17.00
Molasses (kg.)	1.00
Urea	1.00
DCP 18	0.8
Premix	0.5
Total	100
Calculated composition (dry matter basis)	
DM	87.27
OM	12.74
Ash	5.35
CP	18.23
CF	5.50
EE	1.90
ADF	8.12
NDF	40.76
NFE	56.31

<sup>1</sup> Price/baht = price of feedstuff local area in Phayao Province

<sup>2</sup> DM = dry matter, OM = Organic Matter, Ash =, CP = crude protein, EE = ether extract, CF = crude fiber, NFE = nitrogen free extract.

### 3.2.3 Study Area

The experiment was conducted at Phayao Animal Research and Breeding Center. The terrain and climatic characteristics are latitude at 19° 9' north and longitude at 99° 55' east at a moderate sea level of 440 meters, the average tropical savannah temperature of 25°C and an average relative humidity of 73%. (Department of Land Development, 2007)

### 3.3.4 Data collection

#### Body weight

The calves (aged 2 month) were weighed at the beginning of the study and at monthly intervals and the values were recorded in kilogrammes (kg). Average daily gain was calculated by dividing the difference between consecutive body weight values by the number of days in the interval. 18%). Cows were weighed at 08.00 h after period of water and feed with creep feed. Calves remained with their dams during this period.

#### Body proportions

Body proportions was measured for heart girth (HG) and body length (BL) measurements. Hip height (HH) measurements were obtained with a metric measuring stick. The measurements were carried out on the animals in a 'forced station', with anterior and posterior members perpendicular on a flat floor, forming a rectangular parallelogram support base. For each biped, when seen in profile, each limb concealed the other and when seen from the front/back, the members were upright and equally supported on the floor (Hoffman, 1997).



Figure 5 Body proportions was measured for calves

### **Hematology and blood biochemical profiles**

Blood sampling: On the last day of each month, blood samples (approximately 10 mL) were collected from the jugular vein of each bull before the morning feeding in tubes containing sodium heparin as an anticoagulant. Plasma samples were separated by centrifuging at 1,000×g for 20 min, and supernatant obtained was stored at –20°C for further analysis. Glucose, total cholesterol were determined using applicable kits (Jiuqiang Biological Technology Co., Ltd, Beijing, China) and an automated analyser (HITACHI 7020 Automated Biochemical Analyzer; HITACHI, Tokyo, Japan).

#### **3.3.4 Ethics in the use of experimental animals**

The study was conducted in accordance with the standards of laboratory animals for scientific purposes, which are supervised by the Animal Conduct Control Committee for Scientific Work, Phayao University

#### **3.3.5 Statistical analyses**

Initial values were calculated for BW, ADG, FBW, BWG, HG, HH, and BL. Differences between means were tested using one-way ANOVA range test for significance at the  $p < 0.05$  level. Using R 64-bit version 3.6.1. (Dalgaard, 2002)

## Chapter IV

### RESULTS

#### 4.1 Experiment 1 effects of Concentrate Feed Supplementation on the Reproductive Systems, Progesterone and Estradiol Levels in the Khao Lamphun Cows

##### 4.1.1 Chemical composition analysis

###### 4.1.1.1 Chemical composition of the experimental roughage.

It was found from the results of the study that (Table 1) the chemical composition of Ruzi grass consists of dry matter (DM) 90.40%, ash 8.60%, crude protein (CP) 10.20%, crude fiber (CF) 23.40%, ether extract (EE) 2.38%, neutral detergent fiber (NDF) 63.20%, acid detergent fiber (ADF) 29.20% and nitrogen free extract (NFE) 45.82%.

The chemical composition of rice straw consists of dry matter (DM) 92.64%, ash 13.40%, crude protein (CP) 2.52%, crude fiber (CF) 29.63%, ether extract (EE) 2.14%, neutral detergent fiber (NDF) 70.24%, acid detergent fiber (ADF) 51.16% and nitrogen free extract (NFE) 44.96%.

###### 4.1.1.2 Chemical composition of the experimental diets.

The chemical composition of CP 12% consists of dry matter (DM) 93.33%, ash 4.39%, crude protein (CP) 12.25%, crude fiber (CF) 7.77%, ether extract (EE) 2.86%, neutral detergent fiber (NDF) 42.45%, acid detergent fiber (ADF) 6.48% and nitrogen free extract (NFE) 65.81%.

Table 9 Chemical composition of feed for raising Khao Lamphun cows

Item	Roughage		Concentrate
	Ruzi grass	Rice straw	CP 12%
DM	90.40	92.64	93.33
Ash	8.60	13.40	4.39
CP	10.20	2.52	12.25
CF	23.40	29.63	7.77
EE	2.38	2.14	2.86
NDF	63.20	70.24	42.45
ADF	29.20	51.16	6.48
NFE	45.82	44.96	65.81

#### 4.1.2 Body condition score

condition score record, it was found that the cows who did not concentrate diet had mean body condition score of  $3.04 \pm 0.14$  lower than that of cows who did not concentrate dietary supplement  $3.39 \pm 0.21$  were statistically significant ( $P = 0.001$ )

Table 10 Effects of body condition score (BCS) at postpartum nutrition supplementation on change in BCS of primiparous Khao Lamphun cows

item	No Supplementation	Supplementation	P-value*
	(N = 36)	(N = 36)	
Body condition score (BCS):			
BCS at 2-month calving	2.72±0.25	2.82±0.27	0.120
BCS at the end of treatment	3.04±0.14	3.39±0.21	0.001

The numbers within a row with different superscripts are significantly different ( $P < 0.05$ ).

#### 4.3 Percentage of pregnant cows

The effect of supplementation concentrate on pregnancy rate is shown in Table 4. The supplementation concentrate, which is the ratio of pregnancy responses of treated cows to the proportion of pregnancy responses of supplementation cows, at 30, 60 and 90 d was

63.89 % (23/36), 97.22 % (35/36), 100 % (36/36) increased as than no supplementation was 50 % (18/36), 91.67 % (33/36), 100 % (36/36) and was not significantly different.

Table 11 Percentage of pregnant nutrition supplementation in Khao Lamphun cows

After supplementation (day)	No Supplementation (N = 36)	Supplementation (N = 36)	P-value*
30	50 % (18/36)	63.89 % (23/36)	0.193
60	91.67 % (33/36)	97.22 % (35/36)	0.686
90	100 % (36/36)	100 % (36/36)	1.000

The numbers within a row with different superscripts are significantly different ( $P < 0.05$ ).

Days open were expressed as percentages of herd life to standardize measures of reproductive efficiency and relative time dry when cows had variable length of herd life. The cows which after calving in supplementation concentrate had the short Day Open (Fig 1). The mean Day Open length decreased from supplementation at 30 to 90 day.

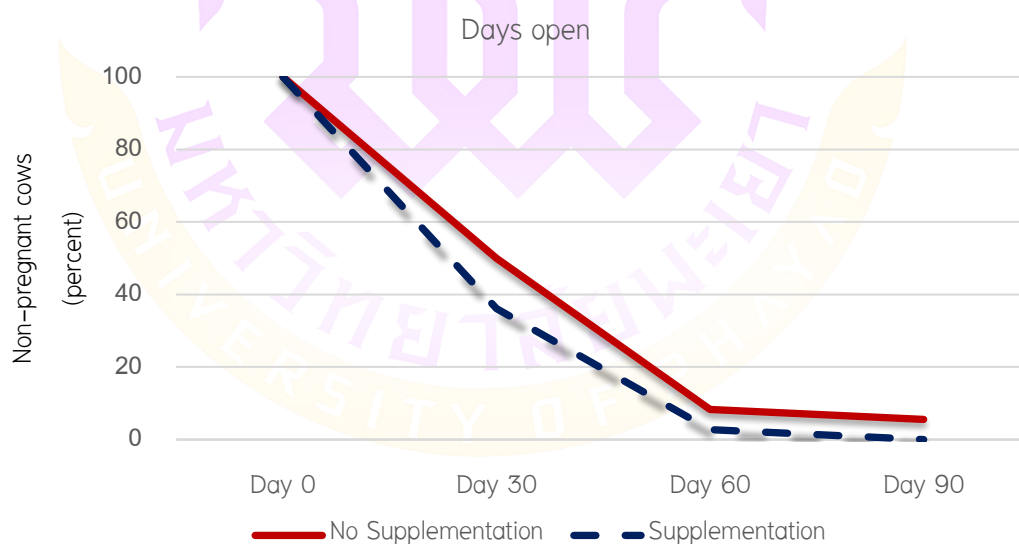


Figure 6 Survival curves for proportion of open day cows by days of breeding season (BS) for supplementation Cp 12% (dashed line;  $N = 36$ ) or non-supplementation Cp 12% (dotted line;  $N = 36$ ) by natural service during a 180-day BS.

#### 4.4 Progesterone after concentrate supplementation

The progesterone level in Khao Lamphun cows at began experiment non supplementation more than supplementation did show any significant difference ( $P=0.01$ ) is  $10.21 \pm 5.18$  ng/ml and  $2.70 \pm 2.84$  ng/ml respectively. Mean progesterone showed an accreting trend from 30 to 60 day and also, an increasing trend up to day 90. There were significant differences between after supplementation 30 day ( $10.55 \pm 12.61$  ng/ml) compared with the amount non supplementation ( $7.53 \pm 6.48$  ng/ml) respectively ( $p = 0.01$ ). Progesterone values were significantly accreting until day 60 and 90 day. There were significant differences between after supplementation ( $24.26 \pm 9.14$  ng/ml and  $15.66 \pm 7.17$  ng/ml) compared with the amount non supplementation ( $16.36 \pm 5.00$  ng/ml and  $10.37 \pm 3.55$  ng/ml) respectively ( $p < 0.001$ ; Figure 2)

Table 12 The progesterone of Khao Lamphun cows

Days	Progesterone (ng/ml)		P-value
	No Supplementation	Supplementation	
0	10.21±5.18	2.70±2.84	0.010
30	7.53±6.48	10.55±12.61	0.015
60	16.36±5.00	24.26±9.14	<0.001
90	10.37±3.55	15.66±7.17	<0.001
Average	12.51±1.56	12.05±2.56	

The numbers within a row with different superscripts are significantly different ( $P < 0.05$ ).

#### 4.5. Estradiol after supplementation concentrate

The estrous cycle is regulated by hormones of the hypothalamus, the pituitary, the ovaries and the uterus. Estradiol concentration increased during follicular phase of estrous cycle. The summary of statistical data for the concentrations of oestradiol in cows in Table 13, The Estradiol level in Khao Lamphun cows at began experiment non supplementation more than supplementation did show any significant difference ( $P=0.001$ ) is  $0.31 \pm 0.19$  ng/ $\mu$ g L<sup>-1</sup> and  $0.27 \pm 0.16$   $\mu$ g L<sup>-1</sup> respectively. Mean progesterone of supplementation group showed an accreting trend from 30 to 60 day and also, an become lower up in day 90.

There were significant differences between after supplementation 30 day ( $0.78 \pm 0.70 \mu\text{g L}^{-1}$ ) compared with the amount non supplementation ( $0.58 \pm 0.31 \mu\text{g L}^{-1}$ ) respectively ( $p = 0.002$ ). But, after supplementation 60 day non supplementation group more than supplementation did show any significant difference ( $P < 0.001$ ) is  $1.06 \pm 0.35 \text{ ng}/\mu\text{g L}^{-1}$  and  $0.80 \pm 0.51 \mu\text{g L}^{-1}$  respectively. Estradiol level were significantly accreting in 90 day. There were significant differences between after supplementation ( $0.63 \pm 0.19 \mu\text{g L}^{-1}$ ) compared with the amount non supplementation ( $0.40 \pm 0.50 \mu\text{g L}^{-1}$ ) respectively ( $P = 0.004$ )

Table 13 The Estradiol of Khao Lamphun cows

Days	Estradiol (ng/ml)		P-value
	No Supplementation	Supplementation	
0	$0.31 \pm 0.19$	$0.27 \pm 0.16$	0.001
30	$0.58 \pm 0.31$	$0.78 \pm 0.70$	0.002
60	$1.06 \pm 0.35$	$0.80 \pm 0.51$	<0.001
90	$0.40 \pm 0.50$	$0.63 \pm 0.19$	0.004
Average	$0.65 \pm 0.10$	$0.63 \pm 0.11$	

The numbers within a row with different superscripts are significantly different ( $P < 0.05$ ).



## Chapter V

### CONCLUSION

#### Discussion

#### 5.1 Experiment 1 Effects of Concentrate Feed Supplementation on the Reproductive Systems, Progesterone and Estradiol Levels in the Khao Lamphun Cows

##### 5.1.1 Chemical composition analysis

##### 5.1.1.1 Chemical composition of the experimental roughage and diets.

Corn germ meal is composed of protein which can be used as an important food source. it contains protein 7.4% (Bureau of animal nutrition, 2016). Corn dust can be mixed in food by up to 60% to reduce production costs without affecting the consumption of cattle (Chairinkum et al, 2013). Fine bran can be used as a source of energy for cattle. The chemical composition of proteins is 12.1% and fat is 4.2% (Bhatt et al.,2005). Cassava is composed almost entirely of carbohydrate which can be used as an important food source. it contains Crude protein 2.3% (Nakhonrachasima Animal Nutrition Research and Development Center, 2009). Cassava can be mixed in food by up to 50% to reduce production costs without affecting the consumption of cattle (Napasirth et al, 2005).

##### 5.1.2 Effects of concentrate supplementation and body condition score progesterone and estradiol to conception rate

Supplemented cows had better body condition score than non-supplemented cows, and this may be accounted for by the provision of extra nutrients by the supplement to meet nutrient demands by the cows thus enhancing microbial synthesis for improved growth and development. Concentrate supplementation in pasture-based systems is usually determined by the average nutritional requirements of the herd, rather than by those of individual cows. Many of the early experiments comparing individualized and flat-rate feeding strategies (Gill and Kaushal, 2000) were conducted with all cows having ad libitum

access to forage and concluded that individualized feeding of concentrate supplements gave no production advantage over flat-rate feeding.

Studies of (Ciccioli et al., 2003) investigating the impact of postpartum nutrition and BCS at calving on reproductive function, postpartum nutrition has been shown to influence (Ciccioli et al., 2003) Additionally, when cattle that received an increased plane of nutrition for greater weight (0.90 kg/day) gains were moved to a maintenance diet, they had elevated ( $P < 0.01$ ) plasma NEFA concentration in relation to cows that were targeted for lower gains (0.45 kg/day) (Ciccioli et al., 2003) This indicates that the maintenance diet may not have provided adequate nutrition for cattle previously receiving an increased plane of nutrition to truly maintain cows BW and BCS.

Results of a more study in Ghana, this study determined the effects of feed supplementation during the postpartum period (Obese et al., 2018), indicated that cows Resumption of postpartum ovarian activity was determined by measuring progesterone concentration in the plasma from weeks 1 to 10. Supplemented cows had a better body condition score (6.2 versus 5.8;  $P < 0.05$ ) and higher partial milk yield (1.94 versus 1.55 L/day;  $P < 0.01$ ) than non-supplemented cows. Sanga cows had a better body condition score (6.2 versus 5.8;  $P < 0.05$ ) but lower milk yield (1.58 versus 1.92 L/day;  $P < 0.01$ ) than the Friesian-Sanga crossbreds.

Results of a more study in Australia, where cows were offered a restricted pasture allowance (Garcia et al., 2007), indicated that cows fed a concentrate supplement based upon an individual requirement produced 7% more fat and protein compared with cows fed at a fixed rate based on an overall herd requirement. This study also indicated significant between-cow variation in DMI ( $CV = 32\%$ ), highlighting the potential for exploiting this variability through individualized feeding strategies.

(Lents et al., 2008) Supplemented cows had a better body condition score than non-supplemented cows. if a cow had a low body condition score have a 10% lower conception rate of postpartum. (Lopez-Gatius et al., 2003). Supplemented cows had high body condition score have a lower open day of postpartum

### 5.1.3 Progesterone and estradiol after supplementation concentrate

Luteinizing hormone plays an important role in reproduction by assisting in the final maturation of the dominant follicle and inevitably leading to ovulation (Hess et al., 2005). A pulsatile release of GnRH is responsible for the production and release of LH. And the estrous cycle is regulated by hormones of the hypothalamus, the pituitary, the ovaries and the uterus (Forde et al., 2011). Estradiol 17- $\beta$  concentration increased during follicular phase of estrous cycle. Noseir (2003) reported that the increase in follicular size was associated with an increase in estradiol 17- $\beta$  concentration, it means that the level of blood estradiol can use to determine stage of follicle development. Putro et al (2014, in press), the level of hormone in the follicular fluid as same as with blood such as thyroid hormone. Follicular fluid (FF) is an avascular compartment separated from the perfollicular stroma by the follicular wall within the mammalian ovary (Abd Ellah et al., 2010; Albomohsen et al., 2011; Nasroallah et al., 2012).

Reproductive success in beef cattle is reliant on proper endocrine function and hormone concentrations. It has been shown that increased concentrations of P4 and estradiol are linked with increased pregnancy maintenance (Atkins et al., 2013). Nutrition can influence hormone concentrations (Hess et al., 2005). Also, supplemental fats have been shown to increase concentrations of serum cholesterol (Lammoglia et al., 1996) and could then increase P4 concentration as cholesterol serves as a precursor for P4 production [Funston, 2004]. Cows with higher serum cholesterol concentrations from 2 weeks prior to parturition until 4 weeks postpartum had a decreased ( $P < 0.05$ ) interval from calving to resumption of ovarian cyclicity (Gudon et al., 1999)

## 5.2. Experimental 2 Effect of creep feeding on grow performance and hematology in calf Khao Lamphun Cattle

### 5.2.1 Chemical composition analysis

5.2.1.1 Chemical composition of the experimental roughage and diets.

### 5.2.2 Growth Performance of khao Lamphun calf

We found that at birth weight khao Lamphun calf in Cp 18 % treatment group ( $18.06 \pm 0.53$ ) and control group ( $17.89 \pm 0.57$ ) no different ( $P=0.206$ ). When khao Lamphun calf were feed with Cp18% they have weaning weight ( $101.92 \pm 13.00$ ) more than control group ( $89.17 \pm 10.43$ ) different ( $P < 0.001$ ). The ADG of treatment group Cp18% ( $0.42 \pm 0.06$ ) better than control group ( $0.36 \pm 0.05$ ) different ( $P < 0.001$ ). Calf was fed 18% crude protein had greater weaning weight and ADG. For creep fed calves over control group was consistent with the reports of Shike et al. (2007), Parish and Rhinehart (2009) and Vinales et al. (2013). Shike et al. (2007) reported 21% higher body weight gain (BWG) in creep fed calves compared to non-creep fed animals. In the present stud.

### 5.2.3 Blood hematological of khao Lamphun calf

The importance of haematological indices in animal husbandry is well acknowledged. Metabolic disturbances usually by inappropriate feeding without overt clinical manifestation could cause reduced performance and productivity (Radostits et al., 2000; Mamun et al., 2013). Such altered metabolic status could be reflected in altered haematological indices. The significantly different haematological indices between treatments in the present study could relate to the different feeding materials and feeding regimes. Mamun et al. (2013) had stated that factors such as nutrition and stress affect blood values of animals. The significantly higher Hb, and RBC in the control animals could indicate higher oxygen capacity of the blood as a result of greater demand of oxygen by the tissues while the higher neutrophil and neutrophil: lymphocyte ratio indicate greater stress profile of non-creep fed calves compared to creep fed animals. The haematological values reported in the present study were however consistent with some published blood parameters of apparently healthy calves.

Nutrition among other factors influences serum total protein, urea, globulin and bilirubin concentrations (Klinkon and Jezek, 2012). Serum protein and urea concentration is hence influenced by the amount of protein in diets. Klinkon and Jezek (2012) stated that increased urea concentration in serum of calves is indicative of increased protein catabolism. On the other hand, creatinine is synthesized during endogenous metabolism in muscles and

do not depend on nutrition (Klinkon and Jezek, 2012). The similarity in serum creatinine levels of experimental units is hence not surprising. AST (SGOT) activity is increased above normal range in pathological situations that cause cell necrosis such as damage to liver cells (Klinkon and Jezek, 2012).

#### **5.2.4 Glucose after supplementation concentrate in calf**

The chemical composition of the blood stream found that the glucose concentration is normal at Milliliters deciliter the normal value of blood glucose is 40–80 milligrams deciliter. (Frandsen et al., 2009) However, the glucose concentration in Lamphun white calves at the age of 2 months was higher than normal and found that the control calves were  $105.33 \pm 25.11$  milligrams of deciliter. Less than calves fed with enriched foods  $109.40 \pm 6.39$  milligrams ( $P > 0.05$ ) Calves under 3 months of age were given a concentrated diet with  $111.20 \pm 9.78$  higher than the control calves with  $94.80 \pm 22.87$  milligrams ( $P > 0.05$ ). Calves aged 4 months; control group had the mg deciliters. Was higher than those receiving enriched diets  $80.60 \pm 6.02$  milligrams ( $P > 0.05$ ). Calves at the age of 5 months were given a concentrated diet supplement with  $80.50 \pm 6.95$  milligrams. Higher than calves in the control group was  $77.33 \pm 3.51$  milligrams ( $P > 0.05$ ). And at the age of 6 months, calves in the control group were  $64.60 \pm 11.82$  milligrams of deciliter. Were higher than the group receiving  $47.20 \pm 16.99$  milligrams of enriched food ( $P > 0.05$ ), respectively (Table 4). The concentration of glucose in the bloodstream indicates the energy balance in the calves. Due to the amount of sugar in the blood stream from fermentation, digestion and absorption of glucose and volatile fatty acids. Especially the pionic acid from the absorption into the blood and then converted into glucose (Metha 2016). The remaining energy will be used for fat synthesis. To accumulate in different organs in the animal body

#### **5.2.5 Cholesterol after supplementation concentrate in calf**

From the study of cholesterol in the bloodstream, Lamphun white calves showed that the calves in the control group had an average cholesterol of  $149.67 \pm 13.32$ , lower than the calves fed with the average supplement of  $163.60 \pm 35.42$  was not statistically different ( $P = 0.547$ ). And the group that received dietary supplement at age 3, 4, 5 and

6 months had the amount of cholesterol. ( $178.80 \pm 7.69$ ,  $179.60 \pm 18.82$ ,  $209.25 \pm 18.60$  and  $214.00 \pm 17.51$  respectively) were higher than the control group. ( $143.20 \pm 7.60$ ,  $144.60 \pm 22.68$ ,  $159.00 \pm 7.00$  and  $173.20 \pm 21.95$  respectively) statistically different (Table 9) The amount of high-density cholesterol (cholesterol high-density lipoprotein, HDL-cholesterol), which is good fats that calves receive. Is a substance that has properties to help boost the immune system in the body Preventing the integration between oxygen and lipid substances. Or fat of the cell wall (Suan Chit, 1992)

## Conclusion

### **Experiment 1 Effects of Concentrate Feed Supplementation on the Reproductive Systems, Progesterone and Estradiol Levels in the Khao Lamphun Cows**

Supplemented cows had better body condition score than non-supplemented cows. Feed supplementation of cows during lactation thus assisted in meeting nutrient requirements for maintenance and maximum production.

### **Experimental 2 Effect of creep feeding on grow performance and hematology in calf Khao Lamphun Cattle**

Creep-fed calves achieved higher body weight and higher percent body weight gain than non-creep-fed calves over the study periods. Creep feeding influenced the haematological and serum biochemical indices of the calves although all values were within normal ranges. Given the seasonal nature of forage availability in the study environment, it is advisable to develop and adopt the practice of creep feeding calves to enhance their growth rate

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Appendix

## APPENDIX A

### Laboratory–Based Analyses (Proximate analysis)

#### Moisture Determination (MO)

Moisture is determined by the loss in weight that occurs when a sample is dried to a constant weight in an oven. About 2g of a feed sample is weighed into a silica dish previously dried and weighed. The sample is then dried in an oven for 650C for 36 hours, cool in a desiccator and weigh. The drying and weighing continues until a constant weight is achieved.

$$\% \text{Moisture} = \frac{\text{wt of sample + dish before drying} - \text{wt of sample+ dish after drying}}{\text{Wt of sample taken}} \times 100$$

Since the water content of feed varied very widely, ingredients and feed are usually compared for their nutrient content on moisture free or dry matter (DM) basis.

$$\% \text{DM} = 100 - \% \text{Moisture}$$

#### Ether Extract (EE)

The ether extract of a feed represents the fat and oil in the feed. Soxhlet apparatus is the equipment used for the determination of ether extract. It consist of 3 major components

1. An extractor: comprising the thimble which holds the sample
2. Condenser: for cooling and condensing the ether vapour
3. 250 ml flask

Procedure: about 150ml of an anhydrous diethyl ether (petroleum ether) of boiling point of 40 – 600 C is placed in the flask. 2–5g of the sample is weighed into a thimble and the thimble is plugged with cotton wool. The thimble with content is placed into the extractor; the ether in the flask is then heated. As the ether vapour reaches the condenser through the side arm of the extractor, it condenses to liquid form and drop back into the sample in the thimble, the ether soluble substances are dissolved and are carried into solution through the siphon tube back into the flask. The extraction continues for at least 4 hrs. The thimble is removed and most of the solvent is distilled from the flask into the

extractor. The flask is then disconnected and placed in an oven at 650C for 4 hrs, cool in desiccator and weighed.

$$\% \text{Ether extract} = \frac{\text{wt of flask + extract} - \text{tare wt of flask} \times 100}{\text{wt of sample}}$$

### Crude Fiber (CF)

The organic residue left after sequential extraction of feed with ether can be used to determine the crude fibre, however if a fresh sample is used, the fat in it could be extracted by adding petroleum ether, stir, allow it to settle and decant. Do this three times. The fat-free material is then transferred into a flask/beaker and 200mls of pre-heated 1.25% H<sub>2</sub>SO<sub>4</sub> is added and the solution is gently boiled for about 30mins, maintaining constant volume of acid by the addition of hot water. The buckner flask funnel fitted with whatman filter is pre-heated by pouring hot water into the funnel. The boiled acid sample mixture is then filtered hot through the funnel under sufficient suction. The residue is then washed several times with boiling water (until the residue is neutral to litmus paper) and transferred back into the beaker. Then 200mls of pre-heated 1.25% Na<sub>2</sub>SO<sub>4</sub> is added and boiled for another 30mins. Filter under suction and wash thoroughly with hot water and twice with ethanol. The residue is dried at 650C for about 24hrs and weighed. The residue is transferred into a crucible and placed in muffle furnace (400–6000C) and ash for 4hrs, then cool in desiccator and weigh.

$$\% \text{Crude fibre} = \frac{\text{Dry wt of residue before ashing} - \text{wt of residue after ashing} \times 100}{\text{wt of sample}}$$

### Crude Protein (CP)

Crude protein is determined by measuring the nitrogen content of the feed and multiplying it by a factor of 6.25. This factor is based on the fact that most protein contains 16% nitrogen. Crude protein is determined by kjeldahl method. The method involves: Digestion, Distillation and Titration.

### Digestion

weigh about 2g of the sample into kjeldahl flask and add 25mls of concentrated sulphuric acid, 0.5g of copper sulphate, 5g of sodium sulphate and a speck of selenium tablet. Apply heat in a fume cupboard slowly at first to prevent undue frothing, continue to digest for 45mins until the digesta become clear pale green. Leave until completely cool and rapidly add 100mls of distilled water. Rinse the digestion flask 2–3 times and add the rinsing to the bulk.

### Distillation

Markham distillation apparatus is used for distillation. Steam up the distillation apparatus and add about 10mls of the digest into the apparatus via a funnel and allow it to boil. Add 10mls of sodium hydroxide from the measuring cylinder so that ammonia is not lost. Distil into 50mls of 2% boric acid containing screened methyl red indicator

### Titration

The alkaline ammonium borate formed is titrated directly with 0.1N HCl. The titre value which is the volume of acid used is recorded. The volume of acid used is fitted into the formula which becomes

$$\%N = \left[ \frac{14 \times VA \times 0.1 \times w}{1000 \times 100} \right] \times 100$$

VA = volume of acid used w= weight of sample

%crude protein = %N × 6.25

### Ash

Ash is the inorganic residue obtained by burning off the organic matter of feedstuff at 400–6000 C in muffle furnace for 4hrs. 2g of the sample is weighed into a pre-heated crucible. The crucible is placed into muffle furnace at 400–6000C for 4hrs or until whitish–grey ash is obtained. The crucible is then placed in the desiccator and weighed

$$\% \text{Ash} = \frac{\text{wt of crucible+ash} - \text{wt of crucible}}{\text{wt of sample}}$$

### **Nitrogen Free Extract (NFE)**

NFE is determined by mathematical calculation. It is obtained by subtracting the sum of percentages of all the nutrients already determined from 100.

$$\% \text{NFE} = 100 - (\% \text{moisture} + \% \text{CF} + \% \text{CP} + \% \text{EE} + \% \text{Ash})$$

NFE represents soluble carbohydrates and other digestible and easily utilizable non nitrogenous substances in feed.



## Appendix B

## Proximate analysis

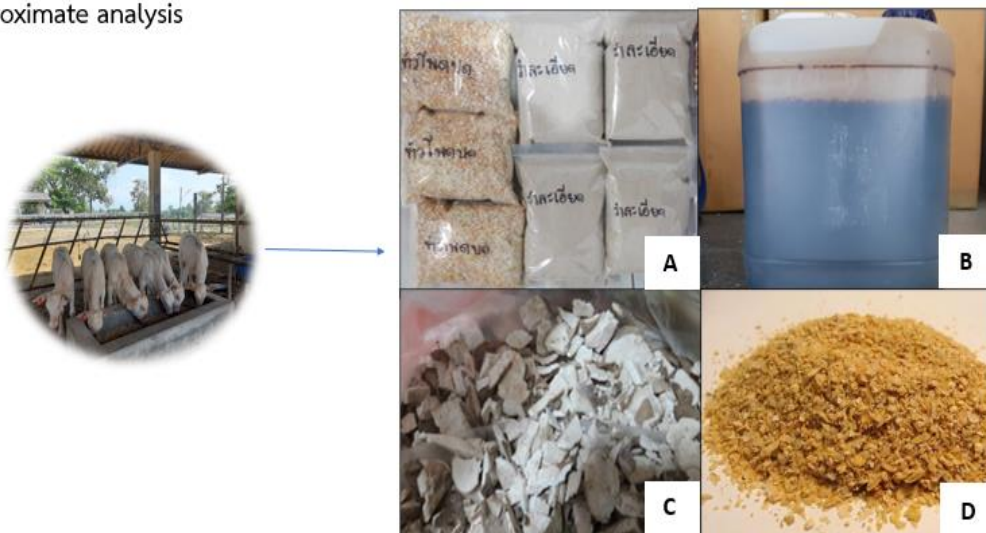


Figure 7 Local ingredients used in the concentrated formula for Khao Lamphun Cattle

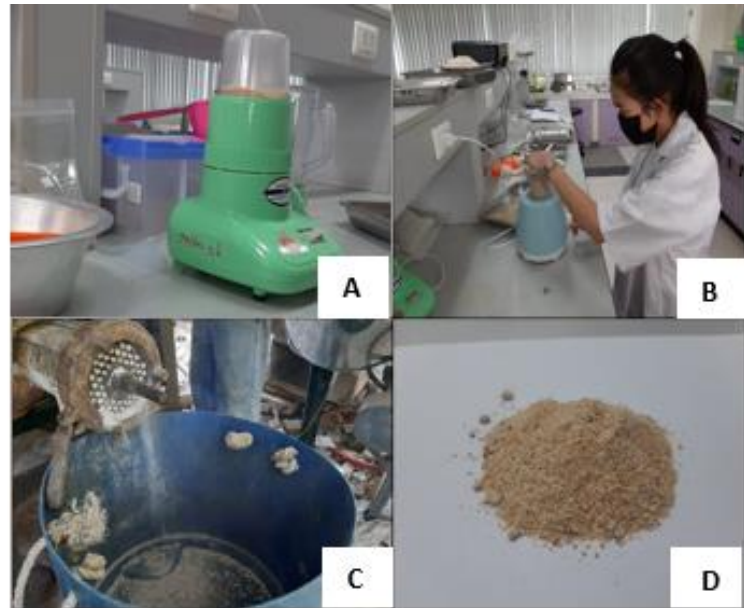
A: Crushed corn and rice bran

B: Molasses

C: Cassava

D: Soybean meal

### Proximate analysis



*Figure 8 How to prepare animal feed ingredients before taking to Proximate analysis to analyze the chemical composition*

A: roughages spinning into small particles (grass)

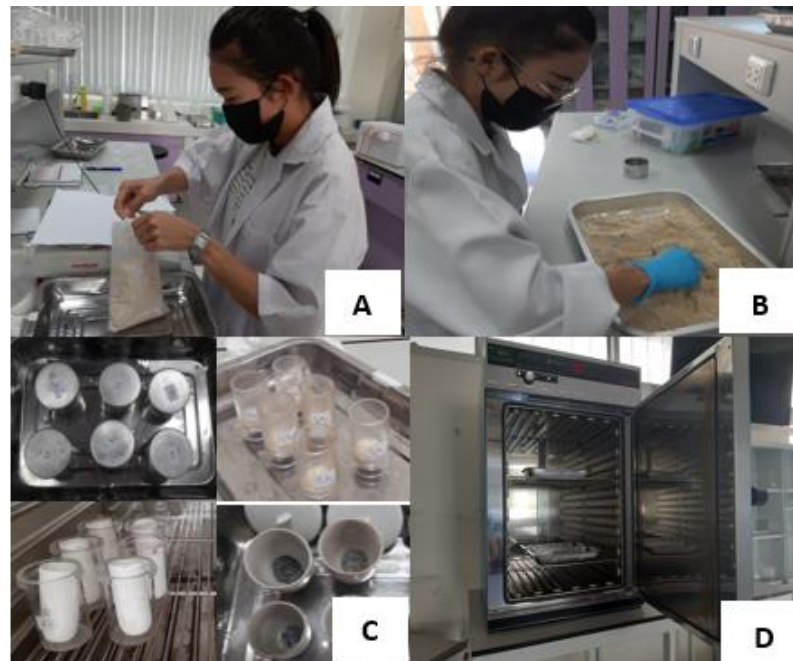
B: Blending of forage raw materials before assembling into a concentrated formula.

C: Cassava spinning into small particles

D: Finely ground animal feed ingredients that can be used for Proximate analysis.



## Proximate analysis



*Figure 9 The process of mixing concentrated food before analyzing the chemical composition*

- A: Weigh the raw material from the blender in proportion to the formula specified in the animal feed.
- B: Mix the proportion of raw food ingredients thoroughly for efficient analysis
- C: Chemical composition analysis
- D: Hot air oven for analysis in part of dry matter, moisture, protein, fat, crude fiber

## Proximate analysis

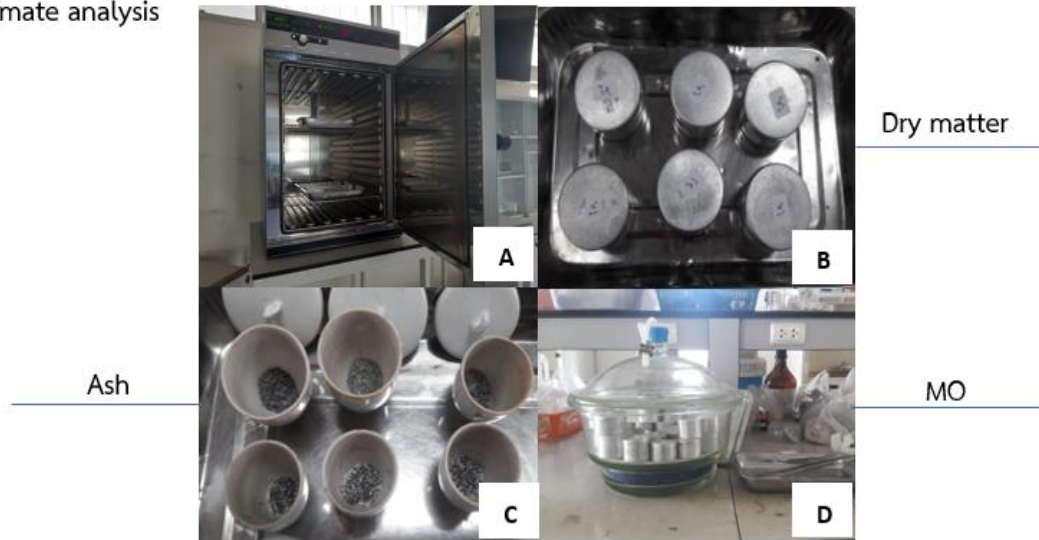
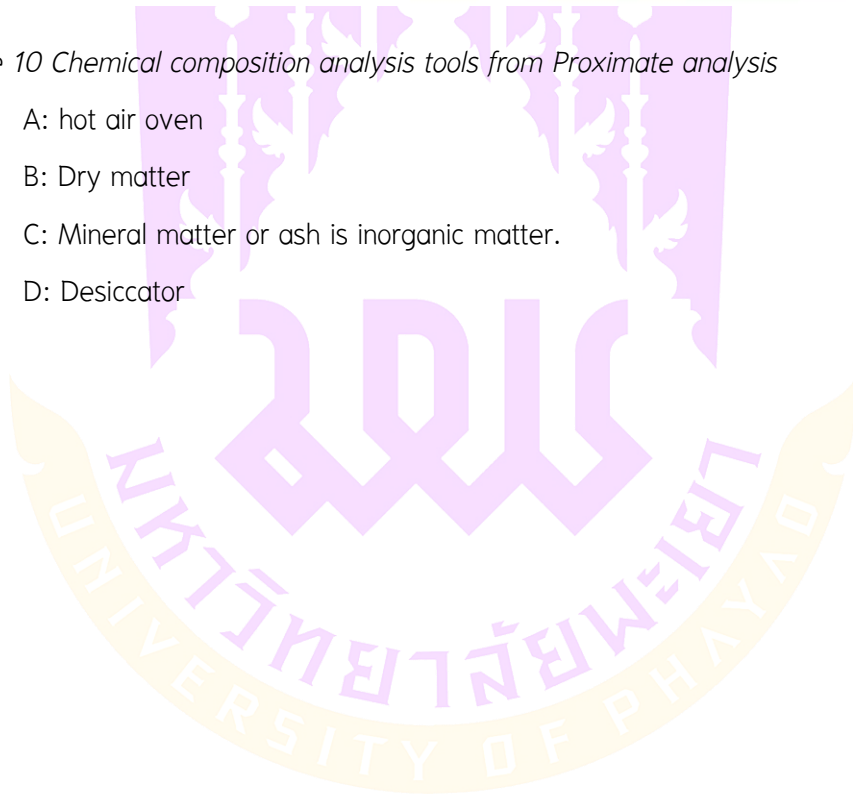


Figure 10 Chemical composition analysis tools from Proximate analysis

- A: hot air oven
- B: Dry matter
- C: Mineral matter or ash is inorganic matter.
- D: Desiccator



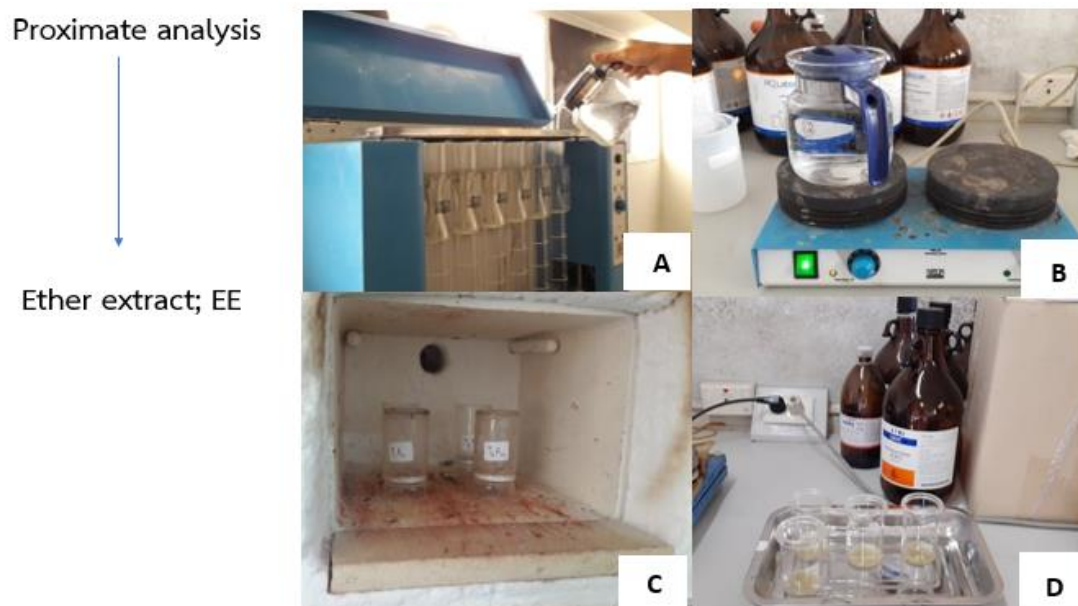


Figure 11 Ether extract content analysis by Proximate analysis

A: Ether extract

B: Boiling hot water to wash chemicals in the fat determination process

C: Burning cups to know the weight of fat

D: Fat from the sample analyzed

Proximate analysis



crude fiber ; CF

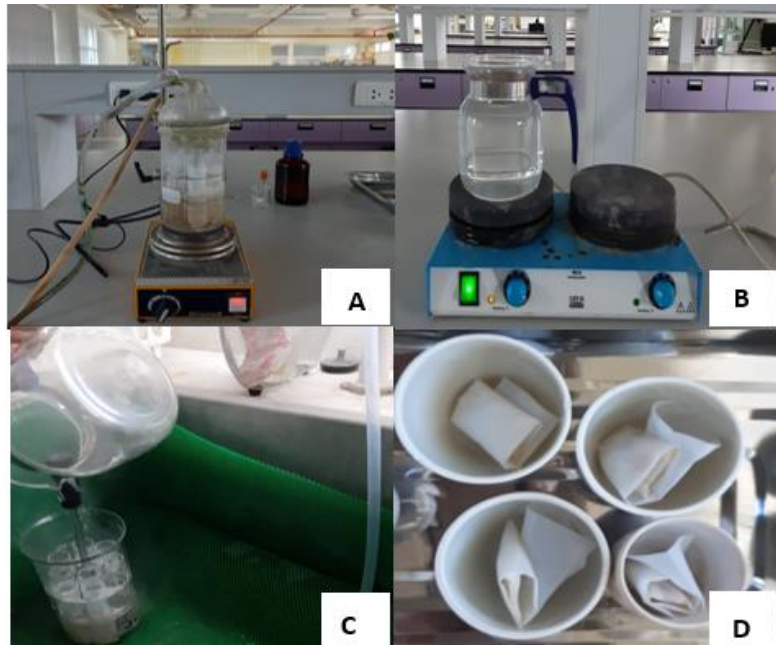


Figure 12 The crude fiber content analysis using Proximate analysis

A: crude fiber

B: Boil hot water to wash chemicals in ADF and NDF analysis.

C: Wash before burning

D: C: Burn at 550 °C



## Appendix II Feed of Khao Lamphun Cattle



Figure 13 Local ingredients used in the concentrated formula for Khao Lamphun Cattle

A: Preparing local ingredients to make a concentrated formula.

B: Mix the food to thicken evenly.

C: Concentrated food from self-mixing

D: Weighing mixed food according to the needs of the cows And Lamphun white

calves

## Roughage

The feed used in the study is a seasonal coarse diet. Each group was fed with of the two diets containing either Roughage were RuZi or Rice straw

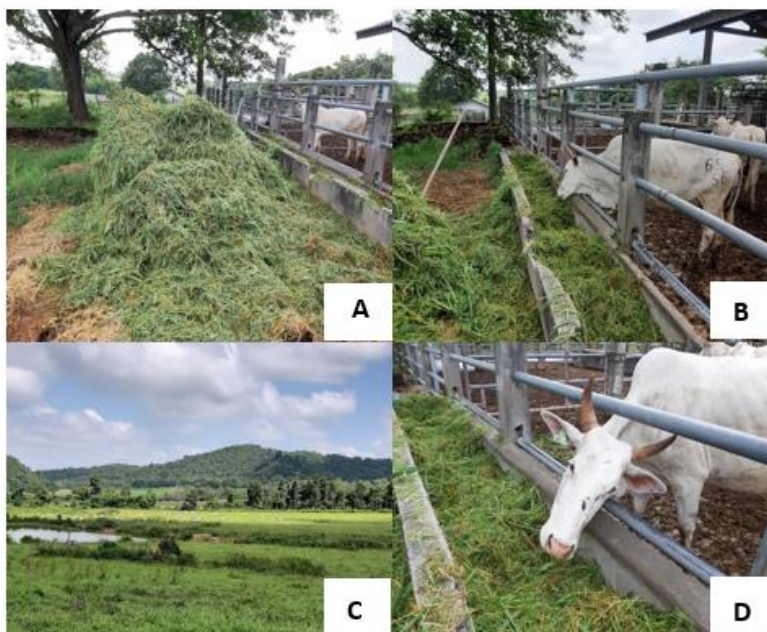


Figure 14 Pasture for raising cow and calf at Lamphun cattle at Phayao Livestock Research and Breeding Center, Phayao

- A: Grass that was cut for the cows before the experiment in the pen
- B: The cow receives grass during the activity
- C: Grass plot for releasing cow grazing
- D: Feeding the cow during cattle stall





Figure 15 Demonstration of stall preparation for use in experimental feeding of Creep calves

- A: Cattle stall, experimental group with Creep feed supplement
- B: stall pen before modification
- C: Appearance of food trough before modification
- D: Stable pen used in the experiment

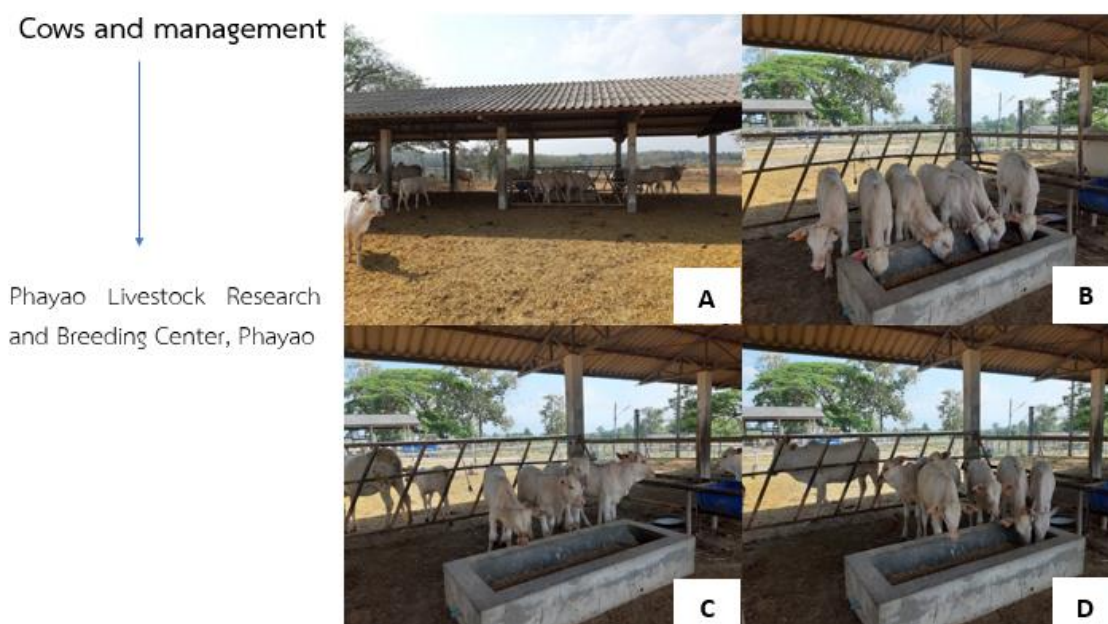


Figure 16 Calf feeding during the experiment. Using Creep feed in calves

- A: Calves during the trials of the group receiving the Creep feed supplement
- B: Calves fed with Creep feed
- C: Stable that cows cannot enter the feeding trough
- D: During the experiment, calves are able to fully feed





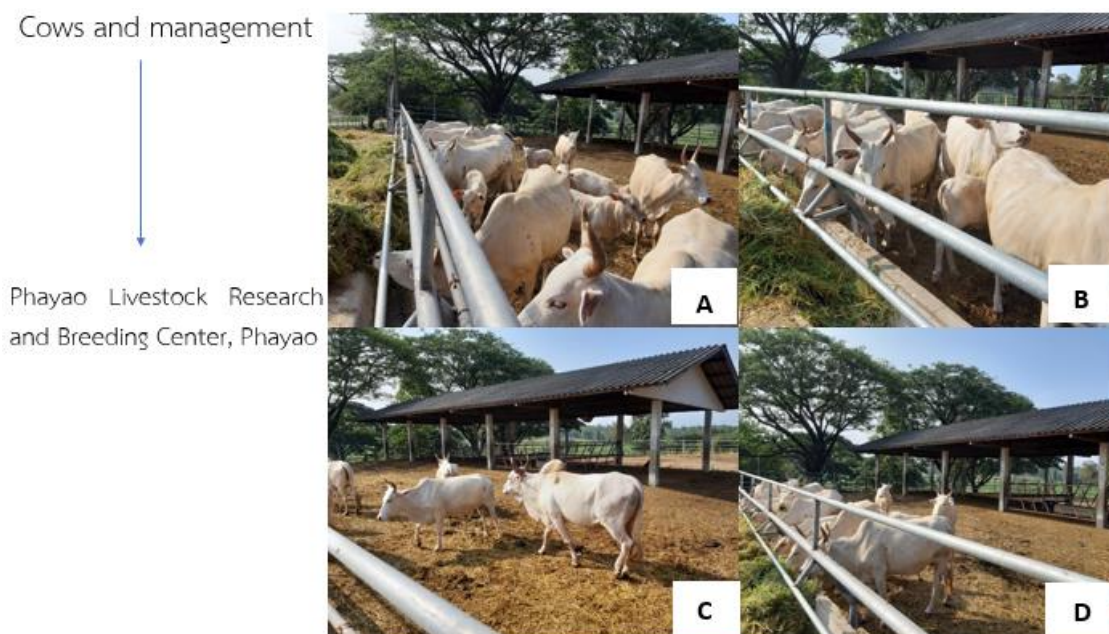


Figure 17 Cattle were exhibited after experimenting to feed Creep feed in Lamphun white calves.

A: Cow and calves are fed Creep feed.

B: Cow and calves after receiving rough feed in the pen

C: Cow whose calves have been fortified for 3 months have more fertility.

D: The cows have better physical condition after receiving the supplement. Clearly shows the distinctive characteristics of the species

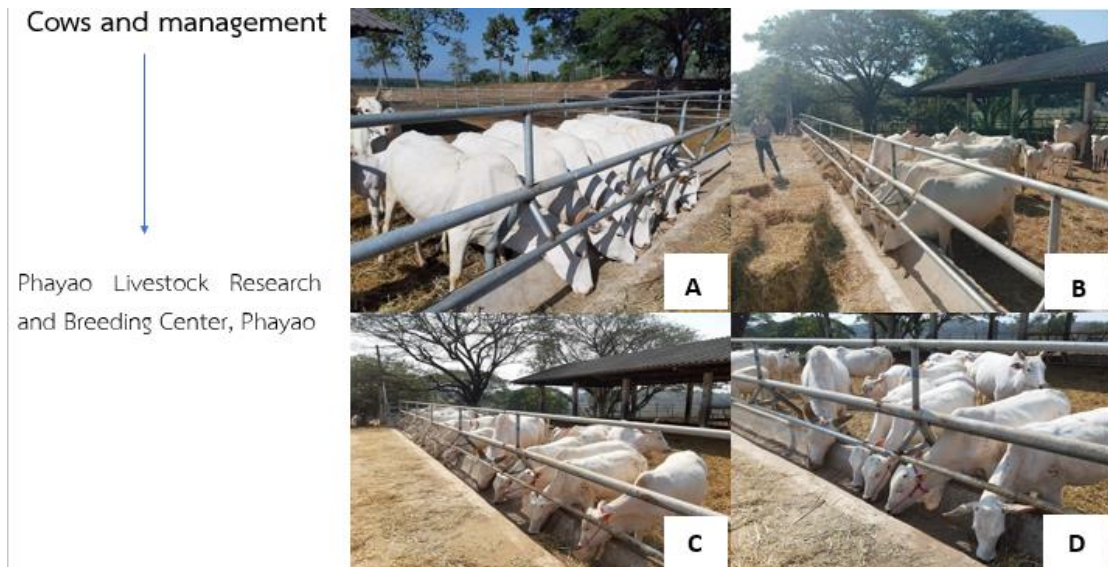


Figure 18 Cattle were exhibited after Creep feed feeding experiments.

A: Mother cows receive daily supplementation of the diet.

B: After supplementing the concentrated food for 6 months

C: The calf is fed with the concentrated feed with the cow And has the same physical structure as the breeder

D: Calves that have been fortified with good physical condition are suitable for breeders.



Study Data

↓

Birth weight  
weaning weight



Figure 19 An evaluation of growth performance by weighing the cows and calves in both groups.

A: Cattle stall

B: Assess the physical condition of the cow and calf

C: weigh calves

D: Characteristics of Weighing scale in Cattle stall





Figure 20 The body proportion assessment of calves in both groups

A: Embedding the cow into the envelope

B: Heart girth of calves

C: Hip height of calves

D: Body lengtht of calves



## Appendix Blood collection in cattle

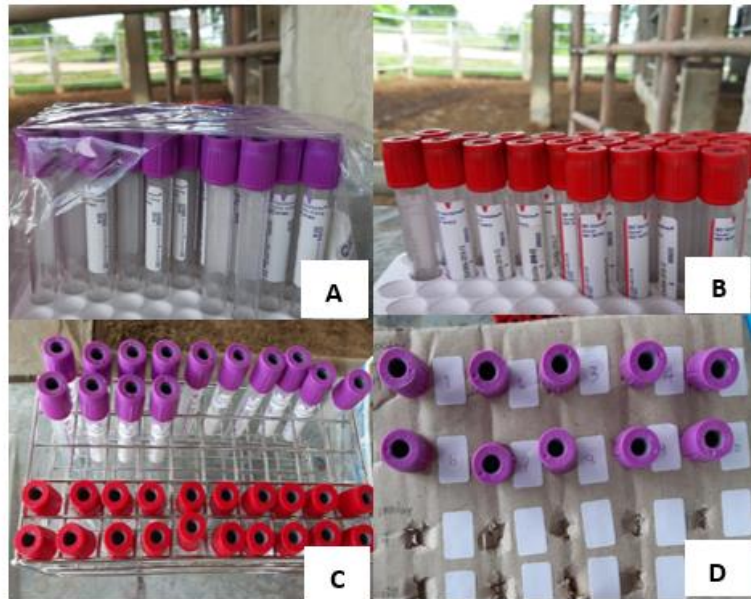
## Blood collection in cattle



Figure 21 Equipment used to draw blood to evaluate hormones in cows. And the health of Lamphun white cows

- A: Cotton
- B: Latex Gloves
- C: needle
- D: Syringe

### Blood collection in cattle



*Figure 22 Blood collection tubes used to draw blood to evaluate hormones in cows. And the health of Lamphun white cows*

- A: EDTA tube
- B: Plain tube (Clotted blood)
- C: Stainless Steel Test Tube Rack
- D: Blood sample

### Blood collection in cattle



*Figure 23 Blood sample collection used to assess hormones in cows And the health of Lamphun white cows*

A: Blood collection

B: Blood collection in a sample tube for analysis of CBC in the blood

C: Placing blood samples for serum separation To detect progesterone and estrogen values

### Blood collection in cattle

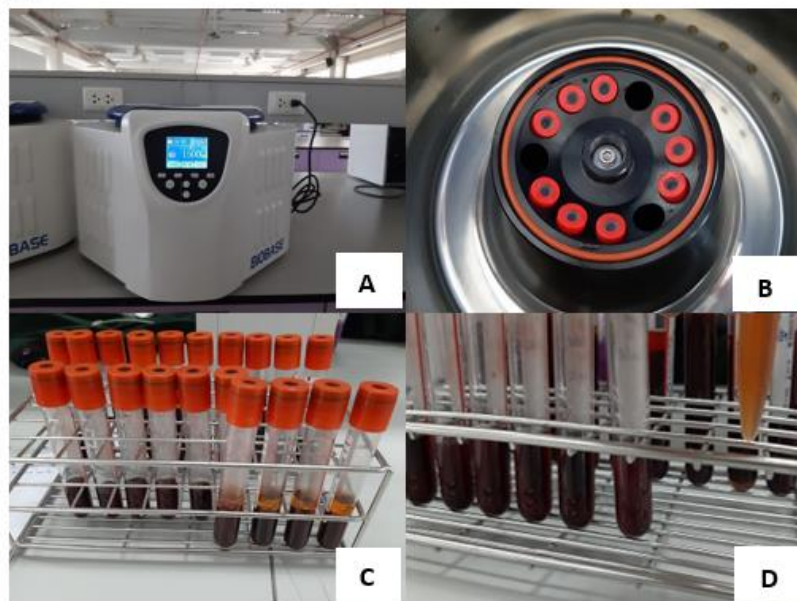


Figure 24 The process of analyzing the blood values to evaluate the hormones in the body of the Khao Lamphun Cattle

A: Centrifuge

B: Placement of blood vessels in centrifuge

C: Lamphun White Cow Blood Serum

D: Serum is packed in Micro Centrifuge Tube for progesterone and estrogen test.



## VITA

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