DEVELOPMENT OF PROBIOTIC YEAST PRODUCT TO IMPROVE RUMEN FERMENTATION AND ENHANCE PRODUCTIVITY IN DAIRY COWS

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การพัฒนาผลิตภัณฑ์โปรไบโอติกยีสต์ เพื่อปรับปรุงประสิทธิภาพการหมักในกระเพาะรูเมน และการเพิ่มประสิทธิภาพการผลิตในโคนม

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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาปรัชญาดุษฎีบัณฑิต
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Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

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การศึกษามีทั้งหมด 3 การทดลอง ดังนี้ 1) การคัดแยก การคัดเลือก และการตรวจสอบอัตลักษณ์ของยีสต์ที่คัดแยกจากสัตว์เคี้ยวเอื้อง ซึ่งประกอบด้วย แพะ กระบือ โคเนื้อ และโคนม เพื่อให้ได้โปรไบโอติกยีสต์ที่ดีที่สุด 2) การเสริมโปรไบโอติกยีสต์ต่อกระบวนการหมักในกระเพาะรูเมน ความหลากหลายของจุลินทรีย์ในกระเพาะรูเมน และ 3) การเสริมผลิตภัณฑ์โปรไบโอติกยีสต์ต่อผลผลิตน้ำนม องค์ประกอบทางเคมีของน้ำนม และค่าทางโลหิตวิทยา

การทดลองที่ 1 ศึกษาการคัดแยก การตรวจสอบอัตลักษณ์ และการคัดเลือกยีสต์ โดยคัดเลือกจากแพะ กระบือ โคเนื้อ และโคนม พิจารณาจากประสิทธิภาพของยีสต์แต่ละสายพันธุ์ คัดแยกเชื้อยีสต์ได้ทั้งหมด จำนวน 91 ไอโซเลต ด้วยวิธีการเลี้ยงเชื้อในอาหารเลี้ยงเชื้อบริสุทธิ์ที่เฉพาะเจาะจงทำให้เชื้อบริสุทธิ์โดยวิธีสติกเพลท ทำทั้งหมดจำนวน 3 ครั้ง ตรวจสอบอัตลักษณ์ด้วยวิธีการทดสอบการย่อยสลายน้ำตาล ชุดทดสอบ API® 20C AUX แล้วเปรียบเทียบผลปฏิกิริยาแต่ละประเภทกับเชื้อในฐานข้อมูล และทำการยืนยันชนิดของยีสต์ด้วยวิธีทางชีวโมเลกุล เปรียบเทียบลำดับของเบสในฐานข้อมูล เอ็น ซี บี ไอ พบว่า เชื้อยีสต์ในจีนัส แคนดิดา คือ แคนดิดา กลาบราต้า (ความเหมือน 99 เปอร์เซ็นต์ของชนิดคู่เทียบ) แคนดิดา ทรอปิคอลลิส (99 เปอร์เซ็นต์) แคนดิดา โรกูซ่า (98 เปอร์เซ็นต์) และอิสซาชีเกีย ออรินทรอลิส (99 เปอร์เซ็นต์) นำยีสต์ทดสอบจำนวน 12 ไอโซเลต สายพันธุ์จีโอ 10 116 และ 19 (คัดแยกจากแพะ) บียู 3 4 และ 7 (คัดแยกจากกระบือ) บีอี 1 2 และ 7 (คัดแยกจากโคเนื้อ) และดีซี 4 14 และ 18 (คัดแยกจากโคนม) มาทดสอบเพื่อคัดเลือกสายพันธุ์ที่ดีที่สุด พบว่า 1) ยีสต์ทุกสายพันธุ์สามารถเจริญเติบโตในสภาพความเป็นกรด-ด่างที่ระดับ 3.5-7.5 แต่สายพันธุ์บียู 3 และดีซี 18 จะมีประสิทธิภาพดีที่สุด ($P<0.01$) 2) ค่าความเป็นกรด-ด่างที่ระดับ 6.5 ยีสต์สายพันธุ์บียู 3 และดีซี 18 มีอัตราการเจริญเติบโตดีที่สุด ($P<0.01$) อย่างไรก็ตามในสภาวะไร้ออกซิเจน สายพันธุ์ดีซี 18 จะมีอัตราการมีชีวิตรอดดีที่สุด ($P<0.05$) และ 3) ยีสต์ 3 สายพันธุ์ คือ บีอี 7 บียู 3 และดีซี 18 พบว่าสายพันธุ์ดีซี 18 ปริมาณการใช้ 20 เปอร์เซ็นต์ของของเหลวที่ใช้ในการหมัก สามารถปรับปรุงประสิทธิภาพจลศาสตร์ในการผลิตแก๊ส ปริมาณแก๊สสะสม และเพิ่มกรดอะซิติก สัดส่วนกรดอะซิติกต่อโพรพิโอนิก

การทดลองที่ 2 ศึกษาระดับความเข้มข้นของผลิตภัณฑ์ยีสต์ที่มีชีวิตต่อกระบวนการหมักในกระเพาะรูเมน ความหลากหลายของจุลินทรีย์ในกระเพาะรูเมน และค่าโลหิตวิทยาในโคนม โดยใช้

โคนมเจาะกระเพาะจำนวน 4 ตัว วางแผนการทดลองแบบ 4 x 4 ลาตินสแควร์ ในแต่ละระยะการทดลองใช้จำนวน 28 วัน สัตว์ทดลองจะได้รับอาหารดังต่อไปนี้ คือ T1 กลุ่มควบคุม (ไม่เสริมผลิตภัณฑ์ยีสต์) และการเสริมผลิตภัณฑ์ยีสต์ 3 ระดับ คือ 50 100 และ 150 กรัมต่อตัวต่อวัน สำหรับ T2 T3 และ T4 ตามลำดับ พบว่า การเสริมผลิตภัณฑ์ยีสต์มีชีวิต ชนิด แคนดิดา กลาบราต้า ไม่มีผลกระทบทางลบต่อค่าโลหิตวิทยา แต่มีผลทำให้ค่าเฉลี่ยความเป็นกรด-ด่างในกระเพาะรูเมนสูงกว่า ($P<0.05$) กลุ่มที่ไม่เสริมและไม่มีผลต่อค่าแอมโมเนีย-ไนโตรเจน ทั้งในกระเพาะรูเมน และในกระแสเลือด ($P>0.05$) ความเข้มข้นของกรดไขมันที่ระเหยได้ และประชากรของจุลินทรีย์ในกระเพาะรูเมนไม่มีความแตกต่างกัน ($P>0.05$) แต่การเสริมผลิตภัณฑ์ยีสต์มีผลทำให้จำนวนโปรโตซัวลดลง ($P<0.05$)

การทดลองที่ 3 ศึกษาการเสริมผลิตภัณฑ์โปรไบโอติกยีสต์ต่อการให้ผลผลิตน้ำนม องค์ประกอบทางเคมีในน้ำนม ปริมาณ โซมาติกเซลล์ และค่าโลหิตวิทยา ใช้โคนมจำนวน 14 ตัว ซึ่งมีระยะ 4 สัปดาห์ก่อนวันคลอด และสิ้นสุดการทดลองเมื่อครบ 4 สัปดาห์หลังวันคลอด โคจะได้รับอาหารทดลอง คือ 1 กลุ่มควบคุม (T1) ไม่เสริมผลิตภัณฑ์โปรไบโอติกยีสต์ และ 2 เสริมผลิตภัณฑ์โปรไบโอติกยีสต์ จำนวน 50 กรัมต่อตัวต่อวัน (2.3×10^9 โคโลนี) โดยผสมกับอาหารสำเร็จรูปทางการค้าให้ในเวลาเช้า อาหารหยาบที่ใช้ คือ ฟางข้าวและหญ้าเนเปียร์สด สำหรับโคทดลอง ก่อนคลอดและหลังคลอด ตามลำดับ เก็บข้อมูลปริมาณอาหารที่กิน และปริมาณการผลิตน้ำนมทุกวัน พบว่า การเสริมผลิตภัณฑ์โปรไบโอติกยีสต์ไม่มีผลกระทบ ($P>0.05$) ต่อปริมาณการกินได้ของวัตถุแห้ง กระบวนการหมักในกระเพาะรูเมน (ค่าความเป็นกรด-ด่าง แอมโมเนีย-ไนโตรเจน และกรดไขมันที่ระเหยได้) ผลผลิตน้ำนม องค์ประกอบทางเคมีในน้ำนม (ยกเว้นค่าเปอร์เซ็นต์โปรตีน มีแนวโน้มเพิ่มขึ้นเมื่อได้รับการเสริมด้วย T2 $P=0.05$) และปริมาณโซมาติกเซลล์ จากการทดลองนี้ชี้ให้เห็นว่า ยีสต์แคนดิดา กลาบราต้า ไม่มีผลกระทบต่อค่าโลหิตวิทยา และสามารถใช้เป็นสารเสริมในอาหารสัตว์สำหรับโคนมได้

สาขาวิชาเทคโนโลยีและนวัตกรรมทางสัตว์ ลายมือชื่อนักศึกษา_____
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ลายมือชื่ออาจารย์ที่ปรึกษาร่วม_____

KRUNG WILACHAI : DEVELOPMENT OF PROBIOTIC YEAST PRODUCT TO IMPROVE RUMEN FERMENTATION AND ENHANCE PRODUCTIVITY IN DAIRY COWS. THESIS ADVISOR : ASSOC. PROF. PRAMOTE PEANGKOUM, Ph.D., 108 PP.

YEAST/PROBIOTIC YEAST/CANDIDA GLABRATA/DAIRY COWS

Three studies were conducted. In experimental I, 91 yeast isolates were collected by using the conventional method, and purifying them by streak plate 3 times. Additionally, the API® 20C AUX Kit and sequencing of the D1/D2 domain of the 26S rRNA gene were used to accurately identify the resulting genera *Candida spp.* and *Issatchenkia orientalis.* The 12 strains of yeast from each ruminants, Dc4 14 18; Be1 2 7; Bu3 4 7 and Go10 16 19 that were selected to determine the best strain of yeast. For the results, first all strains of yeast were grown on pH 3.5-7.5, but both strain Bu3 or Dc18 were better propagated and resistance of TVFAs than other strains ($P<0.01$). On the other hand, the yeast strain Dc18 that was grown had high ability ($P<0.01$) under anaerobic condition incubated. The second, under *in vitro* trial three strains of yeast providing the best growth performance, which was strain Be7, Bu3 and Dc18. Thus, it could be concluded that the addition of live yeast culture, Dc18 with 20% of fermented fluid would improve gas kinetic, gas cumulative, and also increase acetic acid (C_2), acetic acid: propionic acid (C_3) ratio.

In experimental II, four ruminal cannulated dairy cows were used according to a 4 x 4 Latin square design, each period was 28 days. Treatment consisted of control group (T1) was without live yeast product and supplementation of live yeast product amount 3 levels, 50 100 and 150 g/h/d (1 gram product had viable 4.5×10^7 cfu.) for T2, T3 and T4, respectively. The results found that the concentration of live yeast

(*Canida glabrata*) product, which can be concluded that *C.glabrata* had no negative effect on hematology parameters. On the other hand, average ruminal pH had affected the live yeast concentration, although both of NH_3-N and PUN were not significantly different ($P>0.05$). Likewise VFAs showed no significant difference ($P>0.05$) with the live yeast product. In addition, the rumen microbial population did not show any significant difference ($P>0.05$) except on both yeast and ciliate protozoa population when compared with the control ($P<0.05$). Consequently, the concentration of the live yeast product at T2 could be appropriately used for dairy cows.

In experimental III, 14 multiparous transition lactating dairy cows were used in this study. The experiment started 4 weeks before calving and ended 4 weeks after calving. The dietaries consisted of two groups, including the control group (T1), without probiotic yeast and T2 with supplemented probiotic yeast product amount 50 g/h/d (2.3×10^9 cfu) on top dress with SUT® concentrate fed on the morning. Rice straw and fresh Napier grass were used as roughage sources for the pre-calving and post-calving periods, respectively. Consequently, supplementation of the probiotic yeast product did not have a negative effect on DMI, ruminal fermentation (pH, NH_3-N and VFAs), milk yield, milk components yield (milk fat and protein), milk compositions and somatic cell count ($P>0.05$). But, milk protein showed increasing trend ($P=0.05$) with added the probiotic yeast. On week 3 after calving, milk yield and composition were higher with supplemented *C. glabrata* than control group ($P<0.05$). And also yeast *C. glabrata* was not negatively effected on hematology, which is safely used in dairy cows.

School of Animal Technology and Innovation Student's Signature _____

Academic Year 2019 Advisor's Signature _____

Co-advisor's Signature _____

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LIST OF ABBREVIATIONS

CBC	=	Complete blood cell count
ADF	=	Acid detergent fiber
CP	=	Crude protein
C.	=	Candida
DM	=	Dry matter
DMI	=	Dry matter intake
FCM	=	Fat corrected milk
GC	=	Gas chromatography
HCT	=	Hematocrit
HGB	=	Hemoglobin concentration
MCH	=	Mean corpuscular hemoglobin
MCHC	=	Mean cell hemoglobin concentration
MCV	=	Mean corpuscular volume
NDF	=	Neutral detergent fiber
NH_3-N	=	Ammonia nitrogen
OM	=	Organic matter
PLT	=	Blood platelets
PUN	=	Plasma urea nitrogen
RBC	=	red blood cell count
S.	=	Saccharomyces
WBC	=	White blood cell count

CHAPTER I

INTRODUCTION

Yeast additive obtained from *Saccharomyces cerevisiae* has been used for many years as a replacement for antibiotics to improve rumen fermentation and enhance production efficiency in ruminant production systems Ding et al. (2014a), after antibiotics were banned by the European Union (AnadÓN, 2006). Therefore, the animal nutritionists were sought a substitute for antibiotics due to protected of product residues antibiotics, which have been lead to on the consumers, whom concern about safety, quality of animal products and also environment issues. In recent years, the addition of yeast is not only increase productivity in animal, but also to decreases the risk of animal digestive transfer of potential human pathogen, to decrease the antibiotic load and the risk of antibiotics resistances gene transfers until to limit excretion of pollutants (Chaucheyras-Durand et al., 2008), (Chaucheyras-Durand et al., 2012).

The yeast products have been widely used in ruminant feed to improve rumen fermentation and animal performance. It various based *Saccharomyces cerevisiae* have been shown to impact dry matter intake (DMI), rumen pH and nutrient digestibility. Researchers (e.g. Callaway and Martin (1997); Bruno et al. (2009), Desnoyers et al. (2009), and Ding et al. (2014b) were suggested that feeding yeast products may be the most benefit to dairy cows during late pregnancy and early lactation, when these effects of yeast cultures might be most valuable. Indeed, Ding et al. (2014b) reported that *Saccharomyces cerevisiae* had to positive effect on DM and NDF digestibility of forage

and increased rumen total bacteria fungi, protozoa, lactate utilizing bacteria and enzyme fibrotic activities for beef cattle. In the same way, AlZahal et al. (2014) found that the rumen function could be improvement by active dry *Saccharomyces cerevisiae*, and greater numbers of cellulolytic microorganisms within the rumen of dairy cows. Fernando et al. (2010) demonstrated that rumen microbial population dynamics during adaptation to high-gain diet in feedlot cattle, showed that the *Megasphaera elsdenii*, *Streptococcus bovis*, *Selenomonas ruminantium*, and *Provotella bryantii* populations were increased by high-concentrate diet. Thereof, the *Butyrivibrio fibrisolvens* and *Fibrobacter succinogenes* populations decreases were adapted to the high-gain diet. However, Chaucheyras-Durand et al. (2008) showed that effect of yeast vary depending on 2 main factors as follows- 1) biotic factor such as the strain of yeast and its viability, and 2) abiotic factors, for instances the nature of the diet or animal management. Therefore, if we will known in these factors, we can be used yeasts to rumen microorganism, and also that will selected to new generation yeast. Noteworthy, the supplementation of yeast (*Saccharomyces cerevisiae*) was widely studied in recent years by many researchers and also more focus in the dairy cows between high production. But a few data addition live yeast were studied in the transition period of dairy cow, which is played very importance role in the next lactation.

The transition period is defined as 3 wk prepartum until 3 wk postpartum, it is a period marked by changes in endocrine status to accommodate parturition and lacogenesis. Whereas, the dairy scientists and dairy producers tend to neglect the transition cow, particularly prepartum. During in this time, sometime decreased of DMI (5-7 d prepartum) and increase DMI in the early lactation (Grummer (1995); Grant and Albright (1995), Loor et al. (2016)). In studied survey from smallholder

farm, Leelahapongsathon et al. (2016) found that ether cow or farm management factor were associated with the intramammary infection rate and subsequent expression of clinical signs of mastitis in early postpartum cows. However, Campanile et al. (2008) found that the dietary supplementation with *S.cerevisiae* increase organic matter digestibility, milk yield and guarantee higher energy availability, and also lower fat mobilization in buffalo cows. Oliveira et al. (2010) reported that the addition of live yeast strain KA500 (10g/d) in the diet caused a significant reduction in the somatic cell count, and also reported similar with Spaniol et al. (2014) who found that somatic cell count in dairy cow was decreased by yeast addition amount 3 g/head/d. Therefore, if feeding high concentrate ratio for animals, it increase the risks on rumen acidosis and lowest fiber digestibility. Consequences, the metabolic disorder will be occurred in the ruminants owing to in rumen dysfunctions. On the other hand, the management of transition cows is play an important role in the preventing the risk of metabolic disorder, and also Roche et al. (2015) suggested that should controlled feed restriction amount 75-90% on 2-3 wk for before calving, whereas Roche et al. (2013) indicated that the both low and high BCS at calving will increase the risk of diseases. In spite of the supplementation of yeast products were results varied of each studied, researchers had been showed to beneficial effect on rumen fermentation, ruminal pH, VFAs, milk yield and composition and animal healthy. In particularly, yeasts flora in the rumen are autochthonous or native yeast can be positive effected nutrient digestibility and improve ruminal microbial communities. Therefore, in this studies have been selected yeasts to the rumen flora of ruminants animal, and also that were used by transition period of dairy cows.

1.1 Research objectives

1.1.1 To study of isolation, selection and identification of yeast in the rumen from the ruminants- goat, buffalo, beef cattle and dairy cows as an examine the best probiotic yeast.

1.1.2 To study on the supplementation of probiotic yeast on rumen fermentation; ruminal pH, VFAs, microbial communities.

1.1.3 To study on the addition of probiotic yeast on milk production and milk composition.

1.2 Research hypothesis

1.2.1 Yeast strain and sources can be differed effect on probiotic properties, rumen fermentation and microbial communities.

1.2.2 The supplementation of probiotic yeast can be supported to rumen fermentation and microorganism in the rumen.

1.2.3 Addition of probiotic yeast product can be increased of milk production, better milk composition and good health of lactating dairy cows.

1.3 Scope and limitations of the research

This study focus on supplemented of new probiotic yeast, which isolate from the ruminants were utilized as probiotic for dairy cow. Indeed, the 3 step were found the new yeast included by 1) step for isolation and identification, which isolated from buffalo, goat, cattle and dairy cattle and identified by API kit and PCR sequence by, 2) selection step, which included growth on pH condition, rumen stimulation and *in vitro* gas production and 3) Utilization step, trial in metabolism with fistulated dairy cow and

transition period in dairy cow. All of step were followed as figure 1.1.

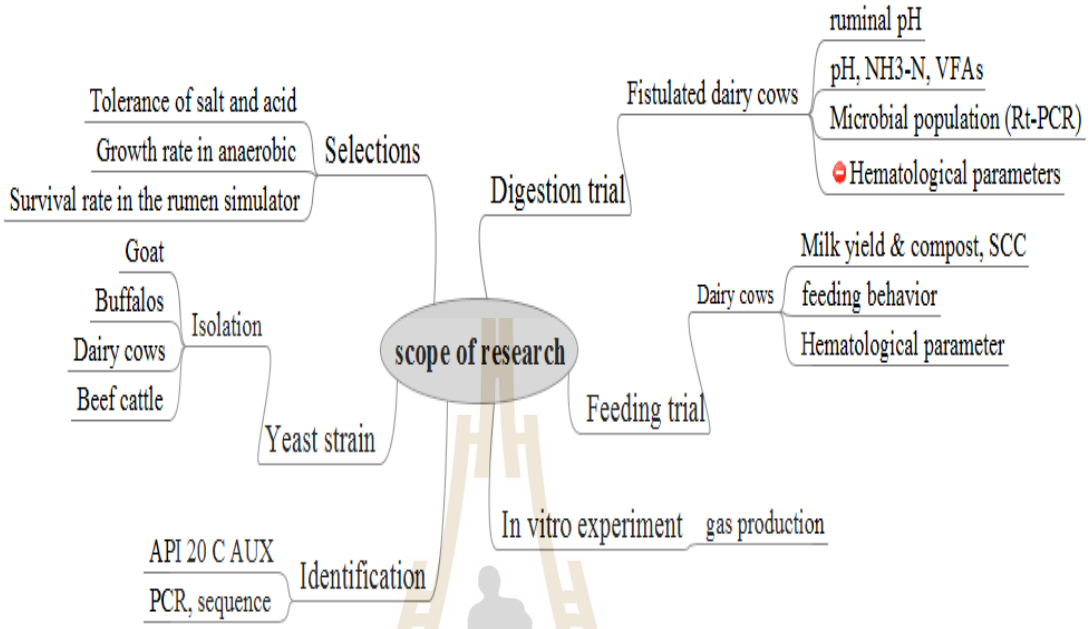

Figure 1.1 Scope of research.

1.4 Expected benefit

According to this research procedures, it could be obtained for expected benefits as follows;

1.4.1 The autochthonous ruminal yeast's isolate from ruminants will be positive effected on ruminal fermentation and rumen microorganism communities.

1.4.2 Bio-feed additives strategy using the live yeast a new strain and innovate for manipulating the ruminal fermentation and improving milk yield and composition for transition period of dairy cows.

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CHAPTER II

REVIEW OF LITERATURE

2.1 Dairy cows production in Thailand

The government sector was being plays a crucial role in the policy formulation and implementation, those of which have resulted in the continual development and prosperity from the past to the present time. Although the number dairy cows has increased, the amount of raw milk is still insufficient for the demand due to the expansion of the consumption of ready-to-drink milk. Thus, Thailand had imported powdered milk to process into ready-to-drink milk to comply with the agreement of the World Trade Organization (WTO). Consequently, the private processing entrepreneurs refuse to purchase raw milk from the dairy farmers. In this case, the government sector needs to get involved to solve the problem by forming such policies-both long and short termed-as increasing the budget for the School Milk Program, etc. However, for the government sector to carry the policies there must be an adjustment in accordance with the world trade economy situation which keeps changing and processing with increasingly intensified competition (Dairy Farming Promotion Organization, 2012). In Thailand, have long-running national school milk program, which have served as useful model in other countries as well as China, India, Japan and Vietnam also have experience with school milk programs at various levels (Kadiresan, 2016). Meanwhile, dairy industry of Thailand refers to the industry entirely involved in Thai input. Regarding strengths, it was found that the dairy

farming occupation in Thailand had been consistently supported by the government and generated before our neighboring countries. We have heat-and disease-tolerant crossbred dairy cattle yielding high production. But the weaknesses are concerned, the cost of dairy farming has escalated due to the increase of fuel prices; as a result, the prices of animal feed mill have also gone higher, and so on. (Dairy Farming Promotion Organization, 2012). Indeed, Dairy Farming Promotion Organization (2019) reported that amount of dairy cow was increased by the total dairy cow, also when separated by region found the Central area has highly other region of Thailand follow as Figure 2.1

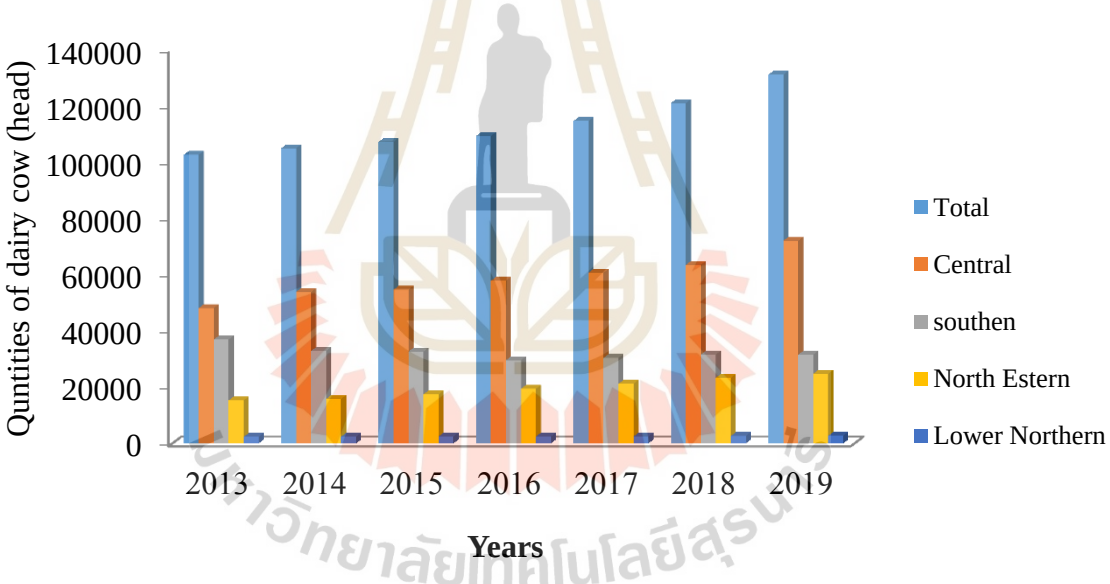

Figure 2.1 DPO's total dairy cow separated by region in Thailand (2013-2019).

Source: Dairy Farming Promotion Organization (2019).

2.2 History of using probiotics for ruminant nutrition

The word "Probiotics" is derived from Greek and means " for life." It was first used by Lilly and Stillwell in 1965 defined probiotic as "Growth promoting factors

produced by microorganisms.” However, Fuller (1989) defined it as “A live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance.” Also, USDA in 1989 as microbial based feed additives that contain “live, naturally occurring microorganism.” Likewise, ILSI (International Life Sciences Institute) Europe Working Group (1998) “A viable microbial food supplement which beneficially influences the health of the host”. Meanwhile, FAO/WHO (Food and Agriculture Organization and World Health Organization) (2001): “Live microorganisms which when administered in adequate amounts confer a health benefit on the host.” Barnett (2007)

In general, most would agree with Direct-fed microbial (DFM) on yeast must be “live”. Thus, they must survie processing, storage and the gut environment (Denev et al., 2007). Whereas, the need to provide a high numbers of live yeast culture has been the subject of many argument. For instances, Dawson et al. (1990) founded that the stimulatory effect of yeast on number of rumen cellulolytic bacteria was refuted when yeasts were autoclaved. Although there have been implications that suggests yeasts were able to grow in continuous rumen culture, their were ether in sterile ruminal fluid or in the rumen of lam were not colonized by yeast (*S.cerevisiae*) (Kung et al., 1997) and (Durand-Chaucheyras et al., 1998). In addition, Newbold et al. (1995) and Chung et al. (2011) showed that to differences between strain of *S.cerevisiae* in their ability to modify the rumen bacterial population and CH_4 emission. In recently, in corn silage, the combination between *Saccharomyces* and *Lactobacillu*s spp. that enhance ensiling and increase fiber digestion although strains of yeast alone would not constitute generation inoculants (Duniere et al., 2015). Furthermore, the goal of many

of these research activities has been to define the application and production strategies that can optimize animal response to live yeast supplement.

2.3 Roles of yeast on rumen fermentation

Approximately 180 species of ruminants animal have a specialized pregastric structure called the rumen, which is strictly anaerobic microorganism (Russell, 2009). The digestive an anatomy and physiology of ruminants is markedly differenced to that of non-ruminant animals. In ruminants, for instance cattle, buffalo, goats and sheep has two additional digestive organs at the anterior end of the tract. The first of these, the rumen, contains in a liquid volume of 60-100 liters (Dairy cattle). This organ is essentially a fermentation chamber, containing about up to 10^{11} of bacteria and 10^5-10^6 of ciliate protozoa per milliliter. Nevertheless, Yeast and aerobic fungi have long been known to be normal inhabitants of the rumen (Hopson (1988) and Fuller (1992)). Indeed, the yeasts were belonging to the genera *Candida* and *Trichosporon* have been isolated in small number from rumen of fistulated cows (Clarke and Di Menna, 1961). Likewise, Lund (1980) illustrated that isolated yeast from rumen content of Musk Oxen in East Greenland founded that the majority belong to *Candida* and *Cryptococcus*, and the count being up to 1.3 x10^5 colonies forming unit (CFU) per gram (Lu et al., 2016). Additionally, digestion of feed in the rumen occurs by a combination of microbial fermentation and physical breakdown during regurgitation of the feed by ruminants. Thus, the rumen ecology-e.g. pH, temperature, microbial communities have been play an important role for activities optimize of microorganism. Consequently, the animal responses have better production and good for health. In addition, Julien et al. (2015) suggested that although a specific interest in

using live yeast increase ruminal total volatile fatty acid (TVFA), Acetic acid (C2), and Butyric acid (C4) for early lactating dairy cow with received diet- low level of rumen degradable protein, decrease that of propionic acid (C3) when used diet have to adequate of rumen degradable protein (RDP). Also, using of live yeast in RDP were deficient diet for early lactating dairy cows. Likewise, Pinloche et al. (2013) found that the supplementation of yeast about 5 g/head/d, it was high rumen pH, total VFA, C3 and lactate than control group ($P<0.05$). While, Marden et al. (2008) demonstrated that to compared during live yeast (5 g/d) and sodium bicarbonate (150 g/d) to stabilize ruminal pH in high- yielding dairy cows were found that although the live yeast prevented accumulation of lactate and allow better fiber digestion, sodium bicarbonate seemed to act only an exogenous buffer for dairy cows. However, Milk yields were increased about 4% when using yeast products of supplemented in water trough (9 ml/h/d) for lactating dairy cows (Rossrow et al., 2014).

Active dry yeast or Baker's yeast had been used for bread throughout, it very important for bread quality (Birch et al., 2013).Whereas, Borchani et al. (2016) showed that yeast cell wall had good potential to used as prebiotic ingredient in food and pharmaceutical product. Likewise, yeast cell are know to be a rich source of vitamins, and some unidentified cofactors that are helpful in increasing microbial activity in the rumen, and live yeast as dietary allow feed additive a better utilization of fed for dairy cows (Williams et al. (1991), Alshaikh et al. (2002), Fonty and Chaucheyras-Durand (2006) and Julien et al. (2015)). Otherwise, the supplementation of yeast to the rumen ecosystem look as influence fermentation and might help the ecosystem to deal with high-concentrate diet (Desnoyers et al., 2009). On the other hand, AlZahal et al. (2014) reported that the active dry yeast addition to feed for dairy

cows can be potentially improved rumen function, which greater number of cellulolytic bacteria in the rumen. Yeast (*Saccharomyces cerevisiae*) was not only positive effected on DM and NDF degradation rate and increased rumen total bacteria, fungi, protozoa and lactate- utilizing bacteria, but reduced lactate producing bacteria (Ding et al., 2014)**.** Therefore, that avoided ruminal acidosis in dairy cows when receiving the high concentrate diet Grant and Albright (1995)**,** Grummer (1995), Chaucheyras-Durand et al. (2008), Guedes et al. (2008) and Yuan et al. (2015)). Bayat et al. (2015) demonstrated added live yeast on rumen fermentation, rumen methane production and milk composition were results indicated that supplementation of live yeast (*S.cerevisiae*) strain A and B had no affected on animal production, rumen fermentation, rumen gas production and milk compositions. While, feeding a yeast culture with *S.cerevisiae* (30 g/d) had a little effect on lameness score, but no influence on reproduction of multiparous cows under heat stress (Bruno et al., 2009). Bruning and Yokoyama (1988) demonstrated that the physical and nutritional characteristics of live and killed brewer's yeast slurries were 44.1 and 43.1 percent of crude protein, as well as ethanol percentages were 6.92 and 1.84 respectively. Moreover, they were supplemented in the ruminally canulated Hereford bull calves. Showing that the ruminal NH_3 concentrations increased to over 70 mg/dl with the 4.5 and 6.9 kg/d dosages of live brewer's yeast slurry, but no more than 35 mg/dl when added with killed brewer's yeast. However, Callaway and Martin (1997) reported that the yeast culture filter provides soluble growth factor for instance; organic acids, B vitamins, and amino acid there stimulate growth of ruminal bacteria that utilize lactate and digest cellulose.

2.3.1 Effect on ruminal pH and acidosis

Feed degradable component involves numerous and complex interrelationships among the different microbial communities. Various types of interactions have been found in the rumen. The rumen acidosis is very low occurs of pH value in the rumen that can be negative effect on health and performance of the animal (Fonty and Chaucheyras-Durand, 2006). Nevertheless, bovine lactic acidosis and laminitis are linked through imbalances in carbohydrate nutrition, after a first stage of high VFAs concentration at pH>6, the lactate is product by the lactate-producing bacteria (*Streptococcus bovis*), but rumen is not lactate-utilizing bacteria. Then, protozoa disappears and others bacteria are reduce quickly. If pH value decline continuous, *Streptococcus bovis* is instead by *Lactobacillus* spp., that also seems start point a spiraling effect with extend lactate accumulation (Nocek, 1997); Enemark (2008)), follow as figure 2.2.

In meta-analysis reported by Desnoyers et al. (2009) showed that yeast supplementation increased on average 0.03 of rumen pH and 2.17 mM on average of volatile fatty acid (Ullah et al., 2014), tended to decrease rumen lactic acid concentration (-0.9 mM on average) and had no affect on acetate-to-propionate ratio. Base on these data they suggested that addition yeast would improvement in the rumen fermentation. But, these effect could be modulated by several different factors such as DMI, percentage of concentrate or NDF it the diet, or species of animal. Otherwise, base on *in vitro* experiment Lynch and Martin (2002) reported that neither *S.cerevisiae* culture or *S.cerevisiae* live cells had any effect on final pH, CH_4, acetate, propionate and butyrate, but the lactate concentration was declined when added

S.cerevisiae culture and live cells at the level 0.73 g/L compared with the control incubation.

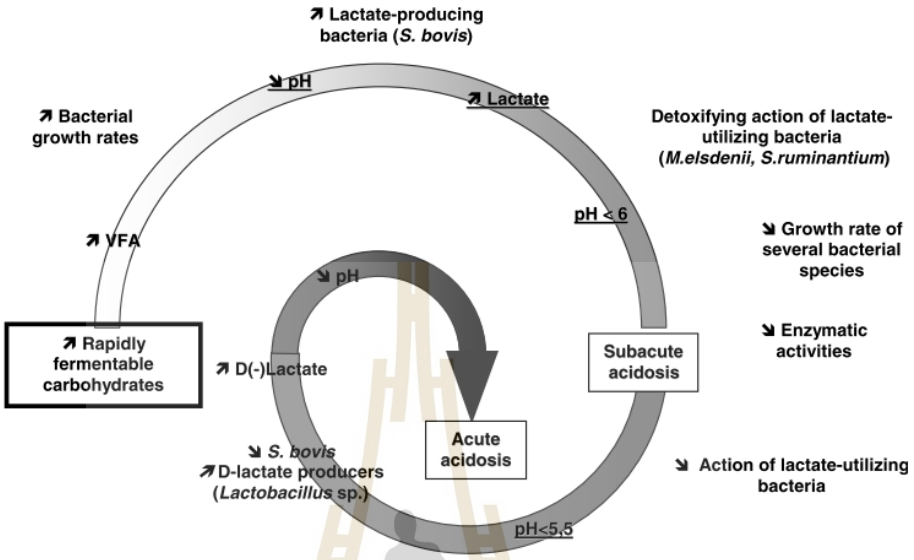

Figure 2.2 Step-by-step microbial mechanism rumen acidosis.

Source: Fonty and Chaucheyras-Durand (2006).

Bach et al. (2007) founded that average rumen pH was greater ($P<0.01$) when was supplemented yeast (*Saccharomyces cerevisiae* strain I-1077) about 5 g/d, equivalent to 10^{10} CFU/d (Table 1). Likewise, Marden et al. (2008) illustrated that added yeast (*S.cerevisiae* strain Sc 47) about 5 g/d could be a pH stabilization (6.14) compared with control diet (5.94) and decreases total lactate concentration 67.3% of control diet. Additionally, when compare with supplemented the sodium bicarbonate, live yeast prevented accumulate of lactate and allowed better fiber digestion, whereas sodium bicarbonate appears to act only as an exogenous buffer. Also, the rumen pH was increased by supplemented yeast (5 g/d) (Pinloche et al., 2013) follow as Table 1.

Table 2.1 Effect of addition of Yeast on Ruminal pH and lactate concentration

Sources	Item	Control	Yeast	p-value	Doses
Erasmus et al.	pH	5.99	6.0	ns	10 g/d
(1992)	Rumen lactic acid (mM)	1.63	1.64	ns	YEA-SACC®
Newbold et al.	pH	7.1	7.2	ns	Yea-acc® 500
(1995)	L-lactate, mmol/d	0.31	0.47	ns	mg
Newbold et al.	pH	6.78	6.78	ns	Biosaf®
(1998)	L-lactate, mmol/d	0.166	0.163	ns	500 mg
Bach et al. (2007)	pH	5.49[a]	6.05[b]	0.01	5 g/d
Marden et al.	pH	5.94[a]	6.14[b]	0.03	5 g/d
	E_h(mV)	-115[a]	-149[b]	0.04	
Pinloche et al.	pH	5.81[a]	6.99[b]	<0.05	5 g/d
(2013)	E_h(mV)	-134.3[a]	-184.4[b]	<0.05	
	D & L Lactate (mM)	13.2[a]	4.0[b]	<0.05	

2.3.2 Effect on intake and nutrients digestion

Williams et al. (1991) demonstrated that using yeast culture ($5x10^9$ CFU/g, or 10 g/d) in the diet for dairy cows on forage degradation and fermentation. That showed the supplemented of yeast culture increased DM intake of the cows by an average of 1.2 kg/d (P<0.062) and milk yield by average 1.4 liters/d. Although the yeast culture in the rumen had effect on ruminal stoichiometry (pH, lactate concentration), an increased rate of forage degradation may be have increased forage intake and productivity of these dairy cows. The main effects of fungal feed additives

are therefore regarded as being intake-driven (Williams and Newbold, 1990) Likewise, Fuller (1992) and Fiems et al. (1993) found that many factors are known to influence appetite, but the ones that have been considered for yeast culture in ruminants have been palatability, the rate of fibre digestion, the rate of digesta flow and protein status. Whereas, Guedes et al. (2008) suggested that added yeasts base on product of the Levucell® SC 10 ME; 1×10^{10} CFU/g in the diet 1 g/d has the potential to reduced the risk of rumen acidosis and increased VFA concentration and fibre degradation of low quality maize silage. In recently, Ding et al. (2014a) and Ding et al. (2014b) studied that supplementation of *Saccharomyces cerevisiae* ($8x10^9$ CFU/h/d though the ruminal fistula) on alfalfa nutrients digestion characteristics of steers were positive effect on DM and NDF digestion rate or effective degradability. However, the responses to yeast was not consisted, depending on dosage, feed time and frequencies, and had a better dry matter intake (25 VS 21.6 kg/d) than control, cows during high gain feed, and strain of yeast (Elghandour et al., 2015). Additionally, AlZahal et al. (2014) reported that the cows received active dry yeast (*S.cerevisiae*) about $8x10^{10}$ CFU/head per day greater total volatile fatty acid and propionate concentration, but lower acetate : propionate ratio than control, whereas the live or dead cells of yeast supplementations via top dressing were declined ruminal lactate and butyrate as propionate (Salvati et al., 2015).

2.3.3 Microbial communities

The growth of cellulolytic bacteria is stimulated by fungal feed additives. Substantial increases in the total viable count (TVC) of anaerobic bacteria in the rumen when ruminants were fed fungal feed additives were yeast culture (30%) and *Aspergillus* spp. (14%) reported by Wiedmeier et al.(1987 cited by Fuller (1992)).

In recent years, AlZahal et al. (2014) founded that the cows were received yeast (8x 10^{10} CFU/head per day) had increased for *S.cerevisiae* (9-fold), *Fibrobacter succinogene* (2-fold), *Anareovibrio lipolytica* (6-fold), *Ruminococcus albus* (1.3-fold) and fungi (Nisbet and Martin, 1991), which suggested an increase in cellulolytic microbes within the rumen. But, the *Megasphaera elsdenii* was reduced by supplement active dry yeast. The reductions this bacteria may reflect lower concentration of lactic acid in the rumen. In the other hand, Julien et al. (2015) reported that live yeast supplementations were not effect on structure of bacteria populations and diversity index (Shannon) in the early lactation cows. Likewise, the number of fiber-digestion bacteria were not effected by added yeast culture (Erasmus et al., 1992). Nevertheless, many researchers found that the total bacteria, cellulolytic bacteria, protozoa population and fungi were significant different reported by Newbold et al. (1995),)Newbold et al., (1996, Newbold et al. ((1998 and Ding et al. (2014) that were listed in the table 2. Otherwise, Fernando et al. (2010) reported that the number bacteria in the rumen was decreased by feeding high-concentrate, for instance, *Butyrivibrio fibrisolvens* and *Fibrobacter succinogen*, there are played an important role fiber digestibility in the rumen.

Microorganisms are used in direct-fed microbial (DFM) for ruminants may be classified as three groups. Firstly, lactic acid producing bacteria (LAB) that including species of *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, *Bacillus* and *Streptococcus*. The second, lactic acid utilizing bacteria (LUB) that strain of *Megasphera elsdenii*, *Selenomonas ruminantium, Propionibacterium* and *Prevotella bryantii*, and also or other microorganisms is yeast products containing *Saccharomyces* and *Aspergillus*. Yeast DFM may reduce hamful oxygen, prevent excess lactate

production, increase feed digestibility and improve fermentation in the rumen that reported by (Seo et al., 2010). Whereas, *in vitro* experiment Nisbet and Martin (1991) found that of used yeast (YEA-SACC) could be stimulated to growth of *Selenomonas ruminantium*, which was also increased to uptake the lactate. However, the concentration of total viable cellulolytic bacterial in the rumen of buffalo calves were increased by with yeast culture supplement that reported by Kumar et al. (1997).

Table 2.2 Effect of Yeast on microbial organism in the rumen.

Sources	**Item**	**Control**	**Yeast**	**p-value**	**Doses**
Erasmus et al.	*Cellulolytic* count, 10^8	3.81	3.60	ns	10 g/d
((1992	of rumen content				YEA-SACC®
Newbold et al.	Total bacteria	2.7	4.3	<0.05	Yea-Sacc®
(1995)	($x10^8$/ml)				500 mg in
	Cellulolytic bacteria	4.3	5.9	<0.05	RUSITEC
	($x10^6$ /ml)				
	Protozoa ($x10^3$ /ml)	4.0	5.6	<0.05	
Newbold et al.	Total bacteria,	3.93^a	5.48^b	<0.05	NCYC
((1996	($x10^8$/ml)				240, 4 g/d
Newbold et al.	Total bacteria	3.46	4.78	<0.05	Biosaf®,
((1998	($x10^8$ /ml)				500 mg in
	Cellulolytic bacteria	1.17	1.74	<0.05	RUSITEC
	($x10^6$ /ml)				
	Protozoa ($x10^3$ /ml)	3.77	3.56	<0.01	

Table 2.2 (Continue.)

Sources	Item	Control	Yeast	p-value	Doses
Ding et al. (2014)	Total bacteria, × 10^{10} copies/ml	7.86	10.07	0.05	SC I-1077 8x10^{9}
	Rumen fungi, ×10^{5} copies/ml	5.99	7.51	0.10	cfu/h/d
	Protozoa, x10^{5} copies/ml	6.64	9.54	0.06	
Jiang et al. (2017)	*Bifidobacterium*, relative (%)	-0.03	1.13	0.01	5.7x10^{7} cfu/cow/d
	Ruminobacter, relative (%)	0.87	1.30	0.02	6x10^{8} cfu/cow/d

2.3.4 Effect on animal health and immune system

Microbial supplementation such as *S.cerevisiae* product have been widely used in ruminant nutrition to manipulate rumen fermentation and improve animal production. But, various *S. cerevisiae* based yeast product have been shown to impact dry matter intake (DMI), rumen pH and nutrients digestibility (Callaway and Martin (1997), Fonty and Chaucheyras-Durand (2006), Hristov et al. (2010), Chaucheyras-Durand et al. (2012)), some team of researchers (e.g. Harrison et al. (1988), Chung et al. (2011), Wanapat et al. (2013)) had suggested that feeding yeast products may be most beneficial to dairy cows during late gestation and early lactation when these effect of yeast culture and dry yeast might be most valuable.

The supplementation of probiotics in the animal diet can be cause alteration in blood biochemistry and cellular immune response as a result of the microorganism's action on the digestive system. Recently, (Spaniol et al., 2014) demonstrated that conclude the *S.cerevisiae* (3 g/d, top dress) in the diet of dairy cows at the does a decrease of had no effect on milk production and composition (Table 2.3). However, there was *S.cerevisae* after 30 d of usage as well as an increase in the concentration of circulating globulin and cytokines [tumor necrosis factor (Baurhoo et al., 2012), interleukin-) 4IL-(4, and interferon (IFN)]. Then, they had suggested a beneficial effect of probiotic yeast on the immune system of lactating cows. Indeed, Yuan Hulbert et al. (2015) reported that the addition with yeast culture plus enzymatically hydrolyzed yeast (YC-EHY) enhanced measures of humoral and mucosal immunity and modulated uterine inflammatory signals and mammary gland health in transition dairy cows. Moreover, Bruno et al. (2009) who found that feeding a yeast culture of *S.cerevisiae* was minor effected on lameness score, but no influenced on reproduction of multiparous cows under heat strees. Indeed, Alibrahim et al. (2010) reported that the supplementation of yeast 2.5 g/cow/d for pre-calving and 10 g/cow/d for post-calving (108 CFU/g), as a results, feeding yeast supplemented had no effect on energy status of lactating dairy cows with high or low BCS at calving, whereas it improved serum insulin concentration. Also, Nocek and Kautz (2006) reported that plasma NEFA and BHBA levels were not effected by supplemented of direct-fed microbial combination between yeast and two strain *Enterococcus*.

Table 2.3 Effect of supplemented with probiotic (BacSol®) on blood and immunological of dairy cows.

Variable	Days	Groups		P-value
		Control	Treatment	
Urea (mg/dL)	0	30.75	32.29	0.05
	45	31.47	29.10	0.05
Total Protein (mg/dL)	0	9.59	9.66	0.05
	45	9.20	10.85	0.08
Albomin (mg/dL)	0	2.86	2.90	0.05
	45	3.18	3.09	0.05
Globulin (mg/dL)	0	6.72	7.05	0.05
	45	6.05	7.78	0.01
IL-4 (pg/mL)	0	81.16	77.16	0.05
	45	85.80	106.10	0.05
TNF-α (pg/mL)	0	162.33	154.71	0.05
	45	160.40	196.60	0.05
INF-γ (ug/mL)	0	25.83	28.42	0.05
	45	25.2	39.9	0.05

IL-4 interleukin 4, TNF-α tumor necrosis factor alpha, INF-γ interferon gamma

Sourece: Spaniol et al. (2014)

2.4 Isolation, identification and selection of yeasts

Clarke and Di Menna (1961) studied that the yeast from the rumen content of cows were founded that yeast belonging to the genera *Candida* and *Trichosporon* have been insolated in small number (80-1.3x10^4 CFU per gram of fresh rumen content) from the rumen fistula cows. Even though Lund (1974) showed that the count of yeasts in the rumen fluid of cattle were amounted being up to 3.5x10^3 CFU/ml, when culture on agar plates at 39^{o}C. Likewise, in the East Greenland, the yeast flora 16 sample of rumen content isolate from rumen content of Musk Oxen was examined by Lund (1980). Founded that variable number of yeast colonies developed on agar plates incubated at 25^{o}C, the counts being up to 1.36x10^5 CFU per g (Lu et al., 2016), and the species of *Candida* and *Cryptococcus* were found majority belonged. Meanwhile, some researcher was selected *Propionibacterium jensenii* on improve weight gains in claves by Adams et al. (2008). However, the supplementation of yeasts appears feed additive for improve of health and production of livestock has been studies for many years. Usually used probiotic include *Saccharomyces cerevisiae* for enhancing the activities, such as rumen microbial communities, nutrients digestibility and production potential of the animals. Indeed, different of strain of *S. cerevisiae* had different effect on rumen bacteria in *in vitro* experimented and in sheep (Newbold et al., 1995). Otherwise, Agarwal et al. (2000) demonstrated that selection of *S. cerevisiae* strain for use as microbial feed additives. In screening, they used *S. cerevisiae* 8 strain for their tolerance to pH 2.0-7.0, and bile salts (0 0.3 0.6 and 0.9%). Although the results showed that two of the strain were tolerance of acid or bile salts, the *S.cerevisiae* NCDC 49 can be enhance IVDMD to be seem and wheat straw, that can be considered best strain.

In recently an researchers from Thailand, that is country locate in tropical Asia, has been considered to be rich in microbial diversity and found a lot of yeast considered by (Nakase et al., 2010). In the other hand, for ruminant animals, Sirisan et al. (2013) demonstrated that yeasts isolated from the ruminal fluid of dairy cattle can be utilize lactic acid, when ether fed high cassava pulp or high concentrate. The results showed that the three most effective yeasts in terms of specific growth rate and generation time were *Pichia kudriavzevii*, *Candida rugosa* and *Kodamaea ohmeri*, which 99 100 and 99% nucleotide identities, respectively. Meanwhile, the isolate yeast from soil was found *Trichosporon asahii* strain GSY10 was the most promising oleaginous yeast for microbial lipid production from molasses, and this strain contained unsaturated fats up to 62.5% reported by (Paserakung et al., 2015). Whereas, Leesing and Karraphan (2011) showed that cell growth and lipid production of yeast depended on the nitrogen and glucose concentration, and they found that cell yeast's *Torulaspora maleeae* Y30 were palmetic acid, stearic acid and oleic acid that are comparable to vegetable oils. The next stage, as far as most worker are concerned, is to determine whether other strains or species of yeasts can be effective of ruminants, owing to no information is available about other species or genera of yeast, and also expect feed additives for ruminant or stimulation of fiber degradation.

Interestingly, the lipases are secreted by many bacteria and fungi. They catalyses not only the hydrolysis but also the synthesis of long-chain acylglycerol. Additionally, *Candida rugosa* is yeast would be lipase secreted, and that is generally regarded as safe (GRAS) status and no adverse effect on human and another form life has been reported as a result of traditional, when used in situ or ex situ purified lipases (Jaeger and Reetz (1998) and (Benjamin andPendey, 1998). While, species of

yeast, *Candida* have been widely used in the production of biosurfactanct from soluble and insoluble carbon sources. In this case, found that the yeast *Candida glabrata* is biosurfactant produced that reported by de Luna et al. (2009). However, Lagneau et al. (1996) suggested that yeasts: *C. tropicallis*, *C. glabrata*, *C. parapsilosis* and *T. asahii* are able to grow above 40^{o}C and there can be consider as potentially phatogenic, cause mastitis in dairy cows.

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CHAPTER III

EXPERIMENT I

ISOLATION, IDENTIFICATION AND SELECTION OF YEAST FROM RUMINANTS; GOAT, BEEF CATTLE, DAIRY COW AND BUFFALO AS AN EXAMINED THE BEST PROBIOTIC YEAST

3.1 Abstract

In this chapter that is the isolation, identification, and selection of yeast from ruminants, along with the beef cattle, dairy cow, goat and buffalo that were considered strain of yeast performance. The ninety-one isolated of yeasts were collected by using to conventional method, which is microbial culture on agar medium and purified them by streak plate among 3 times until to purity colony of yeast. Besides, the API® 200C AUX Kit and sequencing of the D1/D2 domain of the 26S rRNA gene were used for accurate identified that found the genera *Candida spp.*,which is *C. glabrata* (99% of identify), *C. tropicallis* (99%), *C. rugosa* (98%) and also *Issatchenkia orientalis* (99%).

Yeast amount of 12 strains, Dc4, 14, 18; Be1, 2, 7; Bu3, 4, 7 and Go10,16, 19 that were selected for their as to best the strain of yeast. The criteria for test the performance that were consisted of tolerate pH values (3.5-7.5), total volatile fatty acid (TVFAs, 0 0.25 0.5 1 2 and 4 % of broth medium) resistant, to growth on anaerobic condition, and an efficiency of *in vitro* gas productions. As the results, first

to all strains were grown on pH values 3.5-7.5, but the strain of Bu3 and Dc18 were better performance than other strain ($P<0.01$). Second, at the pH 6.5 the resistant of TVFAs the strain Bu3 and Dc 18 were bested to growth, while when addition an organic acids to high concentrate lead to the growth of yeasts were declined. On the other hand, the yeast of strain Dc18 that was high to viable counted ($P<0.01$) under anaerobic condition incubated. And also the third, three of strains providing the best performance, which strain Be7, Bu3 and Dc18 that were considered by *in vitro* gas productions technique. Thus, it could be concluded that to addition live yeast culture, Dc18 with 20% of fermented fluid could improve gas kinetic, gas cumulative, and also increased acetic acid (C_2), acetic acid: propionic acid (C_3) ratio. In addition, the next experiment will be using live yeast of strain Dc18 for fistulated dairy cows on rumen ecology, rumen fermentation and physicochemical parameters for truly understanding the action of live yeast in the rumen.

Keywords: Yeast, *Candida glabrata*, Isolation

3.2 Introduction

Since 1980, the ruminant nutritionists have been interesting used by probiotic in animals, although different strain or genera of probiotic had various influenced on ruminant production (Harrison et al., 1988), (Agarwal et al., 2000), (Kumura et al., 2004)). Newbold et al. (1995) and Chung et al. (2011) were reported that the different strains of *Saccharomyces cerevisiae* (*S. cerevisiae*) in their ability to modify anaerobic bacteria in the rumen and also some strain tended to lower CH4 emissions but increased the risk of acidosis of non-lactating dairy cows. However, Numerous commercial yeast products are available and vary widely in the strain of yeast.

Otherwise, yeasts are able to supply growth factors; organic acid, B-vitamin and amino acid to rumen bacteria (Chiquette, 2009). Meanwhile, the new generation of microbial from the habitat have been interesting for enhance the productivity of animals, for instance Chiquette et al. (2008) founded that supplementing of *Prevotella bryantii* can increased ruminal fermentation products and milk yield of early lactation dairy cows.

Interestingly, the yeast rumen flora may appropriately considered to enhance ruminants production. Likewise, many researchers were done isolated yeast from the rumen, for instance, Musk Oxen, cattle, and dairy cow reported by Clarke and Di Menna (1961), Lund (1974), Lund (1980), Sirisan et al. (2013) and Paserakung et al. (2015). They were found the majority genera of *Candida* spp, *Tichosporon* spp., and *Picia* spp. Marrero et al. (2015) who reported that used yeast *Candida* spp. could improved *in vitro* rumen fermentation of *Provotella bryantii* , which is lactate utilizing bacteria isolated from the ruminal fluid by Rodriguez (2003). Therefore, the yeast rumen flora have been used probiotic for dairy cows, because that are appropriately for tropical ~~ruminants~~ host to increases productivity in animals. In this study was yeasts isolated, identified and selected of yeast from ruminants; beef cattle, dairy cow, goat and buffalos for examined the properties of probiotics at the optimum for dairy cows.

3.3 Objective

The main aim of this study was considered the yeasts isolation, selection and identification from ruminal fluid of ruminants habitat to determining the best probiotic yeast.

3.4 Materials and methods

3.4.1 Procedure of isolation and identification

3.4.1.1 Isolation step

In this study isolation was collected the native yeast from ruminants habitat. There are isolated differently places and animals as following, 1.) beef cattle from slaughterhouse, 2.) dairy cattle and goat were chosen to farm of Suranaree University of Technology, and 3.) buffalos were taken at small holder farm at Nongboonmark district Nakhon Ratchasima, Thailand.

In the morning before fed of animals, the samples were taken from the rumen fluid via a stomach tube that are immediately place on ice. Besides, the rumen fluid 1 ml have been moved into 50 ml of enrichment broth medium, which containing of glucose 70 (g/l), yeast extract 5 (g/l), and peptone 5 (g/l) with adjust pH onto 4.5 and incubate in an incubator shaker at 30^{O}C with shaking at 150 rpm. for 24 h according to Paserakung et al. (2015). Consequently, the enrich samples were diluted by serially dilution sample and also spread onto yeast malt agar (YMA) describe these technique by Cuppuccino (2012). The culture plates were incubated to incubator at 30^{O}C for 48 hours. Yeasts characteristic were separated by colony appearance according to Boekhout et al. (2002), and also purified by the conventional streaking technique on YMA plates. As a results, the purification yeast was transferred to YMA slant incubate at 30^{O}C for 48 hours, then maintenance with Cryoprotectant medium before storage at -80^{O}C until to selection step according to the K.M.P. Biotech.,LTD, Thailand.

3.4.1.2 Identification step

Accordingly, first-biochemical test which step was isolated to characterize 19 sugars fermenting yeasts by using API® 20C AUX Kit, which strip consists of 20 cupules containing dehydrated substrates. Second, genomic identification which mean confirmed by sequencing of domains D1/D2 of the 26S rDNA gene described by Hesham et al. (2014).

3.4.2 Procedure of selection

Each of the yeast ruminant sources that were obtained to stock in an isolation stage had been considered of their performance. Re-streak from stocks on YMA plate were incubated an incubator at 30°C for 48 h for experimental work. Then, the single colony was inoculated in the broth medium, which consists yeast extract, 3.5 g; peptone, 5 g; glucose, 10 g; and deionized water 1000 ml. according to Agarwal et al. (2000), incubated at 30°C for 24 h. Besides, 20 yeast isolates, that were chosen each 5 isolates from beef cattle, dairy cattle, goat and buffalo to the first tested by the temperature at 39°c. Afterward, the good 3 isolated from each animals were chosen to testing performance by the pH (3.5-7.5), *in vitro* tolerance to VFA in medium, and propagation on anaerobic condition.

3.4.2.1 Tolerance to organic acid

Yeast 12 isolates that were tested to tolerance organic acid. The volatile fatty acid (VFAs) that are acetic, propionic and butyric acids were mixed in the ratio of 70:20:10, respectively, that added into broth medium at a concentration for 0 0.1 0.25 0.5 and 1.0 % (v/v) and there adjusted pH by 0.1 N of HCL or NaOH to appropriate as following; 3.5 4.5 5.5 6.5 7.0 and 7.5. Tubes containing 7 ml broth was prepared to each yeasts. Live yeast culture (1 ml.) was mixed by 50 ml sterilize

normal saline and 0.5 ml of this dilute culture was used by the inoculation of each tube. The tubes were inoculated at 39^{o}C for 24 h and, after concern on a vortex mixer, the absorbance at 540 nm was recorded to measure grow of the yeast cultures according to Agarwal et al. (2000). Each yeast treatment was taken by three replications.

3.4.2.2 Growth in anaerobic conditions

This experiment on anaerobic condition for growth of some yeasts isolated adopt from Lund (1974) who has grown under anaerobic conditions. A medium consisting of glucose, 20 g; peptone, 20 g; yeast extract, 5 g and deionized water 1 litter were removed into tubes (10 ml/tube) and immediately before inoculation, 0.5 ml of a solution containing $NaHCO_3$ (10% w/v), cystein HCL (0.5%, w/v) and $Na_2S.9H_2O$ (0.5%, w/v) that were added by each tube. The tubes were inoculated by 0.1 ml yeast suspension (on loopful of a young agar culture suspended in 10 ml deionized water). They were incubated by anaerobic incubator at 39^{o}C for 24 h. The total viable count of the culture was measured by the spread plate technique, and also the colonies were counted after incubate 30^{o}C for 48 hours (h.).

In addition, the best yeast 3 isolates were selected to candidate going to the next step.

3.4.2.3 Yeast as co-active in the rumen by using *in vitro* gas production technique

This experiment was conducted *in vitro* gas production technique at various incubation time intervals. The study was used a design 4x2 factorial in completely randomized design (CRD) with three replications per treatment. A factor has consisted 3 rumen yeast strain, which is composed to RBe7, RBu3, and

RDc18 and B factor was composed by a dose two levels, which are 10 and 20% of the artificial fermented fluid. The single colony of each yeasts rumen candidate was incubated in the broth medium 100 ml, which consist yeast extract, 3.5 g; peptone, 5 g; glucose, 10 g, and deionized water 1000 ml. according to Agarwal et al. (2000) and also incubate for 30^{o}C at 24 h. Afterward, live yeast culture was inoculated in the strain rumen fluid with according to Menke and Steingass (1988). Briefly, the artificial saliva was prepared under anaerobic conditions in a water bath at 39°C with continuous stirring the strained rumen fluid were mixed in a 2:1 ratio (artificial saliva:rumen fluid) to prepare fermentation solution. Thirty-ml. of buffered rumen fluid solution that were dispensed into 100 ml calibrated glass syringes (which pre-warmed in a water bath for 39^{o}C at 1 h.) containing 200 mg substrate, which a total mixed ration (TMR) were constituted to substrates by concentrate (commercial feed 14% of CP) and roughage (rice straw) ratio follow as 60 : 40 ratio. Indeed the concentrate different live yeast culture isolated and two concentrate level as treatments were added to the grass syringes. Then, its were incubated in water bath for 39^{o}C and also that procedure was performed in triplicate.

Three rumen fistulated dairy cows fed with 2.5% of body weight (% BW) DM/day containing rice straw and commercial concentrate (14% crude protein) (60:40) were used as donors of rumen fluid. The ruminal fluid was sampled before the morning feeding from the three dairy cows and then placed in warm (39°C) insulated flasks under anaerobic conditions. All samples were pooled in equal proportions and strained through 8 layers of cheesecloth under anaerobic conditions and then used immediately.

During the incubation, the total gas productions were measured at 2 4 6 8 10 12 24 36 48 and 72 h. Net gas production values were corrected by subtracting blank values from the samples. The cumulative gas production data was fitted to the model of Ørskov and McDonald (1979) as follows : $p = a+b(1 - e^{-ct})$; where p represent the cumulative gas production at time t, c is the rate of gas production (per hour) and $(a+b)$ is the potential gas production. Meanwhile, at the 24 h. of *in vitro* incubation, the sample of rumen fluid will also be taken and used for estimation of ruminal pH, volatile fatty acid (VFAs) by using HPLC according to Samuel et. al. (1997).

3.4.2.4 Analysis of statistics

The experiment was analyzed as a completely randomized design by using the PROC ANOVA of SAS (1998). For the yeast selection, along with pH tolerate, organic acid resistant, and to growth in anaerobic condition that were used as a normal distribution was considered. For *in vitro* gas production experiment that was considered to as following statistical model- $Y_{ij} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon_{ij}$, where Y = observations, μ = overall means, α = main effect A, β = main effect B, $\alpha\beta$ = interaction AB and ε = error. The statistics significant differences between treatments were determined to using Duncan's News Multiple Range Test (DMRT) (Steel and Torrie, 1980).

3.4.3 Location of the study

These experiments were conducted at the Center for Scientific and Technological Equipment Building 10, Suranaree University of Technology, Nakhon Ratchasima, Thailand and the K.M.P.Biotech.,LTD in Chonburi Province, Thailand where were cooperated to research.

3.4.4 Experimental period

The experiments were carried out from June 2016 to March 2017.

3.5 Results and discussion

3.5.1 Isolation and identification

These native yeasts were isolated to ruminal fluid from 4 kind of ruminants, which are Beef cattle (Bc), Dairy cow (Dc), Goat (Go) and Buffalo (Bu). Although the animals were not treated the feeds to upon each farm, all of them were robustness and not a diseased as a host. The yeast 91 isolates that were isolated amount 23 27 23 and 18 isolates from Be, Dc, Go, and Bu respectively. Moreover, the colonies characterize of yeasts as a white circle although there were difference cell shapes in broth medium after incubated at 39^{o}C, 24 hours as following figure 3.1.

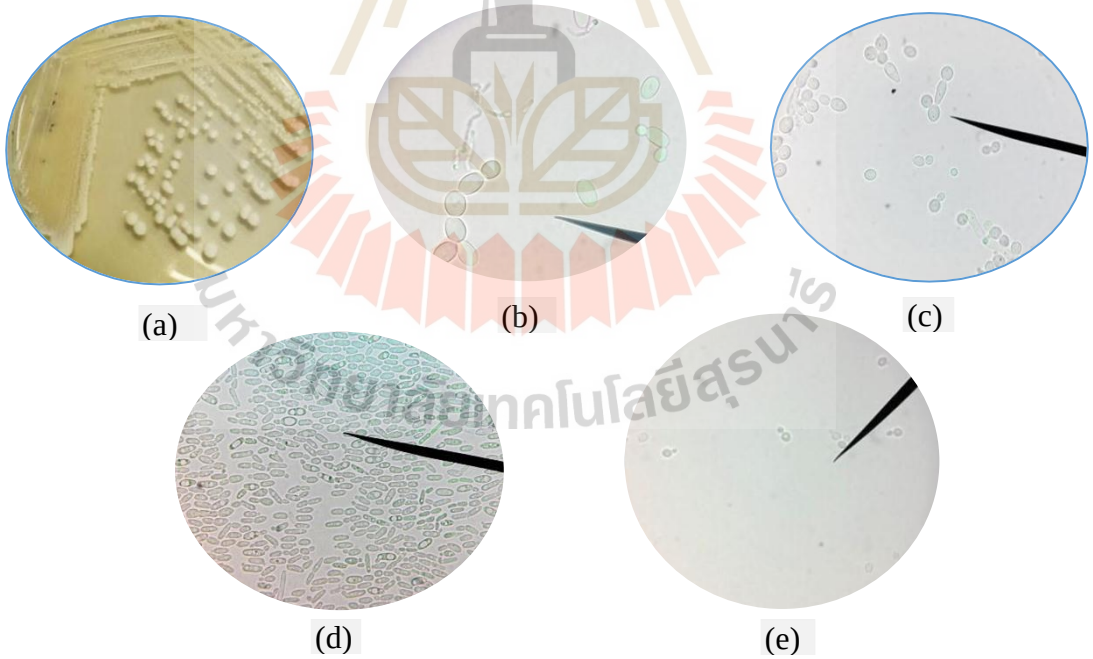

Figure 3.1 Characteristics of yeast that were colonies and budding cell shapes, which were (a) = white colonies on agar medium, (b),(c) and (d) = cell shapes of *Candida tropicalis* as a isolate from buffalo, beef cattle, and goat respectively, (e) = *Candida glabrata* isolate from dairy cattle.

Yeast isolated was identified to used both method by biochemical and molecular sequencing. Firstly, thirty-eight isolates were used to testing by the biochemical test for screen the species of yeast. Consequenly, the isolate that were found *Candida glabrata* (14 isolate), *Candida rugosa* (6 isolate), *Candida krusei* (5 isolate), *Candida tropicalis* (10 isolate) but 3 isolates could not identifies and also an isolate was *Candida albican.* In addition, this identification that was identified by using API® 20C AUX Kit, which was considered to morphological and physiological abilities. But this identified method of yeast species and strain was conventional method, are unreliable and may give uncertain results (Hesham et al. (2014), Guillamón et al. (1998)). Second, the molecular sequencing method was excellently to identified of yeast species and strained. In this study, eight yeasts isolate were identified to able as following Table 3.1. Four species of Candida genera that were found by molecular sequencing method by using the universal gene on the region was D1/D2 domain of 26s rDNA gene that found 98-100% of identify. The genus of yeasts *Candida* were revealed to this study, although *Pichia* spp. or *Issatchenkia orientalis* was found. Similarity of the results, yeasts strain were isolated from animals that found *Candida spp.* and *Picia* spp. for some instance, e.g. reported by Lund (1974) and Lund (1980) they were isolated from Bovine rumen and Musk oxen, and Sirisan et al. (2013) who isolated from dairy cattle.

3.5.2 Selection of yeasts were tested by different pH and organic acid in broth medium

The selection on yeasts were used differently pH and short chain fatty acid or total volatile fatty acid that were adjusted the condition of medium.

Consequently, the growth of yeasts that were affected by pH values in broth. The various

Table 3.1 Yeast identified by molecular sequencing of domains D1/D2 of the 26S rDNA gene.

Isolation sources	Isolated Unknown	GenBank accession No.	Species	Identify (%)
Beef cattle	Be6	HQ860277.1	*Candida rugosa*	98
	Be7	KU862652.1	*Candida glabrata*	100
Buffalo	Bu3	KY928414.1	*Candida glabrata*	99
	Bu7	EF151501.1	*Candida tropicalis*	99
Dairy cattle	Dc14	EF151501.1	*Candida tropicalis*	99
	Dc18	KM103029.1	*Candida glabrata*	99
Goat	Go19	EU479714.1	*Candida tropicalis*	99
	Go20	EU543672.1	*Issatchenkia orientalis*	99

isolate of yeast were grown differ highly significantly ($P<0.01$) that showed on Table 3.1. In this study, although yeasts from rumen were grown in variously pH in broth medium, they were increased to growth on broth medium when adjusted pH at 6.5-7. Besides, the rumen pH with 6.8-7 that are appropriately range for the function of ruminal microbial. Likewise, Russell (2009) noted that the rumen pH is nearly neutral intracellular pH, and the pH gradient lead to a logarithmic accumulation of organic acid anions. While, some strain of yeast (*Sacharomyces cerevisiae*) can be tolerate pH as low as 2 for 6 hours was reported by Agarwal et al. (2000). However, in presence

the authoconous yeast that were isolated from rumen of ruminant can not propagation in pH as 2 they were grown to initially viable as 3.5 to 7.5 of pH condition in broth medium. In addition the pH as 6.5 was not only best the condition to growth for yeast but also an optimizing condition to active for rumen microbial.

The growing yeast in broth medium as a differently organic acid, which total volatile fatty acid (TVFAs) were showed on Table 3.2. The isolate of yeast strain Bu3 and Dc18 were better growth performance than other isolate ($p<0.01$) they can tolerated to TVFAs all of concentrations. While the growth tended to decline when concentration of TVFAs was increased. As this pointed, the organic acids can be passed by the cell wall of yeast, then cells were broken. Likewise, Agarwal et al. (2000) indicated that the mixture of volatile fatty acid in the broth was suppressed to growth of yeast all of concentrate. So, the rumen condition was not multiplying by yeast (*Saccharomyces cerevisiea*) because of the temperature and the chemical composition of rumen fluid were prevented to multiplying (Newbold et al., 1996a). Interestingly, in this present the native yeast can be growth on broth medium was added to mixture volatile fatty acid particularly strain Bu3 and Dc18.

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Table 3.2 Effect of pH (3.5-7.5) in broth medium on the growth of yeasts.

Isolate	pH values					
	3.5	**4.5**	**5.5**	**6.5**	**7**	**7.5**
Dc4	0.194^{d}	0.344^{d}	0.403^{b}	0.406^{cd}	0.433^{c}	0.345^{c}
Dc14	0.185^{d}	0.338^{d}	0.403^{b}	0.369^{d}	0.432^{c}	0.334^{c}
Dc18	0.357^{a}	0.449^{ab}	0.403^{b}	0.501^{b}	0.488^{b}	0.403^{b}
Be1	0.345^{ab}	0.406^{c}	0.373^{bc}	0.420^{cd}	0.434^{c}	0.412^{b}
Be2	0.348^{ab}	0.418^{bc}	0.407^{b}	0.395^{cd}	0.417^{c}	0.320^{c}
Be7	0.315^{b}	0.410^{bc}	0.372^{bc}	0.428^{c}	0.436^{c}	0.401^{b}
Bu3	0.366^{a}	0.478^{a}	0.501^{a}	0.579^{a}	0.523^{a}	0.500^{a}
Bu4	0.214^{cd}	0.329^{de}	0.371^{bc}	0.423^{cd}	0.443^{c}	0.346^{c}
Bu7	0.238^{c}	0.357^{d}	0.395^{b}	0.396^{cd}	0.422^{c}	0.358^{c}
Go10	0.235^{c}	0.169^{f}	0.101^{d}	0.117^{e}	0.096^{e}	0.045^{d}
Go16	0.203^{cd}	0.291^{e}	0.348^{c}	0.376^{cd}	0.379^{d}	0.341^{c}
Go19	0.238^{c}	0.189^{f}	0.115^{d}	0.113^{e}	0.103^{e}	0.064^{d}
SEM	0.012	0.16	0.19	0.02	0.02	0.02
p-value	**	**	**	**	**	**

The letters of superscript in the column that means differently significant (P<0.01)

** p<0.01

Dc = yeast strain isolated from dairy cow, Be = yeast strain isolated from beef cattle, Bu = yeast strain isolated from buffalo, and Go = yeast strain isolated from goat

Table 3.3 Effect of concentration of total volatile fatty acid (TVFAs) in broth medium on yeast growth.

Isolate	TVFAs (% of broth medium)					
	0	**0.25**	**0.5**	**1**	**2**	**4**
Dc4	0.357^{d}	0.359^{d}	0.378^{ef}	0.381^{cd}	0.377^{d}	0.382^{cd}
Dc14	0.355^{d}	0.355^{d}	0.350^{f}	0.371^{de}	0.336^{e}	0.339^{d}
Dc18	0.542^{a}	0.542^{a}	0.553^{b}	0.557^{a}	0.484^{b}	0.454^{b}
Be1	0.447^{bc}	0.447^{bc}	0.467^{c}	0.438^{b}	0.437^{c}	0.410^{bc}
Be2	0.419^{bc}	0.419^{bc}	0.427^{d}	0.414^{bc}	0.379^{d}	0.355^{d}
Be7	0.457^{b}	0.457^{b}	0.445^{cd}	0.435^{b}	0.438^{c}	0.415^{bc}
Bu3	0.567^{a}	0.569^{a}	0.597^{a}	0.582^{a}	0.562^{a}	0.525^{a}
Bu4	0.408^{c}	0.408^{c}	0.389^{e}	0.414^{bc}	0.386^{d}	0.384^{cd}
Bu7	0.413^{bc}	0.413^{bc}	0.377^{ef}	0.399^{bcd}	0.367^{d}	0.343^{d}
G16	0.365^{d}	0.365^{d}	0.364^{ef}	0.341^{d}	0.333^{e}	0.341^{d}
SEM	0.07	0.016	0.015	0.014	0.013	0.011
p-value	**	**	**	**	**	**

The letters of superscript in the column that means differently significant (P<0.01)

** p<0.01

Dc = yeast strain isolated from dairy cow, Be = yeast strain isolated from beef cattle, Bu = yeast strain isolated from buffalo, and Go = yeast strain isolated from goat

3.5.3 Growth in anaerobic conditions

The growth of yeast in anaerobic condition *in vitro* that was showed on Figure 3.2. They were declined to grow all of strain when compare with aerobic condition. It is probably the propagation of yeast that was limited by amount of oxygen in the broth and incubator, which the condition that was 5% of oxygen and 20% of carbon dioxide. While, the yeast of strain Dc18 had considered viable count been to highest by highly of significantly different ($p<0.01$).

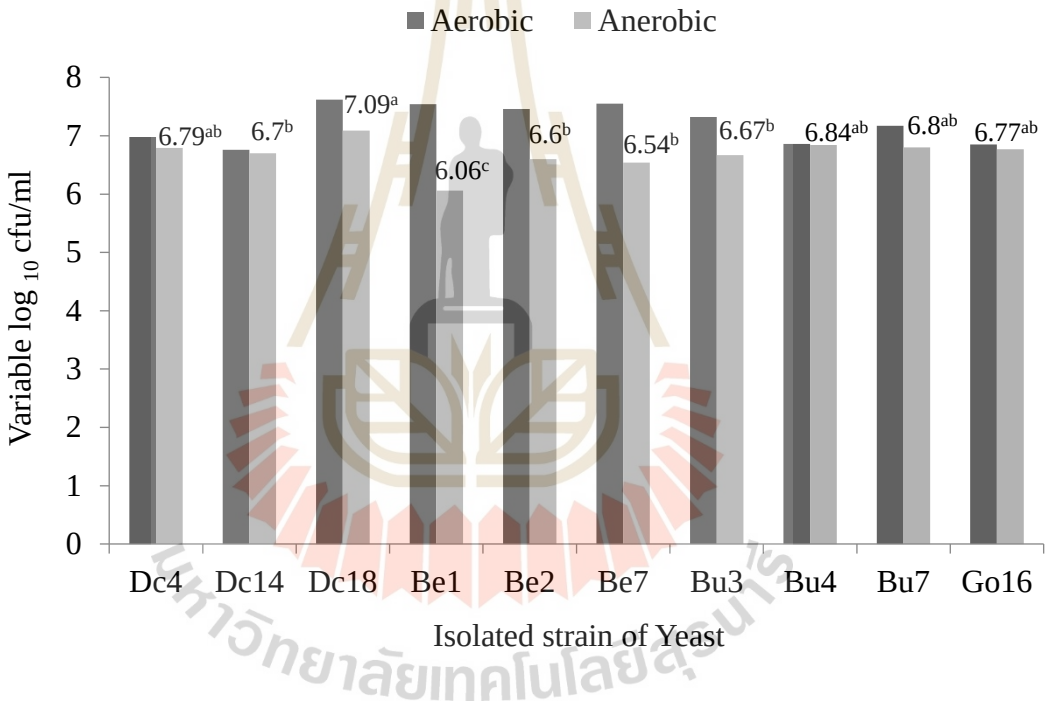

Figure 3.2 To demonstrate of viable count of yeasts variably strain that were incubated in *in vitro* aerobic and anaerobic condition, which incubator was set 5% of O_2 and 20 % of CO_2 , 24 h of incubated.

On the other hand, Lund (1974) reported that the reproduction of yeast in the rumen (a few of air) is limited to growth. Whereas, to various strain of yeasts were differed to O_2 uptake that was indicated by Newbold et al. (1996b). Interestingly, in

this study some strain can be survival, when limitation of O_2 that are used to the step forward as yeast candidate.

3.5.4 Yeast as co-active in the rumen using by *in vitro* gas production technique

Gas kinetics in term of instantly soluble fraction (*a*), insoluble fraction (*b*) and potential extent of gas production (*a*+*b*), were not interaction ($p>0.05$) among main effect, but the main effect factor B (doses of live yeast culture) lead to a, b and c values were highly significant different ($P<0.01$) as follow in Table 3.4. Whereas, the cumulative gas production profiles from the *in vi*tro fermentation of treatments were showed on Table 3.5 and Figure 3.3. Although of gas production at 24 h incubation were highly significant different ($P<0.01$) with positive control, after hr. 36, 48 and 72 were increased by yeast strain inoculum which were relationship with the doses (Table 3.5). Likewise, Blümmel et al. (1997) and Sommart et al. (2000) indicated gas production that was shown to be a good predictor of microbial growth and short chain fatty acid (SCFA) production. Indeed, Marrero et al. (2015) demonstrated that the yeast strain (*Candida* spp.) enhances the ruminal fermentation process and the action of microorganisms on gas production and DM digestibility of oat straw fiber, as the same result with this experiment although addition live yeast strain lower than positive control, within live yeast strain culture different gas responsibility and the VFA (Table 3.3). However, the effect of doses were highly significant different ($P<0.01$) on gas production and the volatile fatty acid. Otherwise, Brossard et al. (2006) who noted the yeast action that may differed to depending on nature and function of rumen microbial.

Table 3.4 Effect of live yeast culture and concentrated level supplementation on gas kinetics that was used by an *in vitro* gas production.

Items	Gas kinetic[1]				GP, ml at 24 h
	a, ml	b, ml	c, ml/h	a+b, ml	
[2]Strain					
YC0	-18.54	124.87	0.14[a]	106.33	99.37[a]
YC1	-11.58	120.37	0.09[b]	108.80	93.93[b]
YC2	-13.77	120.69	0.09[b]	106.92	90.23[c]
YC3	-14.48	121.46	0.09[b]	106.98	91.20[b]
[3]Doses, % of fermented fluid					
10	-8.38[b]	114.67[b]	0.08[b]	106.08	87.04[b]
20	-20.80[a]	129.03[a]	0.12[a]	108.22	100.33[a]
[4]P-Value					
YC vs Dose	ns	ns	ns	ns	ns
YC	0.05	0.06	**	ns	**
Dose	**	**	**	ns	**

[1]*a*, the gas production from the readily dietary soluble fraction; *b*, the gas production from the insoluble fraction, but slow releases; *c*, the gas production rate constant for the insoluble fraction; *a+b*, the potential extent of gas production; GP, gas production at 24 h of incubated (mL/ 200 mg of DM substrate, concentrate 120 g and rice straw 80 g) [2]Strain; YC0 = Yeast culture no inoculum, YC1 = yeast culture inoculum strain Be7 ($5.1x10^7$ cfu/ml), YC2 = yeast culture inoculated yeast strain Bu3 ($4.3x10^7$ cfu/ml), YC3 = yeast culture inoculated yeast strain Dc18 ($4.1 x10^7$ cfu/ml), yeast culture 1000 ml. was consisted to 3.5 g of yeast extract; 5 g of peptone, 10 g of glucose. [3]Doses of supplementation; 10% of artificial rumen fluid, 20% of artificial rumen fluid. [4]YC vs Dose = interaction of yeast strain and level of supplemented, YC = main effect A, Level = the main effect B [a, b, c] Means within a column lacking a common superscript letter differ * P<0.05, ** P<0.01, ns = not significant

Table 3.5 Effect of live yeast culture (main effect factor A) and doses added (main effect factor B) on gas cumulative at 2-72 h incubated.

Items	Gas accumulates, ml/ 200 mg of substrate									
	H2	H4	H6	H8	H10	H12	H24	H36	H48	H72
[1]Strain										
YC0	11.70	34.20	51.00	63.61^{a}	73.12^{a}	80.33^{a}	99.37^{a}	114.66	118.47	119.96
YC1	8.75	21.87	39.30	50.71^{b}	60.16^{b}	68.00^{b}	93.93^{b}	91.74	94.46	95.37
YC2	5.40	21.38	34.75	46.00^{c}	55.31^{c}	64.39^{b}	90.23^{c}	114.06	121.15	124.49
YC3	5.40	25.50	35.56	46.96^{bc}	60.16^{bc}	80.33^{a}	91.20^{c}	121.24	130.18	134.62
[2]Dose, % of fermented fluid										
10	8.39	22.53	34.48	44.61^{b}	53.22^{b}	60.56^{b}	87.0^{b}	105.59	110.77	113.07
20	7.22	28.84	45.82	59.00^{a}	69.30^{a}	77.38^{a}	100.33^{a}	114.49	120.92	123.91
[3]P-Value										
YC VS Dose	**	**	*	0.07	ns	ns	ns	**	**	**
YC	**	**	**	**	**	**	**	**	**	**
Dose	*	**	**	**	**	**	**	**	**	**

[1]Strain; YC0 = Yeast culture no inoculum, YC1 = yeast culture inoculum strain Be7 ($5.1x10^{7}$ cfu/ml), YC2 = yeast culture inoculated yeast strain Bu3 ($4.3x10^{7}$ cfu/ml), YC3 = yeast culture inoculated yeast strain Dc18 ($4.1 x10^{7}$ cfu/ml), yeast culture 1000 ml was consisted to 3.5 g of yeast extract; 5 g of peptone, 10 g of glucose.

[2]Doses of supplementation; 10 % of artificial rumen fluid, 20% of artificial rumen fluid., [3]YC vs Dose = interaction of yeast strain and dose of supplemented, YC = main effect A, Dose = main effect B, * $P<0.05$, ** $P<0.01$, ns = not significant

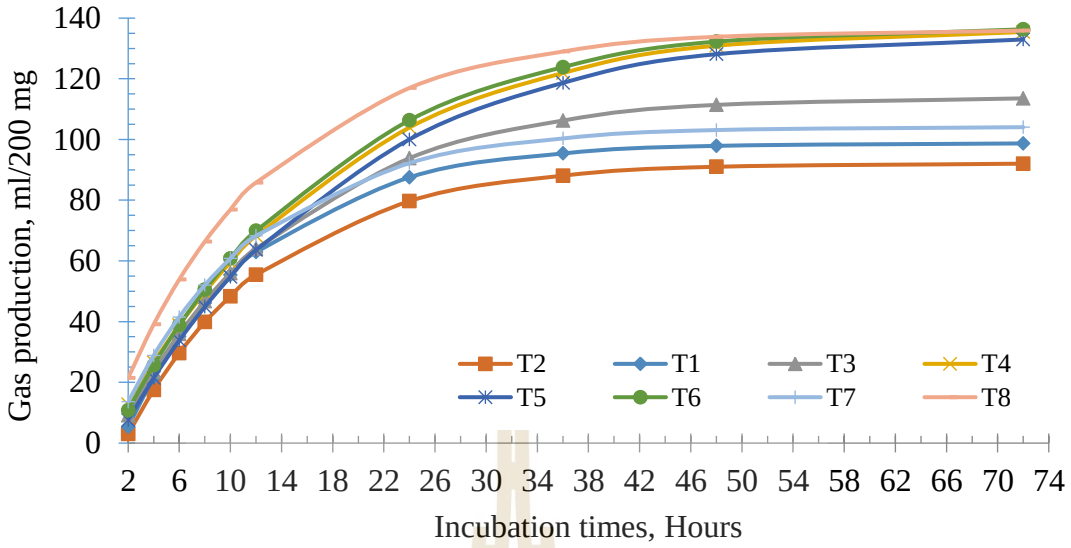

Figure 3.3 Gas cumulative profile of treatments when incubated with by presents of rumen fluid at different incubation times, T1=YC1, T2 = YC2, T3 = YC3, and T7 = YC0 with 10% of fermented fluid, T4 = YC1, T5= YC2, T6 = YC3, and T8= YC0 with 20% of fermented fluid.

Additionally, the effect of live yeast culture and doses on volatile fatty acid that were presented in the table 3.4, and interaction effect among main factor were showed in table 3.5. The results of study that revealed to acetic acid (C_2) and acetic acid : propionic acid (C_3) ratio ($P<0.01$) (Table 3.4) were interacted effect by addition to live yeast strain and doses. Otherwise, the butyric acid (C_4) was highly significant different ($P<0.01$) more than the control (YC0, only the culture no inoculated live yeast). Whereas the total VFAs was not influenced by treatments. These a results were similar with Polyorach et al. (2014) who reported that the total VFA and C_3 increased, while C_2 and C_2:C_3 ratio were decreased with an increasing concentrate level and also Pinloche et al. (2013) that indicated the addition probiotic yeast (5 g/d) was highest VFA than low dose (0.5 g/d). As the same way, in the diets with more than 50% concentrate with added high level live

yeast was improved nutrients digestibility (Figueiroa et al., 2015). Unlikely, Lynch and Martin (2002) who founded the supplementation of *S.cerevisiae* live cell decreased acetate more than *S.cerevisiae* culture. Indeed, although nitrogen was limiting and stopped fermentation (Cone et al., 2009), ammonia nitrogen for microbial growth in vitro has been reported to range between 2-5 mg N/100 ml. (Satter and Slyter, 1974).

Table 3.6 Effect of live yeast culture (effect factor A) and doses added (effect factor B) on volatile fatty acid (VFA).

Items	**VFA (%)**				**Total VFA (mmol/L)**
	C2	**C3**	**C4**	**C2:C3**	
[1]Strain					
YC0	59.22^{a}	29.09	11.70^{b}	2.04	105.65
YC1	57.13^{b}	28.45	14.42^{a}	2.01	104.72
YC2	57.56^{b}	27.83	14.61^{a}	2.08	110.02
YC3	58.15^{ab}	27.80	14.05^{a}	2.10	113.13
Doses, % of fermented liquid					
10	56.61^{b}	28.64^{a}	14.74^{a}	1.98^{b}	105.60
20	59.20^{a}	27.79^{b}	13.01^{b}	2.14^{a}	111.66
P-Value					
YC vs Dose	**	0.05	0.05	**	ns
YC	**	0.10	**	ns	ns
Dose	**	*	**	**	ns

[1]Strain; YC0 = Yeast culture no inoculum, YC1 = yeast culture inoculum strain Be7 ($5.1x10^{7}$ cfu/ml), YC2 = yeast culture inoculated yeast strain Bu3 ($4.3x10^{7}$ cfu/ml), YC3 = yeast culture inoculated yeast strain Dc18 ($4.1 x10^{7}$ cfu/ml), yeast culture 1000 ml was consisted to 3.5 g of yeast extract; 5 g of peptone, 10 g of glucose.

[3]Doses of supplementation; 10 % of artificial rumen fluid, 20 % of artificial rumen fluid.

[3]YC vs Dose = interaction of yeast strain and level of supplemented, YC = main effect A, Level = main effect B, * P<0.05, ** P<0.01, ns = not significant

3.6 Conclusion

Based on these results that were concluded by 3 parts as follow: firstly, the both of isolated and identified yeast from ruminants, which are collected from the dairy cow, beef cattle, buffalo and goat that were found genera of *Candida spp*., which *C.glabrata, C.tropicallis, C.rugosa, C.krusei,* and also *Issatchenkia orientalis*. This the results identified by means of molecular sequencing. Second, the isolated yeast of strain Bu3 and Dc18 were grown on the broth medium, when adjusting the pH values, an organic acid and anaerobic condition to highest viable count were chosen to test on *in vitro* gas production technique. Third, based on *in vitro* experiment, it could be concluded that to addition live yeast culture, Dc18 with 20% of fermented fluid could improve gas kinetic, gas cumulative, and also increased acetic acid (C_2), acetic acid: propionic acid (C_3) ratio. In addition, the next experiment will be using live yeast of strain Dc18 in fistulated dairy cows on rumen ecology, rumen fermentation and physicochemical parameters for truly understanding the action of live yeast in the rumen.

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CHAPTER IV

EXPERIMENT II

TO STUDY THE CONCENTRATION OF LIVE YEAST (*Candida glabrata*) PRODUCT ON RUMEN FERMETATION MICROBIAL POPULATION IN RUMEN FISTULATED DAILY COW

4.1 Abstract

The aim of this study was determine rumen fermentation, microbial diversity, hematology to supplemented by differences live yeast product for ruminal fistulated dairy cows. Four ruminal cannulated dairy cows were used as the experimental unit according to a 4 x 4 Latin square design, each period was 28 days. Treatment consists of control (T1, without live yeast product) and supplementation of live yeast product amount 3 level, 50 100 and 150 g/h/d for T2 T3 and T4 respectively. As the result found that the concentration of live yeast (*Canida glabrata*) product on rumen fermentation and microbial population which can concluded that *C.glabrata* was not negative effect on hematology parameters . On the other hand, average ruminal pH had effected by live yeast concentration although both of NH_3-N and Plasma Urea Nitrogen (PUN) were no significance different ($P>0.05$). Likewise volatile fatty acid (VFAs) was not differ significance ($P>0.05$) by live yeast product. In nylon bag digestion of SUT® concentrate and rice straw including to DM disappeared, effective DM degradability were not

affected (P>0.05) by live yeast level In addition rumen microbial population were not affect excepted both of yeast (high with supplemented live yeast) and ciliate protozoa (low with supplemented live yeast) concentration when compared with control (P<0.05). Consequently, the concentration of live yeast product at 50 g/h/d or T2 could be appropriated to used for transition dairy cow period.

4.2 Introduction

To utilize of yeast products or probiotics that are interested by ruminant nutritionists to more over the past two decades. Because there are altered feed additive instead promoting growth or antibiotics, and also improve rumen fermentation, increased viable count of bacteria (McCann et al. (2017), Uyeno et al. (2015), Lynch and Martin (2002), Newbold (1996a), Newbold et al. (1998)). However, differences strain of yeast were various to stimulating bacteria in the rumen, then when selecting the commercial yeast product that have to ensure for capability to stimulating ruminal bacteria (Newbold et al., 1995)

Currently, most products of yeast are based on *Saccharomyces cerevisiae* or combine with *Aspergillus oryzae*, but there are not new species of yeast that was plays the role an importance to concerned for used in ruminants. A few research of the new species for instance e.g., Lee et al. (2000) who indicated that was used a ruminal anaerobic fungi can improved rumen bacterial population, nutrients digestibility and increased fibrolytic enzyme activities. So, based on alfalfa feed, Marrero et al. (2015) demonstrated that compared native yeast, which *Candida tropicallis* and *Candida norveginsis* they were isolated from rumen of goat that found the *C.norveginsis* can be supported DM fermentation more than the *C.tropicallis*. Otherwise, the most of

effective yeasts, along with *Pichia kudriavzevii*, *Candida rugosa* and *Kodamaea ohmeri* could be used as probiotic for dairy cattle that are lactic acid utilizing yeast (Sirisan et al., 2013). Meanwhile, the new yeast generations that can be used for ruminant, although in Thailand still have not been probiotic specific for ruminant. Therefore, we needed to using native yeast, *Candida glabrata* which was selected from ruminal of ruminant by various the concentration of the yeast product on rumen fermentation and microbial diversity in ruminal fitulated dairy cow.

4.3 Objective

The objective of this study was examined the concentration of yeast product that effected on rumen fermentation and microbial diversity.

4.4 Materials and methods

4.4.1 Experimental design, animals and treatments

The experiment was conducted to recommending by Animal Care and Use Committee of Suranaree University of Technology, Suranaree University of Technology, Nakhon Ratchasima province, Thailand.

The experimental design was conducted to as follows in the 4 x 4 Latin Square Design (LSD) which is 28 days a period. The feed management used the commercial concentrate (16% crude protein) and rice straw was roughage source, also that offered twice times a day (0700 and 1600 h) was feeding. They were gotten fed by limited feeding at total intake 1.5% BW., so as to prevented leak of rumen fluid.

Four rumen fistulated dairy cows were used to examine the effect of concentration of yeast product supplementing on rumen microbial population,

fermentation and nutrients digestibility. To initial body weight of the cows were 489.5±49 kg (mean ± SD). Meanwhile, all cows were housed to individual crate, which in size 2.5x4 meters and also free available of water and mineral block. They were injected by ivermectin which to killed internal and external parasites and AD_3E vitamins before start experiment.

The treatments were used yeast product ($4.5x10^7$ colony forming unit/gram) by arranged a concentrate that were consisted T1, the control diet contained only sterilized starch 150 grams; T2, yeast product 50 g. + 100 grams of sterilized starch; T3, yeast product 100 g.+50 grams of sterilized of starch and T4, yeast product 150 grams. They were mixed with the concentrate before dietary fed on the morning.

The DM digestion of rice straw and SUT® concentrate were evaluated using by nylon bag technique. The samples were used 3 grams (concentrate) and 5 grams (rice straw) in polyester bag that were used 45 µm of pore size and 140x90 mm according to Ørskov and McDonald (1979) and Ørskov et al. (1980). The time for incubates were used at 0 (washing loss), 3 6 9 12 24 48 and 72 hours, which were repeated by each period. In calculation DM disappearance follow by ; $p = a + b(1 - e^{-ct})$ where a; the intercept of degradation curve at time zero (%), b; the fraction of DM degraded when given sufficient time for digestion in the rumen (%), c; a rate constant disappeared of fraction B (h^{-1}), and t is time of incubate. Indeed, effective DM degradation as follow the formula; $p = a + \frac{(b)(c)}{c+k}$ where a, b and c were same means as above explained, k is out flow rate from from the rumen that was obtained from the previous study supported inform by Ørskov and McDonald (1979).

4.4.2 Samples collection, parameters, analysis

The experimental periods were used 28 days a period which 14 day for adjusted the animal and digestive tract and also 14 day for collected the samples. The samples from rumen fluid and blood were collected on day 28 at 0 3 and 6 hours after feeding. The samples of rumen fluid were taken via rumen fistula by hand, which were strained though 4 layer of cheesecloth. The rumen fluid was divided 2 portions, the first- for analyze volatile fatty acids (VFAs) by Gas Chromatography and NH_3-N added 6N-HCL for stop microbial activities according to Bremner and Keeney (1965), the second- to examined microbial population as follows the method and described by Yu and Morrison (2004). Briefly, three step of DNA were extracted including by 1) cell broken, the samples in 2 ml. tube as mixed with sterile zirconia bead. Thereafter, there was broken by the beadbeater machine, 2) Nucleotides precipitated, the precipitation was used 10 M ammonium acetate and dissolve by Tris-EDTA buffer and 3) DNA purify, this step was removing RNA, protein and purification by QIAamp DNA stool mini kit, the procedure as follow kit. Moreover, the primers for amplify in Rt-PCR that were showed on Table 4.1. At the same time on the rumen fluid collected, blood samples that were analyzed a complete blood count (CBC) by used the Blood Analyzer Model Mindray® BC-2800Vet and plasma urea nitrogen (PUN) according to Crocker (1967)

4.4.3 Statistical analysis

All data were analyzed by general linear models (Proc GLM) according to Statistical Analysis System Institute (SAS, 2004). The comparison of treatments were used by Duncan's New Multiple Range Test (DMRT) and trend analysis by Orthogonal polynomial (Steel andTorrie, 1980).

Table 4.1 Primers sequence for run in the real time-PCR quantification.

No.	Items	F/R	sequence	Size(bp)	References
1	*General bacteria*	F	CGGCAACGAGCGCAACCC	130	Denman and McSweeney (2006)
		R	CCATTGTAGCACGTGTGTAGCC		
2	*Ruminococcus flavefaciens*	F	CGAACGGAGATAATTTGAGTTTACTTAGG	132	
		R	CGGTCTCTGTATGTTATGAGGTATTACC		
3	*Prevotella ruminicola*	F	GCGAAAGTCGGATTAATGCTCTATG	78	
		R	CCCATCCTATAGCGGTAAACCTTTG		
4	General anaerobic fungi	F	GAGGAAGTAAAAGTCGTAACAAGGTTTC	120	
		R	CAAATTCACAAAGGGTAGGATGATT		
5	*Succinimonas amylolytica*	F	CGTTGGGCGGTCATTTGAAAC	139	Khafipour et al. (2009)
		R	CCTGAGCGTCAGTTACTATCCAGA		
6	*Prevotella brevis*	F	GCGAACTGGTTTCCTTGAGTGTATT	153	
		R	ACCTTCGAGCTTTAGCGTCAGTTAT		
7	*Fibrobacter succinogenes*	F	GGAGCGTAGGCGGAGATTCA	97	
		R	GCCTGCCCCTGAACTATCCA		
8	*Prevotella bryantii*	F	GAAGGCAGCTCGCTGTAGTGTT	145	
		R	CTTAACGCTTTCGCTTAGCCACT		

Table 4.1 (Continue).

No.	Items	F/R	sequence	Size(bp)	References
9	*Ruminococcus albus*	F	CCCTAAAAGCAGTCTTAGTTCG	176	
		R	CCTCCTTGCGGTTAGAACA		
10	*Selenomonas ruminantium*	F	CAATAAGCATTCCGCCTGGG	71	Stevenson and Weimer (2007)
		R	TTCACTCAATGTCAAGCCCTGG		
11	*Megasphaera elsdenii*	F	GACCGAAACTGCGATGCTAGA	129	Ozutsumi et al. (2006)
		R	CGCCTCAGCGTCAGTTGTC		
12	*Butyrivibrio fibrisolvens*	F	ACACACCGCCCGTCACA	64	Klieve et al. (2003)
		R	TCCTTACGGTTGGGTCACAGA		
13	General methanogens	F	TTCGGTGGATCDCARAGRGC	140	Denman et al. (2007)
		R	GBARGTCGWAWCCGTAGAATCC		
14	Ciliate protozoa	F	GCTTTCGWTGGTAGTGTATT	223	Sylvester et al. (2004)
		R	CTTGCCCTCYAATCGTWCT		
15	*Eubacteria*	F	CCTACGGGAGGCAGCAG	189	Muyzer et al. (1993)
		R	ATTACCGCGGCTGCTGG		
16	*Streptococcus bovis*	F	TTCCTAGAGATAGGAAGTTTCTTCGG	82	
		R	ATGATGGCAACTAACAATAGGGGT		
17	*C.glabrata* Dc18	F	CAGACATGGTGTTTTGCGCC	174	This study, NCBI design primers association no. LC015349.1
		R	AGTATCGCAGTCCTCGGTCC		

4.4.4 Experimental places

The Suranaree University of Technology part of Dairy farm and the Center for Scientific and Technology Equipment Building 9 10 and 14, where were used for field experiment and laboratory, respectively.

4.5 Results and discussion

4.5.1 Feed intake

The dry matter feed intake, which concentrate (commercial feed), roughage (rice straw) and total feed intake were showed in table 4.2. All feed, that were not significant ($P>0.05$). The fistulated cows were gave by limitation of total feed intake at 1.5% BW, because of prevented to leak of rumen fluid. However, the cows were not decreased of body weight and follow as recommend feeding for dairy cow by NRC (2001).

Table 4.2 Effect of quantities of probiotic yeast on feed intake.

Items	Treatments				SEM	P-value
	T1	T2	T3	T4		
Dry matter intake (kg/h/d)						
- concentrate	2.553	2.483	2.530	2.533	0.05	ns
- Roughage	4.94	5.03	4.89	5.23	0.12	ns
- Total	7.49	7.51	7.42	7.76	0.16	ns
Dry matter intake (% BW)						
- Concentrate	0.518	0.498	0.510	0.530	0.08	ns
- Roughage	1.025	1.028	1.000	1.015	0.007	ns
- Total	1.542	1.527	1.510	1.550	0.01	ns

[1] The treatments were compounded by the ratio of live yeast product (g.) and starch sterilized (g.) as follow 1 = 0 : 150 2 = 50 : 100 3 = 100 : 50 and 4 = 150 : 0 Yeast produced ; *Candida glabrata* was used this experiment, which was concentrated by 4.5×10^7 cfu/g

4.5.2 Ruminal pH and Ammonia nitrogen (NH_3-N)

The effect of supplemented live yeast on ruminal pH were significance difference (P<0.05) thereafter animals gave the live yeast at 6 hours post-feeding and also the means value as the same result. This study addition live yeast can increased rumen pH and stabilized of pH in rumen. Likewise Desnoyers et al. (2009) who found that was supplementation of yeast increased rumen pH about +0.03 on average due to decreased lactic acid concentration in the rumen (Ding et al., 2014b). On the other hand, from the presence experiment was not measured by lactic acid although VFAs concentration were not differ significance (P>0.05) (Table 4.4). However the NH_3-N and PUN (plasma urea nitrogen) were not difference (P>0.05) all of hours measured, that are follow as Table 4.3

Table 4.3 Effect of probiotic yeast level of rumen fermentation which included pH, ammonia nitrogen (NH_3-N) and plasma urea nitrogen (PUN).

Items	Hours post feeding	Treatments[1]				SE	Contrast[2]		
		T1	T2	T3	T4		L	C	Q
pH	0	6.91	6.94	6.93	7.00	0.04	ns	ns	ns
	3	6.61	6.67	6.63	6.69	0.04	ns	ns	ns
	6	6.59^{ab}	6.68^{a}	6.54^{b}	6.65^{a}	0.05	ns	ns	ns
	Means	6.71^{b}	6.76^{ab}	6.70^{b}	6.78^{a}	0.04	ns	ns	ns
NH_3-N	0	6.43	6.80	7.15	7.00	0.29	ns	ns	ns
	3	12.47	12.60	12.77	13.13	0.55	ns	ns	ns
	6	7.50	7.80	7.92	8.15	0.15	ns	ns	ns
	Means	8.80	9.07	9.28	9.42	0.30	ns	ns	ns
PUN, mg/dl	0	13.75	15.50	16.00	12.75	1.36	ns	ns	ns
	3	16.00	19.00	18.35	17.25	1.57	ns	ns	ns
	6	16.00	18.00	17.00	16.00	1.35	ns	ns	ns
	Means	15.25	17.50	15.58	15.35	1.05	ns	ns	ns

[1] The treatments were compounded by the ratio of live yeast product (g.) and starch sterilized (g.) as follow 1 = 0 : 150, 2 = 50 : 100, 3 = 100 : 50 and 4 = 150 : 0; Yeast produced ; *Candida glabrata* was used this experiment, which was concentrated by 4.5 x 10^7 cfu/g; [a, b]Means within a row absence a common superscript letter significant difference (P<0.05); [2] L=Linear, Q=Quadratic, C=Cubic; ns= not differ significantly (P>0.05)

4.5.3 Volatile fatty acid (VFAs)

In the study of volatile fatty acid in rumen which was measured on 0 3 and 6 hours after feeding, the supplementation of live yeast were not affected (P>0.05) on total volatile fatty acid, acetic acid (C2), propionic acid (C3), butyric acid (C4), C2:C3 ratio and C2+C4:C3 ration there were showed in Table 4.4. Whereas, the live yeast product for this experiment was used 0 50 100 and 150 grams/head/day, that were concentrated of product as 4.5×10^7 cfu/gram, therefor the animals were gotten by 0 2.3 x 10^9 4.5 x 10^9 and 6.75 x 10^9 cfu/head/day for treatment (T1) 1 2 3 and 4 respectively. The concentrations of live yeast were somewhat similar that affecting on VFAs were not differences. In spite of, Ding et al. (2014b) reported the supplementation of live yeast (*Saccharomyces cerevisiae*) as 8×10^9 cfu/head/day was linear increased for total volatile fatty acid, the molar of C2 C3 C4, valerate and isovalerate. Whereas yeast supplementation was increased of VFA concentration average as +2.7 mM reported by Desnoyers et al. (2009). However, in this study was used by yeast (*Candida glabrata*) strain that selected from rumen of cow as a live yeast added in feed for animal. Otherwise, difference strain of yeast was affected on rumen fermentation and rumen microbial activities (Newbold et al., 1995).

Table 4.4 Effect of probiotic yeast level of volatile fatty acid.

Items	Hours post feeding	Treatments[1]				SE	Contrast[2]		
		T1	T2	T3	T4		L	C	Q
Total VFAs	0	149.34	152.36	148.21	144.37	2.46	ns	ns	ns
	3	154.07	154.95	149.12	149.77	2.52	ns	ns	ns
	6	159.27	159.03	155.96	154.29	3.77	ns	ns	ns
	Means	154.23	155.45	151.10	149.48	2.76	ns	ns	ns
Acetic acid, C_2	0	93.06	94.40	91.67	89.64	1.35	ns	ns	ns
	3	94.79	94.87	91.28	91.46	1.31	ns	ns	ns
	6	97.39	97.12	95.50	94.65	2.06	ns	ns	ns
	Means	95.08	95.47	92.82	91.92	1.47	ns	ns	ns
Propionic acid, C_3	0	32.62	33.45	32.54	31.10	0.71	ns	ns	ns
	3	34.14	34.31	33.00	32.83	0.92	ns	ns	ns
	6	35.30	34.83	33.56	32.68	1.15	ns	ns	ns
	Means	34.02	34.20	33.03	32.20	0.89	ns	ns	ns
Butyric acid, C_4	0	23.66	24.49	24.00	23.63	0.51	ns	ns	ns
	3	25.31	25.78	24.85	25.49	0.57	ns	ns	ns
	6	26.58	27.08	26.90	26.96	0.78	ns	ns	ns
	Means	25.13	25.78	25.25	25.36	0.59	ns	ns	ns
$C_2 : C_3$ Ratio	0	2.87	2.83	2.82	2.88	0.03	ns	ns	ns
	3	2.80	2.80	2.77	2.78	0.05	ns	ns	ns
	6	2.79	2.81	2.85	2.90	0.05	ns	ns	ns
	Means	2.82	2.81	2.81	2.85	0.04	ns	ns	ns

Table 4.4 (Continue).

Items	Hours post feeding	Treatments[1]				SE	Contrast[2]		
		T1	T2	T3	T4		L	C	Q
C_2+C_4 :	0	3.59	3.56	3.56	3.64	0.03	ns	ns	ns
C_3 Ratio	3	3.54	3.56	3.52	3.56	0.06	ns	ns	ns
	6	3.54	3.59	3.65	3.72	0.06	ns	ns	ns
	Means	3.56	3.57	3.57	3.64	0.05	ns	ns	ns

[1] The treatments were compounded by the 1 = Yeast produced 0 g/d with sterilized starch 150 g/d 2 = Yeast produced 50 g/d with sterilized starch 100 g/d 3 = Yeast produced 100 g/d with sterilized starch 50 g/d and 4 = Yeast produced 150 g/d with sterilized starch 0 g/d, Yeast produced ; *Candida glabrata* was used as live yeast this experiment, which was concentrated by $4.5x10^7$ cfu/g; [a, b]Means within a row absence a common superscript letter significant difference ($P<0.05$).

[2] Orthogonal polynomial L=Linear, Q=Quadratic, C=Cubic; ns= not differ significantly ($P>0.05$)

4.5.4 Nylon bag digestibility

The digestion of both rice straw and SUT® concentrate were used by nylon bag technique. In the SUT® concentrate which was found dry matter (DM) disappeared, fraction of a b c and a + b value were not differ significantly ($P>0.05$ as follow Table 4.5 and also all the times incubation were not significant ($P>0.05$) as follow figure 4.1. On the other hand, the effective DM digestion was not effected by treated with yeast product ($P >0.05$). The concentrate digestibility was high rate by all treatments (81-91%) similar to Promkot et al. (2007) who found the soybean meal

(SBM) had been high potential degradability (*a*+*b* value) and rate (c) of DM digestibility. The high digestion of SBM owing to rumen microorganism could be easy attaching and rapidly degraded (Mahadevan et al.1980 cited by Promkot et al. 2007).

Table 4.5 Effect of probiotic yeast level on a, b and c constant value and effective DM degradation of SUT® concentrate in the rumen using by nylon bag technique.

Items	Treatment				SEM	P-value
	T1	T2	T3	T4		
a, %	38.92	43.25	40.38	39.94	2.12	0.93
b, %	48.70	44.90	47.52	51.48	1.15	0.94
c, h^{-1}	0.06	0.06	0.06	0.05	0.006	0.28
a + *b*	87.62	88.15	87.90	91.42	1.98	0.88
Effective DM degradability						
0.02	87.64	88.17	87.92	91.44	1.91	0.79
0.05	87.67	88.20	87.95	91.47	1.91	0.78

a is the intercept of degradation curve at time zero (%), *b*; the fraction of DM degraded when given sufficient time for digestion in the rumen (%), *c*; a rate constant disappeared of fraction B (h^{-1}), 0.02 and 0.05 are mean out flow rate from the rumen.

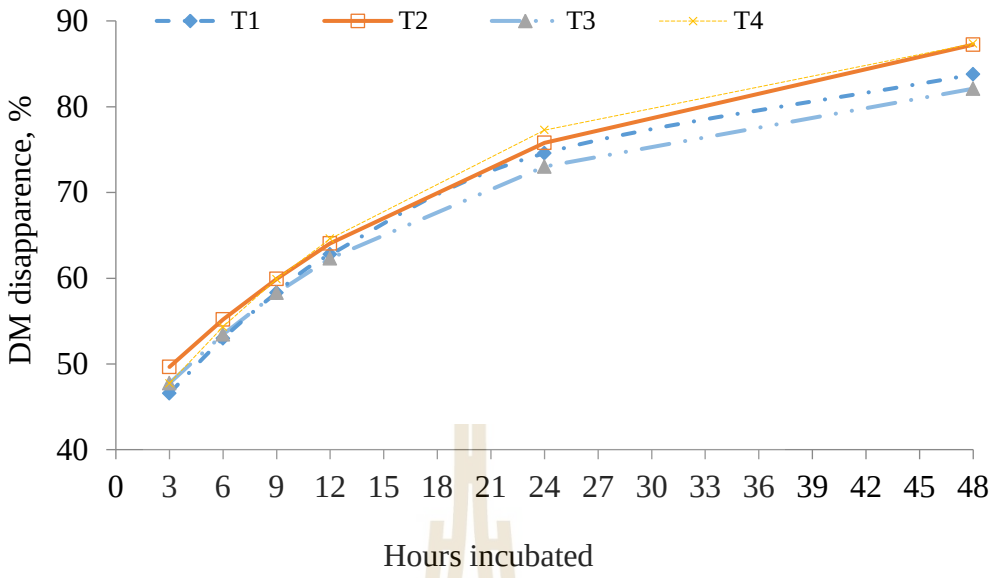

Figure 4.1 Effect of probiotic yeast level on DM disappearance of SUT® concentrate.

As the same time for incubation times, in the roughage which mean rice straw found the supplement yeast product was not effected on DM disappeared and fraction of value a b c and potential rumen degradability ($a + b$) (P >0.05) as follow Table 4.6 and figure 4.2. There was lower DM degraded than the concentrate. Because of the roughage- rice straw had more and more the structural carbohydrate when compared with concentrate feed. However, the effective degradation of DM found small differed between solid out flow rate which was rate 0.05 (P>0.05). While Promkot et al. (2007) indicated that the materials feed were high structural carbohydrate including dried brewer gain, cassava hay and cottonseed meal were low effective digestibility of dry matter when compare with soy bean meal and palm kernel meal. In this study was used rice straw as a sample to tested effective digestibility that was low degraded in the rumen (Figure 4.2). Similarly result with Erasmus et al. (1992) who found that yeast culture was not effect (P>0.05) on DM disappeared of wheat straw after rumen in situ incubation.

Table 4.6 Effect of probiotic yeast level on a, b and c constant value and effective DM degradation of rice straw in the rumen using by nylon bag technique.

Items	Treatments				SEM	P-value
	T1	T2	T3	T4		
a, %	11.25	9.47	8.51	9.95	1.06	0.87
b, %	69.38	69.39	71.83	61.64	3.57	0.85
c, h^{-1}	0.014	0.02	0.02	0.019	0.004	0.95
a + *b*	78.28	78.87	77.60	71.68	4.19	0.95
Effective DM degradability						
0.05	23.92	28.13	24.32	26.42	1.75	0.84

a is the intercept of degradation curve at time zero (%), *b*; the fraction of DM degraded when given sufficient time for digestion in the rumen (%), *c*; a rate constant disappeared of fraction B (h^{-1}), 0.05 and 0.08 are mean solid rate of out flow from the rumen.

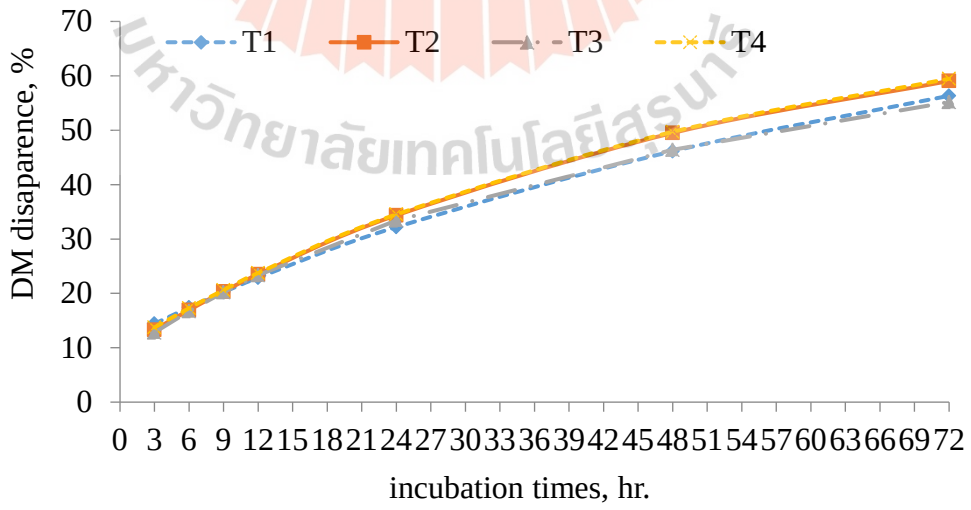

Figure 4.2 Effect of probiotic yeast level on DM disappearance of rice straw.

4.5.5 Rumen microbial populations

The microbial populations in the rumen were showed in Table 4.4. All treatments were not differed significantly statistic ($P>0.05$) excepted by both population of yeast and Ciliate protozoa. Yeast was higher population than control group (T1) ($P<0.05$). Yeast population was high by added live yeast product (T2 T3 and T4), that mean generally in the rumen still had concentrated of yeast about $\log_{10}$ 4.6 copies/ml base on this experiment. While the Ciliate protozoa was decreased by live yeast supplementation, which probably due to yeast might be compete engulf nutrients especially glucose or starch in the rumen. But Ding et al. (2014a) found that live yeast (*S.cerevisiae*, $8x10^9$ cfu/h/d) increased of rumen total bacteria, lactate utilizing bacteria, protozoa and fungi when compare with control group. On the other hand Pinloche et al. (2013) who reported supplement probiotic yeast (*Saccharomyces cerevisiae Sc*47, 10^{10} cfu/g) 5 g/d can improved fibolytic bacteria group (*Fibobacter* and *Ruminococcus*). In this experiment that was lower concentration of live yeast than other researchers, although supplementation of *Canida glabrata* can decrease protozoa population in the rumen. The functions of yeast in the rumen were founded by Newbold et al. (1996b) reported that modes of action of yeast in stimulating rumen fermentation, which included as the first- yeast respiratory activity protects anaerobic rumen bacteria from damage by O_2. The second yeast provides malic and other dicarboxylic acid which stimulate the growth of some rumen bacteria. In spite of, Zhu et al. (2017) found dose of *Saccharomyces cerevisiae* fermentation product (SCFP) could be increased rumen fungi and cellulolytic bacteria, there were decreased lactate producing bacteria.

Table 4.7 Effect of probiotic yeast level on rumen microbial diversities (log_{10}, copies/ml).

Types of microbial	Treatments[1]				SE	Contrast[2]		
	T1	T2	T3	T4		L	Q	C
Yeast; *Candida glabrata*	4.62[b]	5.87[a]	6.04[a]	6.24[a]	0.13	**	**	ns
General bacteria	10.84	10.69	10.76	10.69	0.04	ns	ns	ns
General anaerobic fungi	7.03	6.99	6.96	6.88	0.07	ns	ns	ns
General methanogens	7.66	7.73	7.62	7.58	0.06	ns	ns	ns
Ciliate protozoa	7.60[a]	7.18[b]	7.32[b]	7.22[b]	0.16	ns	ns	ns
Eubacteria	10.97	10.94	10.97	10.91	0.08	ns	ns	ns
Succinimonas amylolytica	8.25	8.35	8.20	8.17	0.09	ns	ns	ns
Ruminococcus albus	8.00	8.00	7.99	7.94	0.06	ns	ns	ns
Ruminococcus flavefaciens	8.32	8.30	8.46	8.20	0.08	ns	ns	ns
Fibrobacter succinogenes	8.33	8.12	8.20	8.10	0.11	ns	ns	ns
Butyrivibrio fibrisolvens	9.70	9.71	9.82	9.62	0.04	ns	ns	ns
Selenomonas ruminantium	8.54	8.52	8.59	8.42	0.05	ns	ns	ns
Megasphaera elsdenii	5.14	5.25	5.26	5.28	0.08	ns	ns	ns
Streptococcus bovis	7.23	7.09	7.08	7.06	0.08	ns	ns	ns
Prevotella brevis	8.59	8.47	8.60	8.41	0.08	ns	ns	ns
Prevotella bryantii	6.74	6.66	6.87	6.94	0.09	ns	ns	ns
Prevotella ruminicola	9.57	9.50	9.47	9.50	0.05	ns	ns	ns

[1] The treatments were compounded by the 1 = Yeast produced 0 g/d with sterilized starch 150 g/d, 2 = Yeast produced 50 g/d with sterilized starch 100 g/d, 3 = Yeast produced 100 g/d with sterilized starch 50 g/d and 4 = Yeast produced 150 g/d with sterilized starch 0 g/d.

[2] Orthogonal polynomial L=Linear, Q=Quadratic, C=Cubic; ns= not differ significantly (P>0.05); ** highly significance difference (P<0.01).

4.5.6 Hematology

The hematological parameters were analyzed along with red blood cell count (RBC), hemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin(MCH), mean cell hemoglobin concentration (MCHC), white blood cell count (WBC) including neutrophils, lymphocytes, monocytes and eosinophils and blood platelets (PLT). The supplementation of yeast strain *Candida glabrata (C. glabrata*) was no effected on complete blood cell count (CBC) which all parameters were normal range as follow Table 4.5. Nevertheless, the cows in this experiment were not found clinical sign of ill after treat with yeast product. Indeed the *C.glabrata* was no blood hydrolysis when culture on blood agar (the result was not showed). However the hematology of dairy cows were differed by age, group and farm management described by Herman et al. (2018), Radkowska and Herbut (2014) and Lumsden et al. (1980).

In human, Dujon (2010), Ahmad et al. (2014) Bolotin-Fukuhara and Fairhead (2014) they were reported that the *C. glabrata* is likely a commensal species of the human digestive tract, but systemic infections of immune compromised patients are often fatal, and also phylogenic of *C. glabrata* is much closer to *S. cerevisiae although* it has under gone major gene and intron loss when compared with *S. cerevisiae*. But in this study, *C. glabrata* was used by dairy cows which is not negative affected on hematology that is probably owing to the ruminant has complexity of the rumen microbial and multi enzyme is function in there. Even though RBC concentration was differed significant statistic ($P<0.05$), yeast *C. glabrata* supplements could be used in dairy cow by top up on concentrate.

Table 4.8 Effect of supplemented by live yeast (*Candida glabrata*) on average of complete blood cell count (CBC).

Items	Treatments[1]				SE	Contrast[2]		
	T1	T2	T3	T4		L	Q	C
RBC, x 10^6 cell/mm^3	6.00[b]	6.50[ab]	7.25[a]	6.00[b]	0.31	ns	ns	ns
Hgb, g/dl	10.00	10.25	11.25	8.75	0.43	ns	ns	ns
HCT, %	30.00	31.75	34.00	27.50	1.12	ns	ns	ns
MCV, fL	52.50	51.00	52.00	52.50	0.42	ns	ns	ns
MCH, pg	17.50	16.25	16.75	16.75	0.50	ns	ns	ns
MCHC, g/dl	29.25	30.00	30.75	29.75	0.65	ns	ns	ns
WBC, log_{10} cell/cu.mm	3.93	4.01	4.05	3.92	0.03	ns	*	ns
% Neutrophil	33.50	35.00	32.25	35.25	0.97	ns	ns	ns
% Lymphocyte	57.75	56.50	58.75	55.00	1.18	ns	ns	ns
% Monocyte	2.25	1.75	2.25	1.50	0.23	ns	ns	ns
% Eosinophil	6.25	5.25	5.75	6.75	0.70	ns	ns	ns
Platelets count, log_{10} cell/cu.mm	5.30	5.22	5.32	5.07	0.05	ns	ns	ns

[1] The treatments were compounded by the 1 = Yeast produced 0 g/d with sterilized starch 150 g/d, 2 = Yeast produced 50 g/d with sterilized starch 100 g/d, 3 = Yeast produced 100 g/d with sterilized starch 50 g/d and 4 = Yeast produced 150 g/d with sterilized starch 0 g/d.

Yeast produced ; *Candida glabrata* was used this experiment, which was concentrated by $4.5x10^7$ cfu/g.

[2] Orthogonal polynomial L=Linear, Q=Quadratic, C=Cubic; ns= not differ significantly (P>0.05); * significance difference (P<0.05).

4.6 Conclusions

Base on this study, the concentration of live yeast (*Canida glabrata*) product on rumen fermentation and microbial population, which can concluded that *C.glabrata* was not negative effect on hematology parameters. On the other hand, average ruminal pH had effected by live yeast concentration although both of NH_3-N and Plasma Urea Nitrogen (PUN) were no significance different ($P>0.05$). Likewise volatile fatty acid (VFAs) was not differ significance ($P>0.05$) by live yeast product. In nylon bag digestion of SUT® concentrate and rice straw including to DM disappeared, effective DM degradability were not affected ($P>0.05$) by live yeast level. In addition rumen microbial population were not affect excepted both of yeast (high with supplemented live yeast) and ciliate protozoa (low with supplemented live yeast) concentration when compared with control ($P<0.05$). Consequently, the concentration of live yeast product at 50 g/h/d or T2 could be appropriated to used for transition dairy cow period.

4.8 References

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CHAPTER V

EXPERIMENT III

EFFECT OF SUPPLEMENTATION PROBIOTIC YEAST PRODUCT ON MILK PRODUCTIVITY PERFORMANCE IN TRANSITION PERIOD DAIRY COWS

5.1 Abstract

This study were conducted, the objective was to addition of probiotic yeast product on milk yield, milk compositions, somatic cell count and hematological parameter. Sixteen multiparous transition lactating dairy cows were used in study. The experiment begun 4 week before calving and ended 4 week after calving. The dietaries were consisted to two groups, including control group (T1), without probiotic yeast and T2 was supplemented by probiotic yeast product amount 50 g/h/d on top dress with SUT® concentrate feed. The yeast product had viability about $4.5x10^7$ cfu/g. Rice straw and Napier grass were used to roughage sources for pre-calving and post-calving period respectively. Individual feed intakes and milk yield were recorded daily. Consequently, supplementation of probiotic yeast product was not effect (P>0.05) on DMI, ruminal fermentation (pH, NH_3-N and VFAs), milk yield, milk components yield (milk fat and protein), milk compositions (except milk protein, % which increased trend with T2 was given). On the other hand somatic cell count was no significance differently (P>0.05).

Base on this experiment probiotic yeast product was not negative effected only on hematological parameters, but increased total WBC, total RBC and PLT in dairy cows.

5.2 Introduction

The transition period is defined as 3 wk prepartum until 3 wk postpartum, it is a period marked by changes in endocrine status to accommodate parturition and lacogenesis. Whereas, the dairy scientists and dairy producers tend to neglect the transition cow, particularly prepartum. During this time, sometime decreased of DMI (5-7 d prepartum) and increase DMI in the early lactation (Grummer, 1995); (Grant and Albright, 1995)). In studies survey from smallholder farm, Leelahapongsathon et al. (2016) who found that ether cow or farm management factor were associated with the intramammary infection rate and subsequent expression of clinical signs of mastitis in early postpartum cows. However, Campanile et al. (2008) found that the dietary supplementation with yeast *S. cerevisiae* increase organic matter digestibility, milk yield and guarantee higher energy availability, and also lower fat mobilization in buffalo cows. Oliveira et al. (2010) reported that the addition of live yeast strain KA500 (10g/d) in the diet caused a significant reduction in the somatic cell count, and also reported similarly with Spaniol et al. (2014) who found that somatic cell count in dairy cow was decreased by yeast addition amount 3 g/head/d. Therefore, if feeding high concentrate ratio for animals, it increase the risks on rumen acidosis and lowest fiber digestibility. Consequences, the metabolic disorder will be occurred in the ruminants owing to in rumen dysfunctions. On the other hand, the management of transition cows is play an important role in the preventing the risk of metabolic disorder, and also Roche

et al. (2015) who suggested that should controlled feed restriction amount 75-90% on 2-3 wk for before calving, whereas (Roche et al., 2013) indicated that the both low and high BCS at calving will increase the risk of diseases.

In spite of the supplementation of yeast products were results varied of each studied, researchers had been showed to beneficial effect on rumen fermentation, ruminal pH, VFAs, milk yield and composition. But yeasts flora in the rumen is autochthonous or native yeast can be positive effected nutrient digestibility and improve ruminal microbial communities. Therefore, in this studies have been addition probiotic yeast flora on milk productivity performance in which were used by transition period of dairy cows.

5.3 Objective

To study on the addition of probiotic yeast product on milk production, milk composition and somatic cell count.

5.4 Materials and methods

5.4.1 Experimental design, animals and treatments

The experiment was conducted to recommending by Animal Care and Use Committee of Suranaree University of Technology, Suranaree University of Technology, Nakhon Ratchasima province, Thailand.

Sixteen multiparous late pregnant of dry period dairy cows which were 4 weeks for expected calving were used in this experiment according to independent group t-test, which lactation number is covariate that were received 2 dietary treatments. All dairy cows were kept in the individual feeding pens during experimental period.

Mineral blocks and clean water will be available *ad libitum* offered for all animals. Treatments consists of control (without yeast supplementation) (T1), and T2 was supplement with probiotic yeast product as the 50 g/head/day on top dress with concentrate feed on the morning. The field this experiment was ending by 4 week after post-calving.

The feeding to animals were used both commercial concentrate (CP 16%) and rice straw as a roughage source for pre-calving, and also the commercial concentrate (CP 21%) and fresh Napier Grass as a roughage source for post-calving. All dietaries were fed two time a day which appropriately feeding requirement was according to NRC (2001). In the post-calving period, all cows were received feeds and supplemented with their respective treatment after milking times. Lactating dairy cows were milked in the morning and afternoon at approximately 05.00 and 15.00 using a milking machine. Physically record for health status and reproductive were observed of retain placenta and heat.

5.4.2 Samples collection, parameters, analysis

Voluntary feed intake was measured daily during of experimental period. Samples of offered and refused diets will be collected every day. Samples of offered and refused diets and feces will be stored at -20^{o}C, oven dried (<60^{o}C) and ground through a 1.0 mm sieve prior to analysis for DM, ash and crude protein (CP) (AOAC, 1995) and NDF and ADF (Van Soest et al., 1991). Individual daily milk yield was recorded on every day of lactation period. Milk samples were collected individually on day 7 14 21 and 28 post-calving of the experiment period, which both at morning and afternoon milking. Individual milk samples were divided into two portions; first portion will be stored at 4^{o}C with a preservative until analyses for contents (fat protein lactose and TS)

using a Milko scan. Second, 1 ml of milk for somatic cell count was measured by Somatic cell Analyzer.

Blood samples was taken from jugular vein of day 1 prior calving and on day 1 7 and 14 post-calving. Sample was analyzed complete blood cell count (CBC) Blood plasma was separated by centrifuge at 3,500×g for 20 min, then plasma will be collected and stored at -20^{o}C for further analysis by the methods as described in and plasma urea nitrogen (PUN) according to Crocker (1967).

5.4.3 Statistical analysis

Weekly average of DMI, milk yield, milk conponents and Somatic cell count (SCC) were analyzed using Proc Mixed of SAS. Week was used as a repeated measurement with cow as the subject. Differences treatment means in pH, NH_3-N and were determined by Proc TTEST of SAS (SAS, 2004).

5.4.4 Experimental places

The Suranaree University of Technology part of Dairy farm and the Center for Scientific and Technology Equipment Building 9 10 and 14, where were used for field experiment and laboratory, respectively.

5.5 Results and discussion

5.5.1 Feed component and Dry mater intake

The feed compositions were showed in Table 5.1 in which used for this experiment each period different offered to feeding. In precalving, the cows were gotten by SUT® concentrate 1 (Conc.1) and rice straw there were as a concentrate and roughage source respectively. But postcalving period, the cows were gotten by SUT

concentrate 2 (Conc.2) and fresh Napier grass (40-45 days of age cutting) there were as a concentrate and roughage source respectively.

Table 5.1 Feed chemical compositions of commercial concentrate, rice straw and fresh Napier grass (DM basis).

Items	Conc.1	Conc.2	Rice straw	Napier grass
Dry matter	95.82	95.99	93.26	92.61
	% of DM basis			
Organic matter, OM	91.59	91.66	83.60	89.95
Ash	8.41	8.34	16.40	10.05
Crude protein, CP	17.29	21.77	3.34	7.62
Neutral detergent fiber, NDF	33.2	23.5	77.50	75.80
Acid detergent fiber, ADF	17.55	14.78	56.26	47.57

Dry matter intake both concentrate and roughage were no differ significant (P>0.05) between control (T1) and supplement probiotic yeast (T2). However, feed intake was decreased trend by week 1 at precalving and week 1 postcalving thereafter increased as follow figure 5.1

5.5.2 Ruminal fermentations

The rumen fermentations were measured consist by pH, NH_3-N and volatile fatty acid (VFAs) on the last day of experiment at 4 hours post feeding which were no significance differently (P>0.0.5) between control group and supplement probiotic yeast there are show on Table 5.2

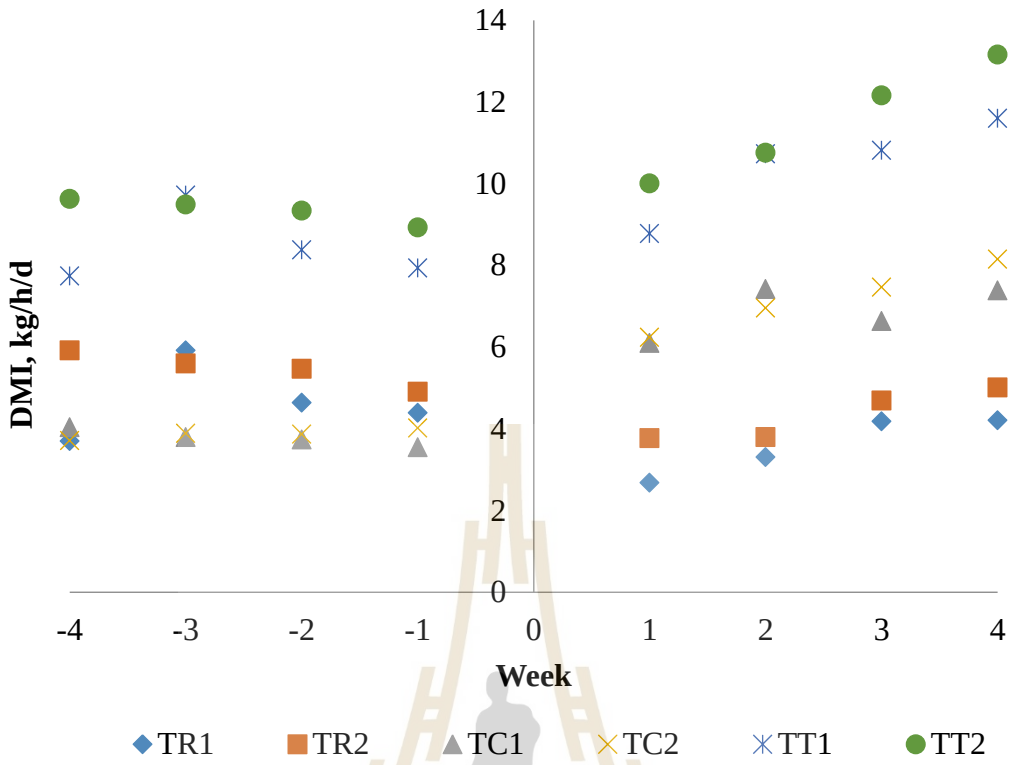


Figure 5.1 Least squares means of Dry matter intake, TR1 was roughage intake of control group, TR2 was roughage intake of supplement probiotic group, TC1 was SUT concentrate intake of control group, TC2 was concentrate intake of supplement probiotic group, TT1 was total intake of control group and TT2 as a total intake of supplement probiotic group.

Table 5.2 Effect of supplement probiotic yeast on ruminal fermentations, pH, NH_3-N and volatile fatty acid (VFAs) after feeding 4 hr.

Items	Treatments[1]				P-value
	T1		T2		
	Mean	SE	Mean	SE	
pH	7.04	0.09	6.93	0.11	0.51
NH_3-N, mg/dL	9.70	1.20	10.20	0.81	0.13
Total volatile fatty acid, mM	78.94	5.02	74.69	7.34	0.68
Acetic acid (C_2), mM	51.14	4.52	47.43	4.77	0.61
Propionic acid (C_3), mM	17.86	0.98	17.92	1.96	0.98
Butyric acid (C_4), mM	9.93	0.39	9.33	0.78	0.58
Acetic acid (C_2), %	64.52	2.02	63.38	0.74	0.55
Propionic acid (C_3), %	22.77	1.31	24.03	0.85	0.42
Butyric acid (C_4), %	21.71	0.87	2.59	0.42	0.90
C_2 : C_3	2.87	0.25	2.66	0.12	0.41

[1] T1 as a control group, T2 as a supplementation of probiotic yeast amount 50 g/h/d, the concentration of live yeast along with 4.5x 107 cfu/gram

SE = standard error of mean

5.5.3 Hematology

The complete blood cell count (CBC) were analyzed along with white blood cell count (WBC) included neutrophils, lymphocytes, gran. red blood cell count (RBC), hemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin(MCH), mean cell hemoglobin

concentration (MCHC), and blood platelets (PLT). The supplementation of probiotic yeast was increased the main indicated concluding to WBC, RBC and PLT when compere with control group (T1). However probiotic yeast was not negative effected on complete blood cell count (CBC) which all parameters were normal as follow Table 5.3. In this study, all hematological parameters fell within the normal range of reference values for healthy dairy cows (Winnicka, 2008 cited by Radkowska and Herbut (2014)). Whereas Radkowska and Herbut (2014) who suggested the hematological parameters in ruminant depend on many factors related to the animal's physiological status and management system, which consisted by housing hygiene and nutrition. Despite the proper management condition are essential for the organism to function normally. White blood cells are the basic cell of the immune system, which determined normal condition of the body. Whereas in this study, WBC was higher when provided probiotic yeast than control group, which probably yeast cell wall supported white cells to improved immunity of cells body. The cell wall of yeast which consisting β-glucan α-galactomanan mannoprotein and chitin (Osumi, 1998). The cell wall compounds were benefited on cell immune, strong cell and helping to against pathogen. Likewise Spaniol et al. (2014) who found that yeast (*S. cerevisiae*) able to enhance immune system for lactating cow.

For the red blood cell count (RBC) that including; hemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin(MCH), which were higher when added probiotic yeast group than control group. Aengwanich et al. (2009) who indicated in Thailand the RBC parameters are nearly relationship on hemoglobin level, erythrocyte count and hematocrit value and also hematological change had related to physiological and pathological of cattle.

However, in this study although RBC and compound of RBC were differing significant between treatments, there were still normal range. Thus supplement yeast (*C. glabrata*) was safety for dairy cow. Indeed the novel probiotic yeast product base of on *C. glabrata* will develop appropriately to continue for ruminants.

Table 5.3 Effect of probiotic yeast supplement on hematology values.

Items	Treatment[1]		SE	P-value[2]		
	1	2		Trt	Wk	Trt*Wk
WBC, x 10^3 per ul.	8.66	12.22	1.74	0.04	0.83	0.04
Lymh, x 10^3 per ul.	3.79	5.25	1.36	0.29	0.78	0.36
Mon, x 10^3 per ul.	0.95	1.20	0.27	0.36	0.71	0.03
Gran, x 10^3 per ul.	3.90	4.98	0.69	0.12	0.89	0.52
Lymh, %	39.66	42.29	6.62	0.69	0.82	0.55
Mon, %	11.49	10.25	1.51	0.42	0.94	0.08
Gran, %	46.7	47.81	7.03	0.87	0.78	0.62
RCB, x 10^3 per ul.	4.94	5.51	0.29	0.06	0.41	0.65
Hgb, g/dL	7.88	9.10	0.31	<0.01	0.19	0.52
HCT, %	25.17	29.08	0.89	<0.01	0.04	0.53
MCV, fL	50.65	53.41	1.31	0.04	0.81	0.77
MCH, pg	15.88	16.55	0.3	0.04	0.81	0.77
MCHC, g/dL	31.16	31.36	0.17	0.27	<0.01	0.64
RDW, %	15.84	15.74	0.28	0.69	0.09	0.11
PLT, $\log_{10}$ per ul.	5.02	5.10	0.04	0.06	<0.01	0.66
MPV, fL	4.96	7.84	0.22	0.66	0.005	0.15
PDW	16.62	16.91	0.35	0.40	0.009	0.91

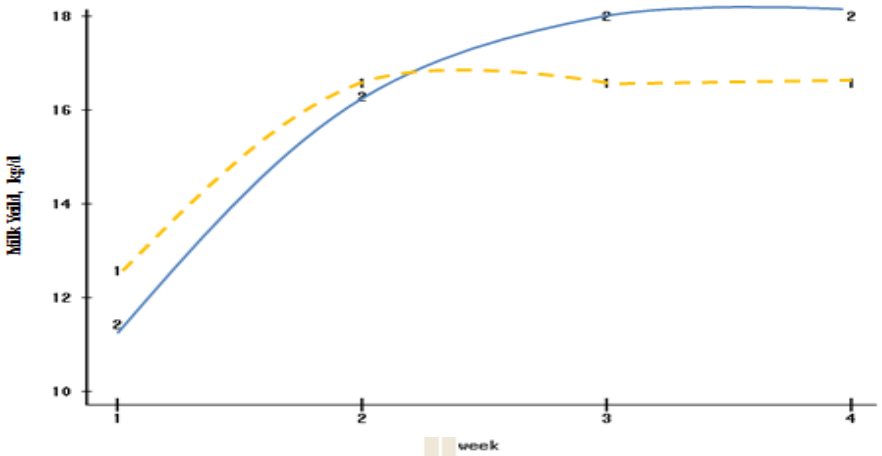
5.5.4 Milk yield and compositions

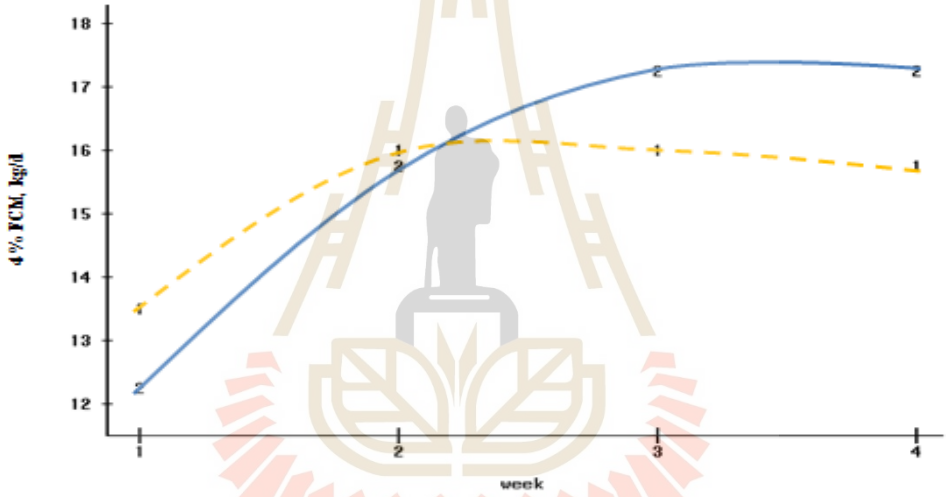
The supplement probiotic yeast was not effected on average of milk yield, 4% FCM, milk components yield (protein and fat), Milk compositions and somatic cell count, although milk protein was increased trend (P=0.05) by probiotic yeast when compare control group as follow Table 5.4. However the main effect of week had effect (P<0.01) on milk yield, 4% FCM, milk components, milk compositions (except milk fat ,and total solid, P=0.06) and somatic cell count as follow table 5.4 and figure 5.2. which were probably due to all cows were early period lactation thereafter their are smallest increasing or decreasing (SCC). Likewise AlZahal et al. (2014) who found that supplemented of direct fed microbial (*E. faecium* and *S.cereviseae*) was no effect on average dry mater intake or milk yield in transition period dairy cows. On the other hand, the *S. cereviseae* as added to dietary which were decreased somatic cell on day15 through day 30 of milking, and also the probiotic based on *S. cerevisiae* when provided to lactating cows is able to enhance their immune system (Spaniol et al., 2014) and somatic cell count in milk was declined by supplemented yeast (Oliveira et al., 2010). Yeast was not only function in the rumen by support activities rumen microbial, decreased O_2, decrease acidosis diseases incidence, but there could be as a source of single cell protein and prebiotic for directly to ruminant (Nocek et al., 2011), (Newbold, 1996a; Newbold et al., 1995; Newbold et al., 1996b)

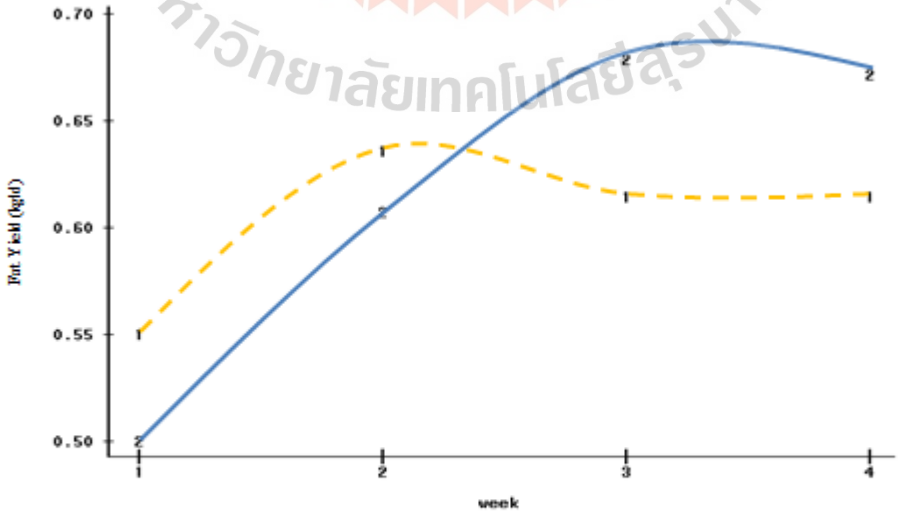
Table 5.4 Effect of supplemented probiotic yeast on milk yield, compositions and somatic cell count (SCC).

Items	Treatment[1]		SE	P-value[2]		
	1	2		Trt	Wk	Trt*Wk
Milk yield, kg/d	15.54	15.93	0.87	0.48	<0.01	0.14
4 %FCM, kg/d[3]	15.34	15.61	0.69	0.38	<0.01	0.42
Protein, kg/d	0.42	0.42	0.02	0.92	0.01	0.28
Fat, kg/d	0.62	0.55	0.05	0.22	0.002	0.66
Milk compositions, %						
Fat	3.90	3.86	0.24	0.38	0.52	0.91
Protein	2.76	2.93	0.08	0.05	<0.01	0.37
Lactose	4.52	4.35	0.10	0.11	0.01	0.32
Solid- not- fat	8.30	8.31	0.19	0.94	<0.01	0.58
Total solid	12.20	12.19	0.36	0.98	0.06	0.77
Somatic cell count, log_{10}/ml	5.62	5.54	0.24	0.72	<0.01	0.60

[1] Treatment was consist 1, control group and 2, supplement probiotic yeast amount 50 g/h/d; [2] Trt = treatment effect, Wk = Week or time effect, Trt*Wk = interaction treatment and week; SE = standard error, [3] 4 % fat corrected milk







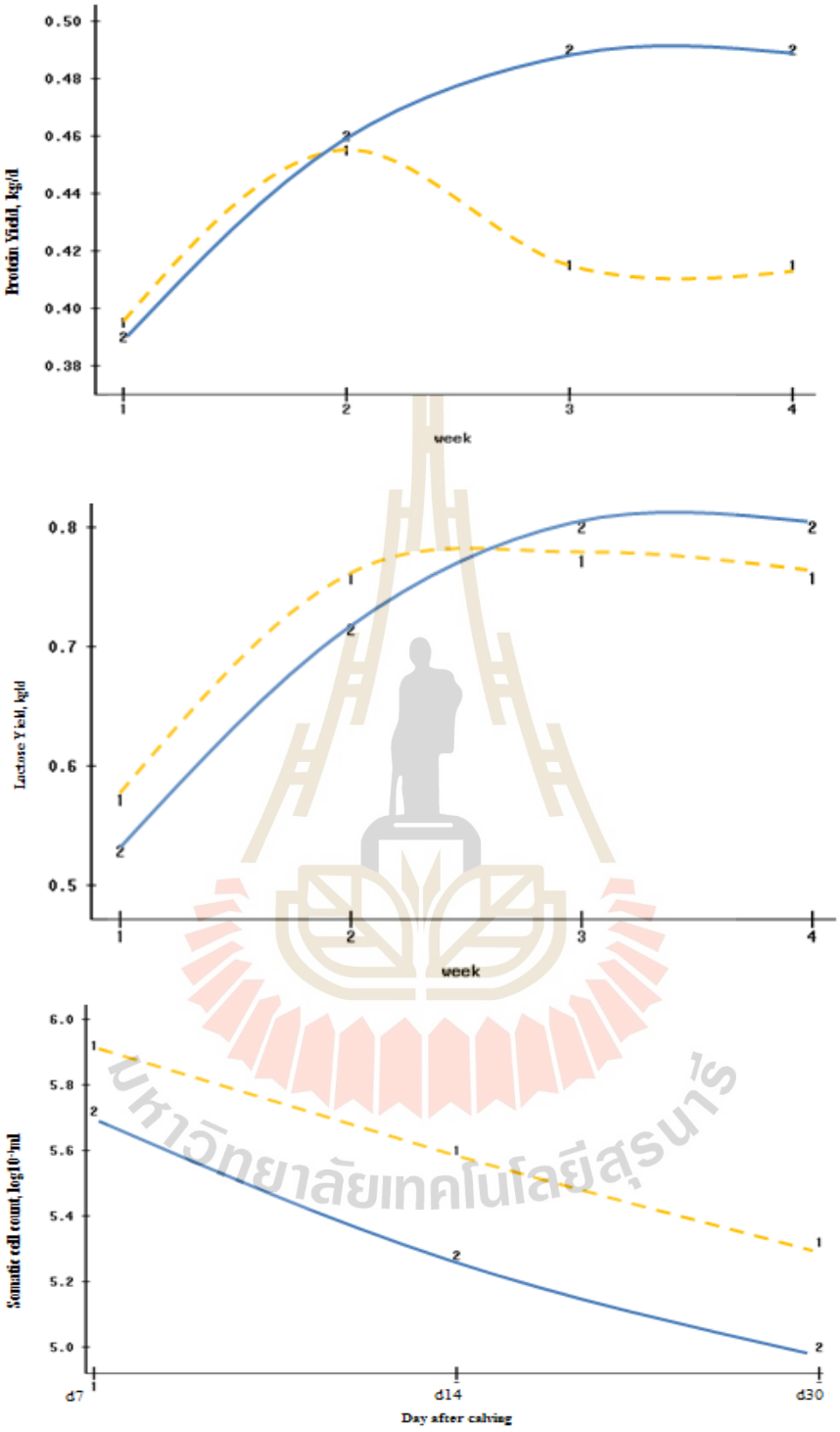


Figure 5.2 Weekly of lest square means of milk yield, 4% FCM, fat yield, protein yield, lactose yield and somatic cell count provided either probiotic yeast (*C. glabrata*) product (solid line) and no supplement (dashed line).

5.6 Conclusion

Consequently, supplementation of probiotic yeast product was not effect (P>0.05) on DMI, ruminal fermentation (pH, NH_3-N and VFAs), milk yield, milk components yield (milk fat and protein), milk compositions (except milk protein, % which increased trend with T2 was given). On the other hand average somatic cell count (scc) was no significance differently (P>0.05) but there was declined to related with the times of day in milk. Base on this experiment probiotic yeast product was not negative effected on hematological parameters, but increased trend total WBC, total RBC and PLT in dairy cows.

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CHAPTER VI

OVERALL CONCLUSION AND IMPLICATION

6.1 Conclusion

Three studies were conducted. The aim of the first study was isolation, selection and identification of yeast from the ruminant, including goat, buffalo beef cattle and dairy cow as an examine the best probiotic yeast. Second study was supplemented by probiotic yeast on rumen fermentation, included by ruminal pH, NH_3-N, VFAs and microbial diversities. Finally to study on the additional of probiotic yeast on milk production, milk compositions and hematological parameters. The results from this studies are summarized as presented below.

The Dc 18 was isolated from dairy cow which was the best isolate. In genera of yeast strain DC 18 was *Canida glabrata* DC 18 to developed probiotic yeast product. Base on *in vitro* trial, it could be concluded that to addition live yeast culture, the *C. glabrata* Dc18 with 20% of fermented fluid could improve gas kinetic, gas cumulative, and also increased acetic acid (C_2), acetic acid: propionic acid (C_3) ratio.

The concentration of probiotic yeast (*C. glabrata* Dc18) was no effect on NH_3-N, volatile fatty acid and rumen microbial diversity excepted both of yeast (high with supplemented live yeast) and ciliate protozoa (low by supplemented live yeast) concentration when compared with control ($P<0.05$). Consequently, the concentration of live yeast product at 50 g/h/d or T2 could be appropriated to use for transition dairy cow period.

Supplementation of probiotic yeast (*C. glabrata* Dc18) product amount 50 g/ h/d which was not effect on DMI, ruminal fermentation (pH, NH_3-N and VFAs), milk yield, milk components yield (milk fat and protein), milk compositions (except milk protein, % which increased trend (P=0.05) with probiotic yeast was given to cows. On the other hand average somatic cell count was no significance differently (P>0.05). Base on this experiment probiotic yeast product was not negative effected only on hematological parameters, but increased total WBC, total RBC and PLT in dairy cows.

6.2 Implication

Now a day, many people concerning of fucntional food have been greater considered. To produce healthy benificial foods (milk and meat) from ruminants, nutritional strategies in order to manipulate rumen fermentation which results in improving the animal products has greater interested. Supplementation of yeast to ruminant animal had a varied results due to defferent strain and genera. Therefore, more understanding of strain of yeast and the function in the rumen could be useful for this strategy application. The novelty of this work is that we explore a of native yeast species which has potential use as in ruminant feeding additive. Therefore, further researchs involving addition multi strain of native yeast, or cochteal with other bacteria will using on experiment in rumen microbiota should be investigate.

BIOGRAPHY

Mr. Krung Wilachai was born on 2nd July 1979 in Kalasin, Thailand. In 1998, he graduated high school level from Muang Kalasin School, Kalasin. In 2002, he obtained his Bachelor's degree in Agricultural Science from the Program of Agriculture, Faculty of Science and Technology, Rajabhat Mahasarakham Institute, Mahasarakham. In 2005, he graduated his a Master of Science in Animal Science from the Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Khon Kaen. During study in master's degree he has gotten to scholarship by Tropical Feed Resources Research and Development Center (TROFREC) under his Advisor's Assoc. Prof. Dr. Chalong Wachirapakorn. Since 2007 he has been working as a lecturer at the Program in Animal Science, Faculty of Agricultural Technology, Mahasarakham Rajabhat University, Mahasarakham. He studied in a field of Animal Production Technology for Ph.D. Program at the School of Animal Production Technology, Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasima from November 2014 to September 2020 with the thesis entitled "Development of probiotic yeast product to improve rumen fermentation and enhance productivity in dairy cows" which supported financial by Thailand Research Fund (TRF) though the Research and Researcher for Industrial (RRi), also cooperated by the K.M.P. BIOTECH CO., LTD., Thailand.