ENHANCING THE EFFICIENT UTILIZATION OF RICE STUBBLE FERMENTED BY WHITE-ROT FUNGI AND UREA AS GOAT DIETS

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การเพิ่มประสิทธิภาพการใช้ประโยชน์จากตอฟางข้าวโดยการหมักด้วยราขาว (WHITE-ROT FUNGI) และยูเรียสำหรับเป็นอาหารแพะ

นายทันสมัย วรพิมพ์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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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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ทันสมัย วรพิมพ์ : การเพิ่มประสิทธิภาพการใช้ประโยชน์จากตอฟางข้าวโดยการหมักด้วยราขาว (WHITE-ROT FUNGI) และยูเรียสำหรับเป็นอาหารแพะ (ENHANCHING THE EFFICIENT UTILIZATION OF RICE STUBBLE FERMENTED BY WHITE-ROT FUNGI AND UREA AS FOR GOAT DIETS) อาจารย์ที่ปรึกษา : รองศาสตราจารย์ ดร. ปราโมทย์ แพงคำ, 152 หน้า.

การศึกษาในครั้งนี้มีวัตถุประสงค์ดังนี้ 1) เพื่อศึกษาระยะเวลาที่ใช้ในการหมักเชื้อราขาว 3 สายพันธุ์ (*Pleurotus ostreatus* (POT), *P. sajor-caju* (PSC) และ *P. eous* (PE)) ต่อการเพิ่มประสิทธิภาพการใช้ประโยชน์ได้จากโภชนะของตอฟางข้าวโดยศึกษาองค์ประกอบทางเคมีและการย่อยได้ในหลอดทดลอง 2) เพื่อศึกษาผลของระดับยูเรียและระยะเวลาที่ใช้ในการหมักตอฟางข้าว ที่ผ่านกระบวนการหมักด้วยเชื้อราขาวที่แตกต่างกัน 3 ชนิด เพื่อหยุดกิจกรรมการทำงานของรา 3) เพื่อศึกษาผลของตอฟางข้าวที่หมักด้วยเชื้อราขาว และนำตอฟางข้าวที่ได้ไปหมักต่อด้วยยูเรีย ต่อการย่อยได้ของโภชนะ กระบวนการหมักในกระเพาะรูเมนและการเจริญเติบโตในแพะเนื้อ โดยการทดลองแรกเป็นการประเมินคุณค่าโภชนะของตอฟางข้าวที่ผ่านการหมักจากเชื้อรากลุ่ม *Pleurotus* โดยวัดจากค่าองค์ประกอบทางเคมี ได้แก่ วัตถุแห้ง (DM), อินทรียวัตถุ (OM), โปรตีน (CP), Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), Acid Detergent Lignin (ADL), hemicellulose, cellulose และเถ้า (ash) นอกจากนี้แล้วยังได้ทำการศึกษาการย่อยได้ของอินทรียวัตถุในหลอดทดลอง ซึ่งตอฟางข้าวที่ใช้ศึกษาจะมีทั้งที่ผ่านการหมักและไม่ผ่านการหมักด้วยเชื้อรา โดยออกแบบการทดลองแบบสุ่มสมบูรณ์ (Completely Randomized Design (CRD)) ซึ่งค่าองค์ประกอบทางเคมีแสดงให้เห็นว่าการหมักตอฟางข้างโดยใช้เชื้อรา *Pleurotus* สามารถเพิ่มปริมาณของโปรตีนและเถ้า ($p<0.001$) เมื่อเปรียบเทียบกับกลุ่มควบคุม ในขณะที่ค่าองค์ประกอบของเยื่อใย ได้แก่ NDF, ADF, ADL, hemicellulose และ cellulose ของตอฟางข้าวที่หมักด้วยเชื้อรามีค่าลดลง ค่าการผลิตแก๊สในหลอดทดลองเพิ่มขึ้นในกลุ่มที่ทำการหมักตอฟางข้าว 25 วันด้วยเชื้อรา โดยเพิ่มขึ้นในช่วงการบ่มที่ 24 และ 96 ชั่วโมง ทั้งนี้ค่าประสิทธิภาพการย่อยได้ การย่อยได้อินทรีวัตถุ และค่าของพลังงานที่ใช้ประโยชน์ได้ ในกลุ่มที่ทำการหมักด้วยเชื้อราก็เพิ่มขึ้นเช่นเดียวกัน งานทดลองที่ 2 ทำการศึกษาองค์ประกอบทางเคมีและการย่อยได้ในหลอดทดลองของตอฟางข้าวที่หมักด้วย *P. ostreatus* (POT), *P. sajor-caju* (PSC) และ *P. eous* (PE) แล้วนำมาหมักต่อด้วยยูเรียที่ระดับแตกต่างกัน คือ 2.5% และ 5% และใช้ระยะเวลาในการหมักที่แตกต่างกัน คือ 7 วัน และ 14 วัน โดยออกแบบการทดลองแบบแฟคทอเรียล (3 x 2 x 2 factorial in CRD) ผลการทดลองพบว่ากระบวนการหมักตอฟางข้าวด้วยเชื้อราไม่ส่งผลต่อค่าของวัตถุแห้ง, ไขมัน และ

cellulose แต่พบว่าค่าของเถ้า และโปรตีนนั้นเพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติ ($p<0.01$) นอกจากนั้นแล้วยังพบว่าค่าของ OM, NDF, ADF, ADL และ hemicellulose มีค่าลดลงอย่างเห็นได้ชัดในกลุ่มที่ทำการหมักด้วยเชื้อรา ($p<0.01$) และการประเมินค่าการย่อยได้ในหลอดทดลองของตอฟางข้าว พบว่ากลุ่มที่หมักด้วยเชื้อรามีความสามารถในการย่อยได้สูงกว่ากลุ่มที่ไม่ได้ทำการหมักด้วยเชื้อรา โดยกลุ่มที่หมักด้วยเชื้อรา POT มีค่าสูงที่สุด ซึ่งพบว่าการใช้ยูเรียที่ระดับ 2.5% และทำการหมักเป็นระยะเวลา 7 วันนั้นมีความเหมาะสมต่อการหมักตอฟางข้าวที่หมักด้วยเชื้อรา งานทดลองที่ 3 ทำการศึกษาผลของตอฟางข้าวที่หมักด้วย POT แล้วนำมาหมักด้วยยูเรีย 2.5% เป็นเวลา 7 วัน (urea treated rice stubble fermented fungi (URSF)) เพื่อเปรียบเทียบกับตอฟางข้าวหมักด้วยยูเรีย (urea treated rice stubble (URS)) อย่างเดียวและตอฟางข้าวที่ไม่ผ่านการหมัก (rice stubble (RS)) เพื่อเป็นอาหารแพะเนื้อ ผลการทดลองพบว่าค่าการกินได้ของวัตถุแห้ง การกินได้ของโภชนะ และการย่อยได้ เพิ่มขึ้นในกลุ่มของ URSF ($p<0.05$) เช่นเดียวกับค่าของแอมโมเนียไนโตรเจนในรูเมนอัตราส่วนของอะซิเตท : โพรพิโอเนต (C2:C3) ในชั่วโมงที่ 2 หลังการให้อาหาร ค่าความสมดุลของไนโตรเจน ระดับไนโตรเจนในเลือด และอัตราการเจริญเติบโตต่อวัน รวมถึงน้ำหนักตัวที่เพิ่มขึ้น แต่พบว่าไม่มีผลต่อค่าความเป็นกรดด่าง กรดไขมันระเหยง่าย และประชากรของจุลินทรีย์ ดังนั้นจากผลการทดลองแสดงให้เห็นว่าการใช้เชื้อราทั้ง 3 สปีชีส์ สามารถเพิ่มประสิทธิภาพการใช้ประโยชน์ของโภชนะจากตอฟางข้าวเพื่อนำมาใช้เป็นอาหารสำหรับแพะได้

สาขาวิชาเทคโนโลยีการผลิตสัตว์
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ลายมือชื่อนักศึกษา _____
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THANSAMAY VORLAPHIM **:** ENHANCING THE EFFICIENT UTILLIZATION OF RICE STUBBLE FERMENTED BY WHITE-ROT FUNGI AND UREA AS FOR GOAT DIETS. THESIS ADVISOR : ASSOC. PROF. PRAMOTE PAENGKOUM, Ph.D., 152 PP.

PLEUTUS SPECIES/UREA TREATED/RICE STUBBLE

The objectives of this study were (i) to evaluate the potential of three species of *Pleurotus* fungi such as *Pleurotus ostreatus* (POT), *P. sajor-caju* (PSC) and *P. eous* (PE) for the nutritive value of rice stubbles using chemical composi-tion and *in vitro* digestibility measurements, (ii) to study the effect of the urea level and duration in treating rice stubbles fermented with different *Pleurutus* fungi, and (iii) to examine the effect of fermented rice stubble with fungi and treated with urea on nutrient digestibility, rumen fermentation, and growth performance in goat meat. The first experiment was conducted to evaluate the nutritive value of rice stubble fermentation by *Pleurotus* fungi. The experiment used a complete randomized design (CRD). The chemical composition illustrated that all of the fermentation by *Pleurotus* fungi treatments were apparently increase ($p<0.001$) in crude protein (CP) and ash contents when compared with the control group. Whereas there was significant decreased in neutral detergent fiber (NDF), detergent fiber (ADF), lignin detergent fiber (ADL), hemicellulose, and cellulose contents of rice stubble by fungal fermentation. *In vitro* gas production was significantly increased at day 25 of fermentation in all fungal treatments for 24-96 h incubation. Moreover, effective degradability (ED), organic matter digestibility (OMD), and metabolizable energy (ME) were also increased in all *Pleurotus* fungi treatments. A second experiment used a 3 x 2 x 2 factorial design. The

results showed that the DM, EE, and cellulose contents were not affected by fungal fermentation. The content of ash and CP were significant increased with processing by fungi in treatments ($p<0.01$). Whereas the content of OM, NDF, ADF, ADL, and hemicellulose were apparently reduced in rice stubble fermentation by all of the fungal ($p<0.01$). *In vitro* degradability increased the rumen degradability of rice stubble as indicated by the higher rate of the degradation constant and the potential degradability in all of fermented fungal treatments than unfermented stubble, but seems to be greater in *P. ostreatus* (POT) treatment. The level of urea indicated that 2.5% more suitable for treated rice stubble and at 7 days seem to be properly treated substrates. A third experiment to determine the effect of the fungi treatment on nutritive value of rice stubble treated urea as 2.5% and 7 days was best for use in the diet of goat meat. The results showed that rice stubble fermented with fungi and treated urea was significantly increased ($p<0.05$) in dry matter intake, nutrient intakes, and digestibility in goat meat. For ammonia nitrogen (NH_3-N) concentration, C2:C3 ratio for 2 h post feeding in the rumen, nitrogen (N) balance, blood urea nitrogen (BUN), and average daily gain (ADG) also increased. But no influence on the pH value, total volatile fatty acid (VFA), and bacteria population. These results indicated that using the fungi treatment of rice stubble improved the nutritional value for ruminant nutrition.

School of Animal Production Technology Student's Signature _____

Academic Year 2016 Advisor's Signature _____

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Thansamay Vorlaphim

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LIST OF ABBREVIATIONS

ADF	=	Acid detergent fiber
ADG	=	Average daily gain
ADL	=	Acid detergent lignin
AOAC	=	Association of Official Analytical Chemists
BW	=	Body weight
CP	=	Crude protein
D	=	Day
DM	=	Dry matter
DMI	=	Dry matter intake
EE	=	Either extract
FAO	=	Food and Agriculture Organization
g	=	gram
GC	=	Gas chromatography
GE	=	Gross energy
GLM	=	General Linear Model
h	=	Hour
kcal	=	Kilo calorie
kj	=	Kilo joules
L	=	liters
ME	=	Metabolize energy
MEI	=	Metabolize energy intake

mg = milligram

mmol = millimole

N = nitrogen

NDF = Neutral detergent fiber

NH_3-N = Ammonia nitrogen

NRC = National Research Council

OM = Organic matter

OMD = Organic matter digestibility

PE = *Pleurotus eous*

POT = *Pleurotus* ostreatus

PSC = *Pleurotus* sajor-caju

SAS = Statistical Analysis System

SEM = Standard error of mean

VFA = Volatile fatty acid

$W^{0.75}$ = Metabolic weight

CHAPTER I

INTRODUCTION

1.1 Rationale of the study

Agriculture plays a significant role in the world to feed the growing human population. Therefore, land for crop production will be used more intensively for human food production and consequently animal production will rely on feeding the by-products from the food produced for human consumption. This is especially in the case of rapidly growing economies in several parts of Asia, increasing also the demand for meat and milk at a high rate. Thus, many countries in this area urgently need to increase their livestock production.

Agricultural residues such as rice straw, maize stover, oil palm fronds, wheat straw, and sugarcane bagasse, are abundantly available in many countries (Wan Zahari et al., 2003; Devendra, 2009; Sarnklong et al., 2010); however, those parts of the plants that are regarded as waste often contain a relatively high concentration of plant cell walls approximately 80%. Plant cell walls consist of high lignocellulosic complex in which lignin, hemicellulose and cellulose are tightly bound to each other via covalent and non-covalent bonds (Jeffries, 1994). Cultivate residues are contending as low crude protein content of approximately 3 to 4% and high content of crude fiber of approximately 35 to 48% (Devendra, 1997), cause low digestibility and feeding values for ruminants (Karunanandaa et al., 1995; Karunanandaa and Varga, 1996; Islam et al., 2000; Wan Zahari et al., 2003; Albores et al., 2006). Rice generates a relatively large

amount of crop residues, approximately 80% of the world's rice is grown by small-scale farmers in many developing countries including South East Asia and it is common to use rice straw for animal feeding. Devendra and Thomas (2002) mentioned that rice straw is the principal crop residue fed to more than 90% of the ruminant livestock in this area. The calculated utilization of rice straw for animal feed in South East Asia, including China and Mongolia, was 30-40% of the total rice straw production (Devendra, 1997). Rice straw is especially important during periods when other feeds are inadequate. In general, the maximum intake of rice straw by ruminants is about 1.0 to 1.2 kg per 100 kg live weight (Devendra, 1997). The problems of farmers who raise ruminants in summer are using rice straw replace grass. Although in current rice is harvested with a mechanical harvester affected to we cannot separate apart of straw and stubble. Rice stubble is a part of rice production system and we are not using it, rice stubble compound lignin bind cellulose and hemicellulose consistency than rice straw. Lignin in plant cell walls is blocking cellulose and hemicellulose, so that these carbohydrates are less accessible for rumen microbes. In general lignin consists of 3 building blocks namely p-coumaryl alcohol (p-hydroxyphenyl propanol), coniferyl alcohol (guaiacyl propanol), and sinapyl alcohol (syringyl propanol) (Vanholme et al., 2010; Bugg et al., 2011). Lignin, therefore, blocks the accessible microbial in the rumen to digest fibrous compound. As such, lignin removal increases the accessibility of carbohydrates for rumen microbes.

There are many applications have been studied to improve crop residues utilization such as physical, chemical, and biological treatments; thus, using white-rot fungi to break lignin with cellulose or hemicellulose bonds (Chen et al., 1995; Mahesh and Mohini, 2013; Bento et al., 2014; Nasehi et al., 2017). Fungi in white-rot fungi

group produce enzymes which contain lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase these microbes are well known (Sánchez, 2009; Bugg et al., 2011). This is likely to increase nutrients digestibility and increased crud protein content due to increasing fungi biomass. Combination of several methods may be achieving maximum utilization of nutrients. Kinfemi et al. (2009) suggested that *Pleurotus ostreatus* increase in the crude protein (CP) from 12.25% for the control to 17.04% in cowpea husk. *Pleurotus sajor-caju* increased in CP content and fibrous digestibility (Jafari et al., 2007). Cultivation of *Pleurotus Eous* fungi on paddy straw was higher yield than other treatments it's implied that fungi can be digested fibrous materials as high efficiency (Samsudin et al., 2013).

1.2 Research objectives

1.2.1 To study types and period time fermentation of white-rot fungi as for improve rice stubble digestibility.

1.2.2 To study effect of rice stubble fermented with white-rot fungi on rumen fermentation, types and amount of microbes in the rumen and growth performance and nutrient digestibility of meat goats.

1.3 Research hypothesis

1.3.1 Rice Stubble fermented with *Pleurotus sp.* and treated with urea will be improved digestibility.

1.3.2 Goats fed with rice stubble fermented *Pleurotus sp.* and treated with urea will be increased weight grain.

1.4 Scope and limitation of this study

1.4.1 Three species of white-rot fungi will be used in this study (*Pleurotus eous, P. sajor-caju* and *P. ostreatus*).

1.4.2. Crossbred meat goats from goat farm of Suranaree University of Technology were used in the studies of optimizing improves rice stubble digestibility and growth performance of meat goat by fermentation of white-rot fungi.

1.5 Expected results

1.5.1 To know the type of whit-rot fungi be suitable to improve rice stubble digestibility.

1.5.2 To know the effects of white-rot fungi treated rice stubble on nutrients digestibility, rumen fermentation, types and amount of microbes in the rumen and growth performance of meat goat.

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CHAPTER II

LITERATURE REVIEW

2.1 Characterizes of Crop and Livestock Production System

The system of crop and livestock mixed farming are featured by interdependency between crop and livestock production activities (Ostergaard, 1995). It is the major system for smallholder farmers in many developing countries to produce (Ostergaard, 1995; Blackburn, 1998).

The main objective of farmers engaged in mixed crop - livestock farming is to gain complementary benefit from an optimum mixture of crop and livestock farming and spreading income and risks over both crop and livestock production (Lemma, 2002; Solomon, 2004; Teshome, 2009). In the mixed crop livestock farming systems, livestock supply important inputs to crop cultivation, especially organic fertilizer and traction. Livestock are often the main source of cash that farmers can use to buy agricultural inputs. On the other hand, crops provide livestock with feed in the form of crop residues and by-products from crop production, which are transformed into worth products like meat, milk, and traction (ILCA, 1992). The crop residues ability to use as livestock feed are greatest in integrated crop/livestock farming systems (Kossila, 1988; Getachew, 2002; Lemma, 2002). The animals are require crop residues to supply feeds during the dry seasons; while they are also necessary to crop. In this situation, it is very likely that changes in the way and time farmers harvest their crops and manage

the residues offer a number of possibilities for increasing both crop and livestock production.

2.2 Production system and feeding efficiency

Three factors to get highest benefit of animal production are genetic makeup, nutrition and management (Sethumadhavan, 2004). The effective of livestock feeding in systems in Thailand vary depending on the animal species/ type, and the feeding system. Feed conversion ratio can be used to estimate compound or concentrate livestock feed requirements, particulaly for non-ruminant species such as pig, poultry and fish. However, ruminant feeding systems are mainly reliant on local agro-industrial by-products including the natural grasses found in the traditional crop, rice and livestock - based mixed farming systems. The mostly, or 95%, of extensive beef production systems use no cereal grain or concentrate feed supplements (Sommart et al., 2012). However, in the case of beef-dairy cattle, a shortage of feed, both in terms of quantity and quality, is a main limitation and is expected to pose larger obstacles as farm sizes increase. The shortage of high quality roughage forces dairy farmers to use high concentrate supplements combined with rice straw, crop residues, agro-industrial co-products and/or low quality roughage. This ensures in low feed intake, low digestibility, low energy utilization and thus low production efficiency as well as air and water environmental stress for instance N, P and enteric methane emissions.

2.2.1. Crop residues

Crop residues are a main source of livestock feeds in Thailand for ruminants. Crop production areas and productivity are extremely associated with the

annual yield of their by - products and residues. Main crop residues for Thailand are arised from rice, corn, cassava, sugar cane, oil palm, soybeans, coconuts and pineapples. Many crops provide feed ingredients directly to livestock such as corn and cassava. Some crops generate more than one product and by-products, such as soybeans that provide the soybean meal used in non-ruminant feeds while supplying soybean hull and stems used in ruminant feeds. The main crop residues are rice straw and stove derived from rice harvesting, and sugar cane tops and corn stove. Cassava leaves and palm oil fond and residues are also sources for animal feed. Due to a lack of data, it is difficult to accurately estimate the quantity of crop residues used in livestock feeding (Sommart et al., 2014).

2.2.2 Roots, tubers and other by-products

Generally, Thailand is the largest exporter of cassava. Cassava chip or cassava pulp feeding technologies have been developed and currently 20% of dairy cattle rations include cassava in order to decrease feed costs (Sommart et al., 2000a,b). Other crop by-products produce in Thailand are also plentiful such as baby corn waste, corn cobs, tomato waste, seafood industry waste, can fruit waste, fish processing waste etc. However, formal data on their availability, their productivity and utilization in animal feeding are lacking.

2.2.3 Grasses and forages

Grasses are elementary sources of roughage for feeding ruminants in Thailand. Sources of grasses for livestock comprise communal pastures, natural pastures, forest grazing, roadside grazing, and natural grasses under paddy and upland

crops, fallow lands and introduced improved pastures. Most dairy farms depend on improved pasture, in addition to crop residues. Buffalo and goats depend on native grasses.

Even though Thailand has introduced substantial galore of leguminous species with the objective that the availability of protein/nitrogen feed inputs increase, so that benefiting the livestock sector, legumes play a minor role in livestock feeding. The Leucaena (*Leucaena leucocephala*) is an important forage tree in Thailand, grown elementary in the Central, North and Northeastern of Thailand. Its fresh fodder is use for ruminant feeding, while dry leucaena leaf meal is use in non-ruminant feeds.

2.3 Rice production in Thailand

World rice production areas were 1,000.06 million rais which decreased from 1,010.81 million rais in year 2014/15 or decreased 1.06%. The rice production was 478.25 million tons which slightly reduced from 478.54 million tons in last year or reduced 0.06%. The mean yield was about 478 kilogram per rai which increased from 473 kilogram per rai in last year or increased 1.0%. The enhancing production countries were China, Bangladesh, Vietnam, Myanmar, Philippines and Brazil. In contrast, the countries with decreased production were India, Indonesia, Thailand and Japan OAE (2016). In Thailand found rice production area in 2015 was reduced from 2014 because the less rainfall in the early of rainy season and the under rainfall average of the overall actual rainfall in most regions. These situations caused delayed in farmers' cultivation. Furthermore, the cultivation in some areas could not proceed. In addition, the declining price leaded farmers switched their planting to other crops with a good price condition like sugarcane for example. The production yield per rai

slightly decreased because the lower level of rainfall was insufficient to grow (OAE. 2016).

2.4 General goats production in Thailand

Goat plays an important role in the rural economy of many developing countries in Asia including South of Thailand. Nowadays, goat has been expanded throughout countries due to the lowering of land for large ruminant animals such as cattle and buffalo. Thus, small ruminant animals such as goat and sheep have been increasing interested by the farmers. Number of goat production in Thailand in 2013, 2014 and 2015 are approximately 440277, 468413 and 539583 heads (DLD, 2016). It seems that goat population trends to be gradually increased and the raising location has been widely distributed throughout Thailand. The advantage of rearing goat may be relied on the fact that goats are easily reared and they are required a small size of pens when compared with large ruminant animals.

Table 2.1 Area of rice production 2010-2015 in Thailand (1000 Rais)

Year	Planted Area	Harvested Area
2010	80,676	75,747
2011	83,405	74,729
2012	81,038	74,729
2013	77,135	73,027
2014	96,280	66,685
2015	62,315	59,308

Source: OAE (2016).

2.5 Feeding systems for goats

The diet for ruminant consists of roughages and concentrates. The most important diet is roughage which responsible for at least 50 - 100% depends on the quantity and quality of roughages. Goats are typically consumed browse from the top downward on a plant, therefore, assumes they are an effectively consumer of biological herbicide for regulating undesirable plants and shrubs.

Goats are good browsers and can selectively eat a wide variety of shrubs, woody plants, weeds and briers (Teixeira et al., 2011). The management of goat production depends on the available sources of roughages for example pasture, hay, haylage and silage. Fresh grass and hay are an excellent roughage sources for goats. Preservation method such as silage or haulage can be successfully used in goat rearing, however, there is limiting to use preserved roughage for young goats due to the incomplete rumen function in this stage of growth. It has been shown that goats are easily accepted novel feed when supply it during pregnancy.

2.6 Ruminant animals feed

Livestock feeds provide the basic nutrients required for animal production, including energy, proteins, minerals, and vitamins. Feed for ruminant animals may be broadly classified as concentrate and roughage depending on their composition.

2.6.1 Concentrates

Concentrates are feeds that contain a high density of nutrients, usually low in crude fiber content (less than 18% of dry matter) and high in total digestible nutrients. Concentrates may be fed in raw or milled forms as individual feeds or may be blended or formulated into balanced rations for particular production purposes.

Concentrates may be high in energy, referred to as energy concentrates, or high in protein, with over 20% crude protein, referred to as protein concentrates.

Table 2.2 Population of goat in 2013-2015 in Thailand (heads)

Years	Central	North east	North	South	Total
2013	157,112	14,613	32,921	235,631	440,277
2014	174,295	16,252	34,681	243,185	468,413
2015	209,155	19,822	38,876	271,730	539,583

Source: DLD (2016).

2.6.2 Roughages

Roughages or forages are the edible parts of plants with a low density of nutrients, with crude fiber content over 18% of DM and low in total digestible nutrients. Forages can provide feed for grazing animals or that can be harvested for feeding that includes the classes of feed such as fresh, herbage, hay and silage, browse, and straws. Forage consists largely of carbohydrate in the form of fiber, and its digestion is accomplished through the enzymic action of the rumen microbes. Forages are a potential feed for ruminant animals, as ruminants are best adapted to the utilization of plant cell walls for conversion of fibrous feed sources into milk and meat products.

2.7 Nutritive quality of rice stubble

Rice stubble consists predominantly of cell walls, comprised of cellulose, hemicellulose, and lignin. To break down these components cellulase, hemicellulase and ligninase are required (Schiere and Ibrahim, 1989). These enzymes are not

produced by the animals themselves but the reticulorumen of ruminants maintains microorganisms that do produce cellulase and hemicellulase. However, lignin cannot be broken down in the rumen due to the lack of ligninase. Even if lignin could be degraded in the rumen it would not provide much energy for the animals. Theoretically, lignin located between the cellulose microfibrils is regarded as the most abundant natural aromatic organic polymer that plays a role in resisting compressing forces, providing protection against consumption by insects and mammals, and also inhibiting the rate and degree of microbial degradation (Iiyama et al., 1990). Silica, one element of the rice cell walls, can be present in high concentrations ranging from 5% to 15%, depending on the rice variety (Vadiveloo, 1992) and the availability of this mineral in the soil (Agbagla-Dohnani et al., 2003). Silica reduces palatability and the degradability of rice straw in the rumen due to its direct action in preventing colonization by ruminal microorganisms (Bae et al., 1997; Agbagla-Dohnani et al., 2003). The role of silica on the quality of rice straw was also reviewed by Van Soest (2006), in an attempt to put into perspective the problems of silicon metabolism. Besides cell wall polymers, rumen organisms need other nutrients for growth and metabolism (Hoover, 1986). Since rice straw does not contain enough sugars, amino acids and minerals for efficient microbial growth, feeding ruminants with only rice straw, without any supplementation of the other required nutrient sources, will result in poor performance of the animals (Doyle et al., 1986). The combination of low intake, low degradability, low nitrogen content and an unbalanced mineral composition means that rice straw alone may not even meet the animal's maintenance needs. Poor degradability is caused by a series of factors (Schiere and Ibrahim, 1989). The fiber is very difficult to degrade, which is partly an intrinsic characteristic of the

straw fiber. The degradation of the straw fiber is also complicated by the poor functioning of the rumen due to the unbalanced availability of nutrients, the low protein content, the lack of easily available energy and the low content of essential minerals such as P and S. Hence, due to the low degradability and the poor rate of degradation, animals will tend to consume less. The mechanism regulating voluntary intake of low quality feeds, such as rice straw, is still not fully understood. The generally accepted theory of feed intake regulation for poor quality roughages is that the capacity of the rumen to process the feed is the major factor determining voluntary feed intake (Conrad, 1966; Baile and Forbes, 1974). The rumen processing capacity is characterized by rumen fill, the rate of degradation of potentially degradable matter and the rate of passage out of the rumen. Devendra (1997) summarized that the main determinants of intake and degradability of rice straw depend on their morphological characteristics, such as the proportion of the different plant parts (leaves and stems), their chemical composition and the distribution of the different chemical components in the tissues, their relative amounts of cell contents and cell walls and the physical and chemical nature of the cell walls.

These factors influence the chewing behavior of animals and the extent of fragmentation in the reticulo-rumen. Rice straw contains a relatively high proportion of leaf (60%), compared to other cereal straws such as barley (35%), oats (43%) and wheat (20-41%) (Theander and Aman, 1984). Leaves of rice straw contain less NDF than the stems, but more ash and acid-insoluble ash, resulting in a lower *in vitro* dry matter digestibility (IVDMD) of the leaves (50-51%) compared to the stems (61%) (Vadiveloo, 2000). In goats, Phang and Vadiveloo (1992) observed an *in vivo* dry matter digestibility of 56.2% for rice leaf and 68.5% for the stem. However, treatment

with a 4% urea solution for 21 d increased the IVDMD of the leaf fraction more than that of the stem fraction (Vadiveloo, 2000). Since rice straw consists of approximately 60% leaves (Vadiveloo, 1995), which are less degradable than stems, improving the feed value of rice straw should focus on improving the degradability of the leaves.

Different technologies have been investigated to improve the feeding value of such by-products. Physical treatments, such as steaming, grinding and pelleting have been reported to increase the intake and digestibility of oil palm fronds and hence the performance of cattle (Wan Zahari et al., 2003). Alkali treatments, especially those with NaOH or NH_3, have been reported to improve the intake and/or digestibility of rice straw (Sarnklong et al., 2010), maize stover (Oliveros et al., 1993), oil palm fronds (Wan Zahari et al., 2003) and sugarcane bagasse (Amjed et al., 1992); however, such physical and chemical treatments can be expensive, harmful to users or environmentally unfriendly (Van Soest, 2006).

2.8 Plant cell walls

The plant cell wall is a complex macromolecular structure that surrounds and protects the cell. Cell walls are important features of plant cells that perform a number of essential functions, including providing shape to the many different cell types needed to form the tissues and organs of a plant. The composition of cell wall varies largely between plant species, tissues within the plant and also between different stages of growth. Plant cell walls are usually divided into two categories: primary walls that surround growing cells or cells capable of growth and secondary walls that are thickened structures containing lignin and surrounding specialized cells such as vessel elements or fiber cells (Figure 2.1)

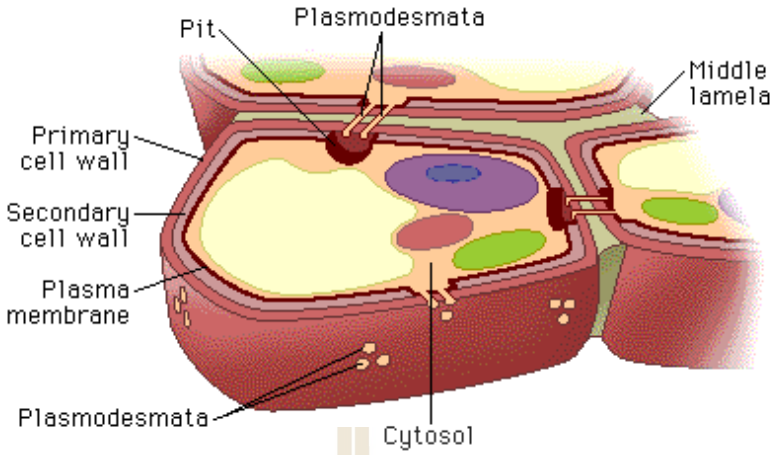


Figure 2.1 Schematic representation of a plant cell and wall development (http://www.phschool.com/science/biology_place/biocoach/plants/walls.html).

Plant cell walls contain a wide range of additional compounds that modify their mechanical properties and permeability. The major polymers are cellulose, hemicellulose, pectin and lignin, which limited ability to digest by animals. However, have bacteria and other microbial populations in their digestive tracts can ferment these compounds partially into usable nutrients for ruminant animals (Figure 2.2). Plant cell walls typically consist of about 35-50% cellulose, 20-35% hemicellulose and 10-25% lignin by dry mass (Sticklen, 2008).

2.8.1 Cellulose

The cellulose chains are organized together into progressively more complex assemblies at increasing size scales. The chemical structure of cellulose, which is a linear polymer of β-(1, 4)-linked D-glucose monomer units, is in fact quite simple. Typically, cellulose chains in primary plant cell walls have degrees of

polymerization (DPs) in the range from 5000 to 7500 glucose monomer units, with the DP of cellulose from wood being around 10,000 and around 15,000 for cellulose from cotton. The basic repeating unit of cellulose is cellobiose, the β-(1,4)-linked disaccharide of D-glucose. Although cellulose functions as the rigid, loadbearing component of the cell wall, the rigidity of the cellulose microfibril is strengthened within a matrix of hemicelluloses and pectins (Figure 2.3).
(www.bio1151.nicerweb.com/Locked/media/ch05/cellulose.html).

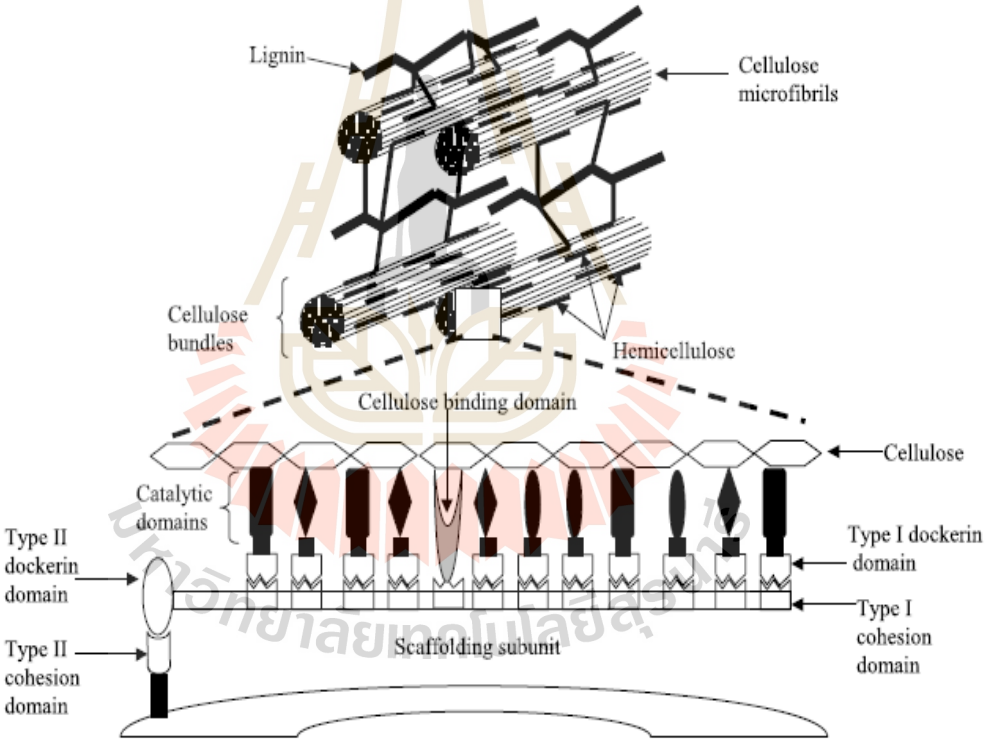


Figure 2.2 Idealized representation of fiber and its component cellulose, hemicellulose, and lignin (Krause et al., 2003).

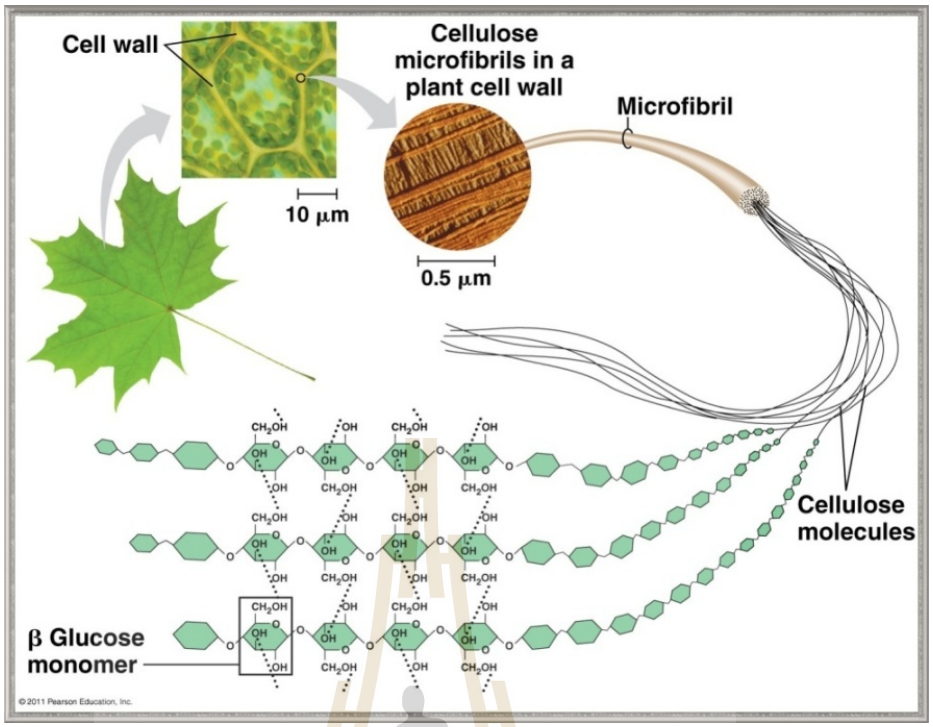


Figure 2.3 Schematic presentation of cellulose structure.

(www.bio1151.nicerweb.com/Locked/media/ch05/cellulose.html).

2.8.2 Hemicellulose

Hemicellulose polysaccharides are found in all terrestrial plants, from woods, grasses and cereals. They were originally defined as those plant polysaccharides that could be separated from cellulose by extraction with alkali-water solutions. Hemicelluloses are closely associated in plant tissues with cellulose and lignin, and they are most often structural polysaccharides in these tissues. Hemicellulose in plants is a mixture of polysaccharides that are soluble in dilute acid. In secondary walls of plant cells, it is characterized by a linear xylan core polymer that consists of repeating units of β-1, 4 linked xylose residues. Hemicelluloses are named according to the main sugar monomer unit in their backbone structure. Hemicelluloses are generally classified according to the main sugar residue in the backbone, e.g.,

xylans, mannans, and glucans, with xylans and mannans being the most prevalent (Figure 2.4). Thus, xylans are polymers with D-xylose units in the main chain and those with D-mannose, L-arabinose and D-galactose are referred to as mannans, arabinans and galactans, respectively. Xylan is the major component of hemicellulose and is, after cellulose, the second most abundant polysaccharide in nature. Xylans account for 30-35% of the cell wall material of annual plants (grasses and cereals), 15-30% of hardwoods and 7-10% of softwoods (Wilkie, 1979; Ladisch et al., 1983). Due to the significant presence of xylans in plants it serves as a major constituent of animal feed.

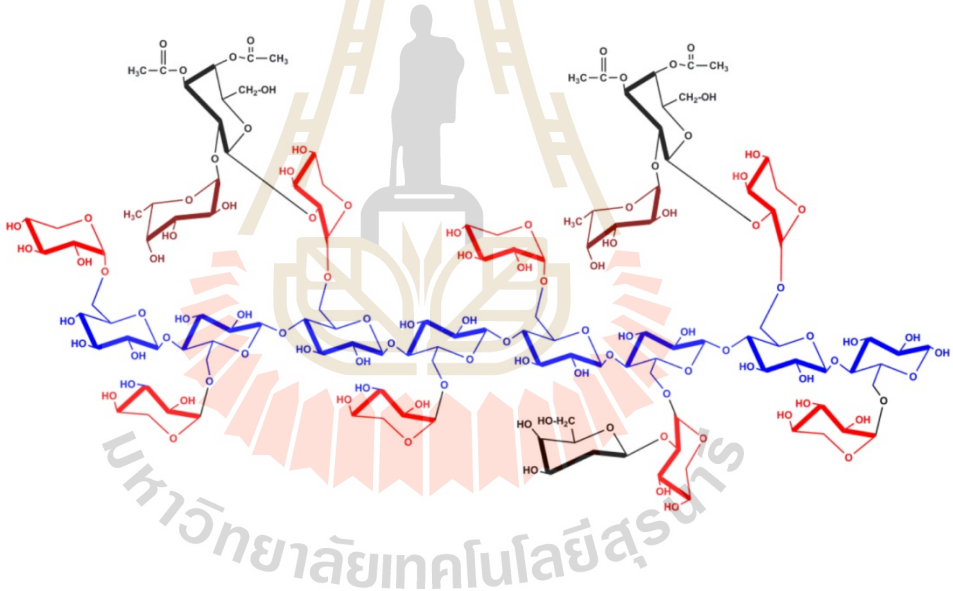


Figure 2.4 Hemicellulose structure (Ochoa-Villarreal et al., 2012**)**

2.8.3 Pectin

Pectins are important both as cell wall components and as industrial gelling agents. Pectic polysaccharides are structurally complex and heterogeneous (Schols and Voragen, 1994; Schols et al., 1994), they consist of a backbone of (1→4) α-D-galacturonosyl residues interrupted with typically a 10% substitution of (1→2)-α-

L-rhamnopyranosyl residues. A fraction of the rhamnosyl residues are branch points for neutral sugar side-chains that contain L-arabinose and D-galactose. Pectins are noncellulosic acidic cell wall polysaccharides and are divided into three classes: homogalacturonan, rhamnogalacturonan I, and rhamnogalacturonan II. Pectins function as a sol-like matrix, providing water and ion retention, support and facilitation of cell wall modifying enzymes, cell wall porosity, cell-tocell adhesion, cell expansion, cell signaling, developmental regulation, and defense.

2.8.4 Lignin

Lignin is one of the most plentiful organic polymers in plants, just behind cellulose. It is the exclusive chemical composition of gymnosperm and angiosperm. The content of lignin in wood and Gramineae is 20-40% and 15-20 %, respectively. Lignin is the name of a group of substances; their inhomogeneity is manifested in different species of plants, length of growing season, and different parts of the plants. Even in the different morphologies of cells of the same xylem or different cell wall layers, the structures of lignin are not the same (Jiang, 2001).

Lignin is an intricate created for confounded phenylpropane units nonlinearly also haphazardly linked; three primary monomers would coumaryl alcohol, coniferyl alcohol and sinapyl alcohol. Because of the different monomers, lignin can be divided into three types (Figure. 2.5): syringyl lignin polymerized by syringyl propane, guaiacyl lignin polymerized by guaiacyl propane, and □ydroxyl-phenyl lignin polymerized by □ydroxyl-phenyl propane. Usually, gymnosperm mainly contains guaiacyl (G) lignin; the dicotyledon mainly contains guaiacyl-syringyl (GS) lignin; the

monocotyledon mainly contains guaiacyl-syringyl-hydroxy-phenyl (GSH) lignin (Wei and song, 2001).

At a time, lignin in plant was categorized into softwood, hardwood, and grass lignins. In light of those structure about lignin, Gibbs divided lignin into G lignin and GS lignin. G lignin is most formed through dehydrated oligomerization of coniferyl alcohol, and its structure is homogeneous. This kind of lignin has negative.

Maule interaction because less than 1.5 % of syringaldehyde and about 5 % of p-hydroxybenzaldehyde were produced when oxidized by nitrobenzene. Ultimate lignin in softwood belongs to G lignin, which is copolymerized by guaiacyl and has a positive Maule interaction. GSH lignin is the result of the dehydrated oligomerization of coniferyl alcohol and sinapyl alcohol; the lignin is content 17-23 %. The ratio of syringyl propane to guaiacyl propane is 0.5-0.1; it also contains 7-12 % ester groups. P-Coumaryl alcohol in it is linked to lignin in the form of ester (Gao and Tang, 2004)

2.9 Lignin degradation

2.9.1 Lignin degrading enzymes

Due to the branching, bulky three-dimensional structure and the C-C and C-O ether linkage heterogeneity of lignin, hydrolytic enzymes (like those responsible for the catabolism of linear cellulose and short-branching hemicellulose polysaccharides) unable to breakdown lignin (Abdel-Hamid et al., 2013). Likewise, low-potential oxidoreductases, such as the plant oxidases that initiate lignin polymerization, cannot oxidize the non-phenolic aromatic lignin subunits. These obstacles have constrained the evolutionary diversity of lignin-degrading microbial phenotypes to a limited set ofspecialized fungiand bacteria. Within lignin-degrading

fungi and bacteria, several enzyme classes have been identified that are proposed to have ligninolytic activity (Bugg et al., 2011; Abdel-Hamid et al., 2013; Adam and Stephen, 2014). While details surrounding the enzymology and distribution of these oxidative enzymes are beginning to be elucidated, the knowledgebase of ligninolytic enzymes still lags far behind the knowledge of cellulases.

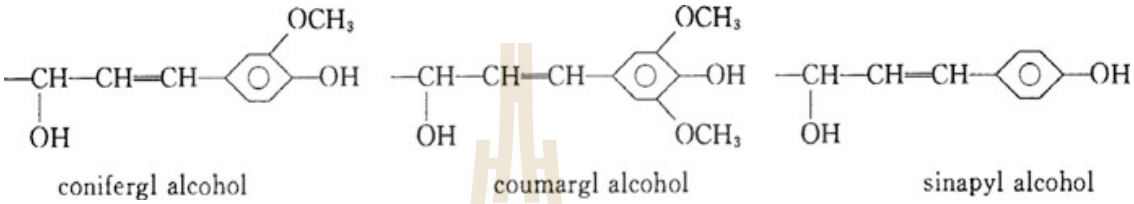


Figure 2.5 Basic structural unit of lignin (Wei and song, 2001)

2.9.2 Fungal lignin enzymology

The knowledge and recognition encompassing fungal lignin degradation surpasses its bacterial counterpart and is the basis for most ligninolytic investigate. Modification and degradation of lignin has been most widely studied in the basidiomycetes, in especially the white-rot fungi and to a lesser extent the brown-rot fungi (Gilbertson, 1980; Boyle et al., 1992; Adam and Stephen, 2014). Phanerochaete chrysosporiumis the model organism for lignin degradation by white-rot fungi, so anyway many other species have been studied such as, *Pleurotus ostreatus, Coriolus versicolor, Cyathus stercoreus, and Ceriporiopsis subvermispora* (Martinez et al., 2004; Abdel-Hamid et al., 2013; Adam and Stephen, 2014). The activity of these organisms are depend on families of peroxidases and laccases have been primarily concerned as ligninolytic enzymes.

2.9.3 Fungal lignin enzymology

The innitial ligninolytic enzyme isolated, lignin peroxidase (LiP, EC 1.11.1.14) was disconnected from *P. chrysosporiumand* found to be capable ofoxidizing sites of particularly high redox potential, including the moderately-activated aromatic rings of non-phenolic model lignin compounds which can comprise up to 90% of the polymer (e.g., β-O-4 linkages) (Tien and Kirk,1984; Miki et al., 1986). Wood-rot fungi produce two more extracellular ligninolytic peroxidases: manganese-dependentperoxidase (MnP, EC 1.11.1.13) and versatile peroxidase (VP, EC1.11.1.16) (Gold et al., 1984; Martinez et al., 2004). MnP is unique from LiP in that it relies upon the generation of Mn3+ as a diffusible charge-transfer mediator and cannot oxidize non-phenolic lignin model compounds (but can reduce amines, dyes and phenolic lignin model compounds) (Gold et al., 1984; Paszczyński et al.,1985; Wariishi et al., 1991). VP are fittingly named, as they are capable of both LiP and MnP (manganese independent and dependent) catalytic activities, cleaving high redox potential nonphenolics, as well as lower potential aromatics and amines (Martinez et al., 2004; Pérez-Boadaa et al., 2005).

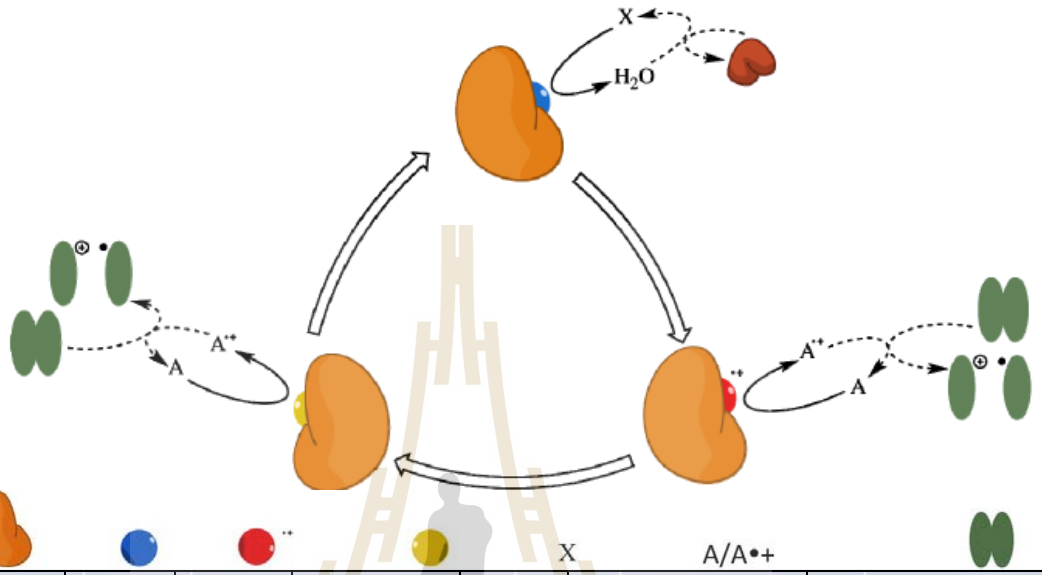
The enzyme cycle of the ligninolytic peroxidases is very similar to other peroxidases where the heme group reacts with hydrogen peroxide to form an oxo-ferryl intermediate (Figure 2.6.). However, there are two basic qualifications special to the ligninolytic peroxidases. These enzymes have a heme environment conferring an increased redox potential and restricting locales particular to non-phenolic aromatics or Mn^{2+}in LiP or MnP, respectively (Martinez et al., 2004). For MnP the decreased Mn^{3+} is an extensively acknowledged diffusible mediator, fit for oxidizing targets distanced from the enzyme active site (Kuan et al., 1993; Kishi et al., 1994). The

emission of oxalic acid and other organic acids that chelate Mn^{3+} in stable complexes extends the scope of the MnP oxidative action (Bugg et al., 2011). It has been proposed that veratryl alcohol can act as a diffusible mediator for LiP, in spite of the fact that the veratryl alcohol cation radical seems to have a short half-life (Khindaria et al., 1995) Dye-decolorizing peroxidases (DyP, EC 1.11.1.19) make up the most as of late found class of heme-peroxidases occurring in fungi and bacteria, which impart no arrangement or structural similarity with other plant, fungal or bacterial peroxidases (Sugano et al., 2007; Liers et al., 2009).

The peroxidases (LiP, DyP, MnP and VP) (Figure 2.6) react with hydrogen peroxide to form oxo-ferryl intermediates (red and yellow circles), while laccases contain a four-copper active site that reduces oxygen to water to gain oxidative potential. While LiP and VP (and possibly DyP) have the reductive potential to directlyoxidize non-phenolics, MnP and laccases must use mediators to attack non-phenolics.

DyPs got their moniker from their can oxidize the high-redox potential anthraquinone dyes in addition to typical peroxidase substrates (Sugano et al., 2007). In supplement to these peroxidase ligninolytic enzymes, white-rot fungi emit adornment enzymes such as aryl-alcohol oxidase (veratryl alcohol oxidase; EC 1.1.3.7) from *P. eryngii* and glyoxal oxidase (EC 1.2.3.5) from *P. chrysosporiumthat* generate hydrogen peroxide required by the peroxidases (Kersten and Kirk, 1987; Guillén et al., 1992). Other from this many fungi secrete oxidoreductases (such as quinone oxidoreductase [EC 1.1.5.1] and cellobiose dehydrogenase [EC 1.1.99.18])

equipped for decreasing the radical methoxy-groups of lignin-derived compounds (Kersten and Kirk, 1987; Guillén et al., 1997).



LiP (DyP)	Fe(III)	Fe(IV)	Fe(IV)	H_2O_2	Non-phenolics, phenolics, Veratryl Alcohol	Non- phenolics, phenolics
MnP	Fe(III)	Fe(IV)	Fe(IV)	H_2O_2	Mn^{2+}/Mn^{3+}	Phenolics, Other Mediators
VP	Fe(III)	Fe(IV)	Fe(IV)	H_2O_2	Non-phenolics, phenolics, Mn^{2+}/Mn^{3+}, Veratryl Alcohol	Non-phenolics, Phenolics, Other Mediators
Laccase	4(Cu2+)	4(Cu+)	2(Cu+) 2(Cu2+)	O2	Phenolics, Mediators (ABTS, HBT, etc…)	Non- phenolics, phenolics

Figure 2.6 The catalytic cycle of ligninolytic peroxidases and laccases differ in their oxidizing substrate (X), their target reducing substrates/mediators (A) and their electron accepting metal co-factors (colored circles).

The use of fungi and/or their enzymes that metabolize lignocelluloses is a potential biological treatment to improve the nutritional value of straw by selective delignification, as mentioned in the review by Jalc (2002). Nevertheless, it is currently

too early to apply this method in developing countries due to the difficulties and lack of technology to produce large quantities of fungi or their enzymes to meet the requirements. There are also a number of serious problems to consider and overcome (Schiere and Ibrahim, 1989). For example, the fungi may produce toxic substances. It is also difficult to control the optimal conditions for fungal growth, such as pH, temperature, pressure, O_2 and CO_2 concentration when treating the fodder. With recent developments in fermentation technology and alternative enzyme production system, the costs of these materials are expected to decline in the future. Hence, new commercial products could play important roles in future ruminant production systems (Beauchemin et al., 2004). White-rot fungi treatment*:* White-rot fungi, belonging to the wood-decaying *basidiomycetes*, as lignocellulolytic microorganisms are able to decompose and metabolize all plant cell constituents (cellulose, hemicellulose, and lignin) by their enzymes (Eriksson et al., 1990). Many species of white-rot fungi which are effective lignin degraders have been used to assess their ability to improve the nutritive value of fodder for ruminant nutrition (Yamakava and Okamnto, 1992; Howard et al., 2003). Their extracellular lignin-modifying enzymes consist of lignin-peroxidase (LiP), manganese-dependent peroxidase (MnP), laccase (phenol oxidase) and H_2O_2-producing oxidase (aryl-alcohol oxidase; AAO and glyoxaloxidase) (Kirk and Farrell, 1987; Arora et al., 2002; Novotny et al., 2004; Arora and Gill, 2005; Lechner and Papinutti, 2006). Some white-rot fungi are able to decompose free phenolic monomers and to break the bonds with which lignin is cross-linked to the polysaccharides in rice straw (Chen et al., 1996), enhancing IVDMD (Karunanandaa et al., 1992; 1995; Karunanadaa and Varga, 1996a, b; Fazaeli et al. (2006). Karunanandaa et al. (1995) reported the effect of incubation of rice straw for 30 days

with three white-rot fungi, showing that *Pleurotus sajor-caju* enhanced IVDMD, in both leaves and stems of rice. However, entire rice straw (leaf and stem) treated with *Cyathus stercoreus* had the highest IVDMD compared to the other fungi (Karunanandaa et al., 1992). Using white-rot fungi to increase the degradability of straw is often at the expense of easy assessable carbohydrates, such as cellulose and hemicellulose, resulting in less degradable feed for ruminants (Karunanandaa et al., 1995; Karunanandaa and Varga, 1996a, b; Jalc, 2002). In fact, cellulose and hemicellulose losses during the initial part of incubation with fungi are rather common, but losses due to mycelial growth depend on the fungus species. After the initial period of incubation, some white-rot species preferably attack lignin, without degrading cellulose and hemicellulose. Rodrigues et al. (2008) were able to extract the enzymes from white-rot fungi that are responsible for breaking down the bonds in lignin and within the matrix of cell wall carbohydrates, but without also extracting enzymes affecting hemicellulose and cellulose. Using these enzymes on wheat straw the *in vitro* NDF degradability (IVNDFD) increased. Although the use of fungi to improve the feed value of rice straw is not new, progressing research and new knowledge offers new challenges and possibilities. Fungi can be selected that preferably attack lignin and not the structural carbohydrates in the cell walls. Once these species are identified, mycologists can breed even better strains. The most desirable situation would be that the mushrooms of the fungi are edible and can be harvested by farmers, after which the remaining straw can be fed to their herd. There are some edible white-rot fungi, like *Pleurotis ostreatus*. However, much research is needed to achieve these goals. The most suitable white-rot species have to be identified and breeding programs will possibly be needed to improve their

characteristics. Also, the optimal conditions to incubate straw with a fungus have to be investigated, not only with the purpose of harvesting quality mushrooms, but also achieving optimal feeding quality of the remaining straw-fungi mixture. To achieve optimal feed qualities of the straw, incubations with fungi in combination with other treatments, such as physical and chemical treatments, have to be investigated.

In cellulolysis, the 1, 4-beta-D-glycosidic linkages in cellulose are broken by either chemical or enzymatic hydrolysis. Here only enzymatic hydrolysis will be introduced (Onuki, 2006). Cellulose molecules can be broken into glucose by various cellulase enzymes found naturally in grazing animals such as cows and sheep. These enzymes can also be harvested from genetically engineered fungus, along with xylanase and hemicellulase enzymes, which can be used on plant feedstock to produce sugars for fermentation (Karhumaa et al., 2006).

Nowadays there many researcher were studied about white-rot fungi and their enzyme. The use of fungi and/or their enzymes that metabolize lignocelluloses is a potential biological treatment to improve the nutritional value of straw by selective delignification, as mentioned in the review by Jalc (2002). Barrasa et al. (1995), Fazaeli et al. (2006), Barrasa et al. (1995) were studied Ligninolytic enzyme on wheat straw from *Phanerrochaete chrysosporium*, *Pleurotus* fungi, and *Trichoderma versicolor* respectively. Moreover, Rodrigues et al. (2008), Eun et al. (2006), Zhu et al. (2005), Giraldo et al. (2007) and Rai and Mudgal, (1996) were studied *Trichoderma spp* on wheat straw and rice straw.

Tuyen et al. (2012) studied 6 types of fungi found a net loss in DM, OM, NDF, ADF, and ADL, and consequently in cellulose and hemicellulose, but a net gain in CP of all substrates. However the fungi caused a loss of nutrients. In general, *P. eryngii*

incubation resulted in the lowest ($p<0.05$) loss of all nutrients in all substrates except for the loss of ADL and HC in the maize stove and the hemicellulose in rice straw. *P. ostreatus*, on the other hand, caused the highest ($P<0.05$) loss of all nutrients in maize stover and rice straw. *L. edodes* incubation resulted in the highest losses of nutrients in oil palm fronds and *C. subvermispora* incubation led to the highest losses of ADL and HC in sugarcane bagasse ($p<0.05$). *C. subvermispora and L. edodes* caused a higher loss of ADL in sugarcane bagasse compared to *P. eryngii and P. ostreatus* ($p<0.01$), but the reverse was observed for maize stover. All fungi caused a high loss of ADL in rice straw (41.1-67.6%) with *P. eryngii* causing the lowest loss ($p< 0.01$), while the other fungi resulted in similar losses.

The high lignin degrading capability of *C. subvermispora, L. edodes, P. eryngii and P. ostreatus* in maize stover, rice straw and sugarcane bagasse observed in the present experiment is generally in agreement with that reported previously (Kim et al., 1998; Okano et al., 2007, 2006; Rahman et al., 2011; Taniguchi et al., 2005; Wan and Li 2010); however, the selectivity for delignification in the substrates varied among the different fungi. *P. ostreatus* incubated with rice straw was more selective for lignin degradation than *C. subvermispora* (Taniguchi et al., 2005). In contrast, the *C. subvermispora* strain in the present study showed higher lignin degradation selectivity than the *P. ostreatus* strain. Using maize stover as substrate, Wan and Li (2010) reported lignin degradation of up to 40% after treatment with *C. subvermispora* for 42 days. Yang et al. (2010), on the other hand, showed no effect of *P. ostreatus* treatment (for 30 days) on the proportion of lignin in maize stover. These findings are different from those in the present study.

A high loss of lignin accompanied by high losses of dry matter and organic matter was observed for the treatment with *P. ostreatus* in all substrates, except for sugarcane bagasse. Similar results have been reported for rice straw (Sherief et al., 2010; Taniguchi et al., 2005) and wheat straw (Bhuvnesh et al., 2011; Tuyen et al., 2012) treated with this fungus. Thus, even though *P. ostreatus* is an effective lignin degrader, it is not a potent fungus to be used for improving the nutritive value of fibrous by-products as feed for ruminants (Jung et al., 1992).

2.10 Basics of urea treatment of straw

The low quality roughages nutritive value of rice straws and stoves can be improved by many methods of treatment. Urea treatment is a method of choice for use at farm in the tropics as it is best adapted to the conditions of smallholder farmers (Chenost, 1995). The main advantages of using urea for crop residue improvement is ease of transport, handling, and do not pose any risk to those handling and using it (Sundstøl and Coxworth, 1984). Moreover, fertilizer grade urea is readily available and relatively cheap compared to either aqueous or anhydrous ammonia. There are two-stage process of urea treatment consisting of ureolysis, where urea is converted to ammonia and the effect of generated ammonia on the cell walls of the roughage being treated (Chenost, 1995). The hydrolysis of urea (ureolysis) proceeds according to the following reaction: NH_2 (CO) NH_2+ H_2O →2NH_3+ CO_2 (Sundstøl and Coxworth, 1984). The important thing to improve the use of crop residues for ruminants is to overcome the barriers to rumen microbial fermentation of lignocelluloses. The two well-known factors of rice straw that limit bacterial digestion in the rumen are its high level of lignification's and low contents of nitrogen, vitamins and minerals. So that, in

principle, there are two approaches, which should be taken in combination, straw delignification treatment and nutrient supplementation.

2.11 Methods of urea treatment

There are a lot of variations in the methods of treatment of poor quality roughages with urea. However, the principal method consists of dissolving urea in water and sprinkling it on layers of straw. The level of urea used varies, but it is commonly between 4%-5% of air dried mass of the straw/stove, and the amount of water used also varies from as low as 0.2 liters per kg of straw to as high as 1 liter per kg of straw (Sundstøl and Coxworth, 1984; Chenost, 1995).

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CHAPTER III

EFFECT OF *PLEUROTUS* SPECIES PRETREATMENT OF LIGNOCELLULOSE ON A REDUCTION IN LIGNIN AND THE BIODEGRADATION OF RICE STUBBLE IN DRY SEASON IN DIFFERENT PERIOD TIME FOR FERMENTED

3.1 Abstract

The aim of this study was to examine the bioconversion of rice stubble fermentation with *Pleurotus ostreatus* (POT), *Pleutus sajor-caju* (PSC) and *Pleurotus eous* (PE). The rice stubbles was inoculated with the fungi and incubated in the dark cupboard in the laboratory at 30°C and 75% relative humidity (RH). The chemical composition and *in vitro* degradability of untreated rice stubble and treated rice stubble with *Pleurotus* species were analyzed at day 20, 25, 30, 35 and 40th inoculation. Results shown that all of fermentation by *Pleurotus* fungi treatments were apparently increased ($p<0.001$) in crud protein (CP) content when compared with the control. Whereas significant decreased in neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), hemicellulose, and cellulose contents of rice stubbles by fungal fermentation. *In vitro* gas production was significantly increased at

day 25th fermentation in all fungal treatments from 24-96 h incubation. The estimated organic matter digestibility (OMD) of *Pleurotus* species fermented at 25 days was improved from 52.02% to 62.12%, 63.75%, and 65.27% (control, PSC, PE and POT) respectively. For the estimated of ME was similarly trend with organic matter digestibility 7.44 MJ/kgDM, 8.95 MJ/kgDM, 9.19 MJ/kgDM and 9.43 MJ/kgDM (control, PSC, PE and POT). It was implied that the period time was effected to fungi fermentation.

Key Words : Rice stubble, *Pleurotus* species, Time fermentation, Chemical composition, Digestibility.

3.2 Introduction

The waste-products from agricultural such as rice straw, maize stover, oil palm fronds, and sugarcane bagasse, are abundantly available in many countries (Methu et al.,2001; Wan Zahari et al., 2003; Sarnklong et al., 2010; Ahmed and Babiker, 2015); however, there are high neutral detergent fiber (NDF) and lignin contents, but low protein contents, cause low digestibility and feeding values for ruminants (Karunanandaa and Varga, 1996; Islam et al., 2000; Wan Zahari et al., 2003; Albores et al., 2006; Malik et al., 2015). Agriculture is very important in the world to feed the growing human population. Therefore, land for crop production will be used more massively for human food production and consequently animal production will trust on feeding the by-products from the food produced for human consumption.

Many crop residues from the human food industry have in common a high biomass, low crude protein content of approximately 3 to 4% and high content of crude

fiber of approximately 35 to 48% (Devendra, 2009). The problems of farmers who raise ruminants in summer are using rice straw replace grass. In currently rice straw was employed as ruminant feed over 87% of the roughage feed (Malik et al., 2015; Peripolli etl al., 2017) as well as rice is harvested with a mechanical harvester affected to the rice straw product consists of rice stubble (rice stubble is a part of rice production system and we are not using it), which high of lignin compound. Rice stubble compound high of lignin binds cellulose and hemicellulose consistency than rice straw. Therefore, using white-rot fungi to break lignin with cellulose or hemicellulose bonds is an alternative method. This is likely to increase nutrients digestibility and may be a combination of several methods to achieve maximum utilization of nutrients.

Fungi in white-rot fungi group produce enzymes which contain lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase these microbes are well known (Bugg et al., 2011). Usual in plants, lignin, cellulose and hemicellulose are compound together as lignocellulose. In general plants consist cellulose 30-45%, lignin 13%, but in hardwood cellulose 45-56%, lignin 18-30% (Chen, 2014). The gold of this experiment was studied species of *Pleurotus* fungi to degrade lignocellulosic materials, to improve the utilization of rice stubble as feed.

3.3 Objectives

The objective of the present study was determined the period of each type fungi digestible of rice stubble and studied the chemical composition and nutrients digestibility of rice stubble *in vitro* gas production.

3.4 Materials and methods

3.4.1 Fungal species and spawn preparation

In this experiment was used three sub experiments depend on types of *Pleurotus species* such as, Experiment 3.1= *Pleurotus ostreatus*, Experiment 3.2 = *Pleurotus sajor-caju*, and Experiment 3.3 = *Pleurotus eous*. The levels of fungal were 0 and 2% of substrate according to (Survase, 2012), and period time at day 20, 25, 30, 35, and 40th after inoculation.

3.4.2 Preparation of substrate and method of cultivation

Rice stubble was collected after harvesting of the grains in Thailand (Nakhon Ratchasima). The feedstuff was chopped by chopper machine into pieces of 2-5 cm length and water was added to approximately three times the weight of the stubble and left overnight for the water to penetrate into the inner structures of the stubble and allow steam to effectively destroy the contaminated fungal spores. The stubble was weighed into plastic bag containers 200 g of rice stubble each and autoclaved, again at 121ºC for 1 h, after the first autoclaving. The autoclaved containers were cooled in an aseptic room at 20ºC and the substrate was inoculated aseptically with 5 g of previously prepared spawn. The containers with inoculated straw were incubated in triplicate along with the control (autoclaved but un-inoculated straw) at 25-30 ºC for 0, 20, 25, 30, 35 and 40 days in the air-conditioned chamber.

3.4.3 Chemical analysis

Samples (control and fermented stubble) were dried immediately in an air-forced oven at 70ºC to constant weight to determine the dry matter (DM) content

before being ground over a 1 mm screen using a Wiley hammer mill. Ash content was determined by combustion at 550ºC for 3 h in a muffle furnace. Ash-free neutral detergent fiber (NDF) was analyzed by a modified method of (Van Soest et al., 1991) with addition of a heat stable amylase, and ash-free acid detergent fiber (ADF) and acid detergent lignin (ADL) were analyzed by the method of (Goering and Van Soest, 1970). The content of hemicellulose was calculated as the difference between NDF and ADF and cellulose as the difference between ADF and ADL. Nitrogen content was measured by (AOAC, 1995) and the crude protein (CP) content was calculated as N - 6.25. The loss of DM and other nutrients due to the incubation with fungi were calculated from the difference between the control and the fermented containers and expressed as a percentage of the total nutrient in the control.

3.4.4 Animals

Four fistulated crossbred goats (about 25 kg weighs) were used for rumen application in *in vitro* gas technique. The animals feeding twice daily with a diet containing rice stubble (60%) and concentrate (40%) and using factorial in Completely Randomized Design (CRD).

3.4.5 *In vitro gas* technique

3.4.5.1 Reagents preparation (Menke and Steingass, 1988)

- **Buffer solution**

- Ammonium bicarbonate (NH_4HCO_3) 4 g

- Sodium bicarbonate ($NaHCO_3$) 35 g

- Dissolve in water and bring up to 1 L in volumetric flask.

- Increase volume of buffer solution as required.

➢ **Macro-mineral solution**

- Sodium hydrogen phosphate, dibasic (Na_2HPO_4) 5.7 g
- Potassium phosphate, monobasic (KH_2PO_4) 6.0 g
- Magnesium sulfate, heptahydrate ($MgSO_4 \cdot 7H_2O$) 0.6 g
- Dissolve in water and bring up to 1 L in volumetric flask.
- Increase volume of buffer solution as required.

NOTE: Buffer and Macromineral solution can be stored refrigerated for up to 3 months and at room temperature for up to 1 month.

➢ **Micro-mineral solution**

- Calcium chloride, dehydrate ($CaCl_2 \cdot 2H_2O$) 13.2 g
- Manganese chloride, tetrahydrate ($MnCl_2 \cdot 4H_2O$) 10.0 g
- Cobalt chloride, hexahydrate ($CoCl_2 \cdot 6H_2O$) 1.0 g
- Ferric chloride, hexahydrate ($FeCl_2 \cdot 6H_2O$) 8.0 g
- Dissolve in water and bring up to 100 mL in volumetric flask.

NOTE: Micro-mineral solution can be stored refrigerated for up to 12 months.

➢ **0.1% (wt/vol) Resazurin**

- Dissolve 0.1 g of resazurin 100 mL water.
- Store in dark (amber coloured) bottle at 4°C (infridge).

3.4.5.2 Substrate preparation

Substrates were dry at 55°C until dry (~48 h) and ground with mill through 1 mm screen after that weigh 0.5 g of substrate into each syringe.

3.4.5.3 Medium preparation

**This recipe is for 1 L, increase volume as required

-Weigh out 2.5 g tryptone and dissolve completely in 500 mL water

-Add 0.125 mL micromineral solution

-Add 250 mL buffer solution and 250 mL macromineral solution

-Add 1.25 mL 0.1% resazurin solution

Place container with medium in water bath (39°C) and flushed with CO_2 through solution for 45 minutes. Put in 0.313 g L-cysteine hydrochloride and 0.313 g sodium sulphide and add directly to medium and flushed with CO_2 through solution for another 15 minutes or until solution turns grey to clear. A purple/pink color indicates the presence of oxygen. Keeping the medium in water bath and headspace saturated with CO_2 until medium+inoculums, then transfer to incubation syringe. At this point rumen fluid can be collected.

3.4.6 Source of rumen fluid for *in vitro* incubations

Inoculum for the batch culture was obtained from four ruminally fistulated meat fed a diet consisting of 60% rice stubble and 40% concentrate. Rumen fluid was collected from different sites within the rumen approximately 2 h after the morning feeding, strained through 4 layers of cheesecloth into a flask and flushed with oxygen-free CO_2. Rumen fluid was transported in insulated flasks to the laboratory within less than 1 h of collection. Added rumen fluid to medium in a ratio of 1:4 (rumen fluid:medium). Anaerobic buffer medium 20 mL, (Goering and Van Soest, 1970) containing tryptone, buffer, macro and micro mineral solution, resazurin, and water. Forty-five milliliters of rewarmed media and 5 mL of inoculum were added

anaerobically to the 100 mL syringes by flushed with oxygen free CO_2, after that incubated at 39°C for 72 h. The incubation was repeated with two runs. Blanks (rumen fluid plus anaerobic buffer medium) were also incubated using 4 replications for correction of gas production and disappearance, respectively.

3.4.7 Sample collection and processing

At pre-determined time points, headspace gas production (GP) were measured at 2, 4, 6, 9, 12, 16, 24, 36, 48, 60, 72 and 96 h post incubation, using *in vitro* gas production of (Ørskov and McDonald, 1979). Pressure values, corrected by the amount of substrate OM incubated and the gas released from negative controls, were used to generate volume using the equation of Mauricio et al. (1999) as:

Gas volume = 0.18 + (3.697 × gas pressure) + (0.0824 × gas pressure^2)

The kinetic parameters of GP were calculated using the equation of France et al. (2000) as:

$$A = b \times (1 - e^{-c(t-L)})$$

Where A is the volume of GP at time *t*; b is the asymptotic GP (mL/g DM); *c* is the rate of GP (/h), and *L* (h) is the discrete lag time prior to gas produced.

3.4.8 Statistical Analysis

All data obtained from the experiment were statistically subjected to curve fit program and significant differences between treatments were determined using Duncan's News Multiple Range Test (DMRT) (Steel and Torrie, 1980) and orthogonal contrast was used for trend analysis.

3.4.9 Experimental site

The experiment was conducted at Suranaree University of Technology, Nakhon Ratchasima, Thailand.

3.4.10 Duration

The duration of the present experiment was from January to June 2015.

3.5 Results and Discussions

3.5.1 Experiment 3.1

Chemical composition of untreated and treated rice stubble with *Pleurotus ostreatus* (POT) was shown in Table 3.1. The Crude Protein (CP) and ash contents of the fungal treated substrates increased from day 20th incubation when compared with control. POT Fungal treatment reduced DM, OM, EE, ADF, NDF, and cellulose ($p<0.05$) at day 20th incubation, but DM and EE were increased after day 25th fermentation were not affected by fungi treated. ADL and hemicellulose were significantly decreased ($p<0.01$) at day 25th by POT fungi incubation. These results similar with Jafari et al. (2007) studied *P. ostreatus* treated rice straw found the constituents of OM, hemicellulose, ADF, NDF, and ADL were apparently decreased (($p<0.05$) when compared with untreated fungi. This result can explain that fungi was used fiber for growing so if we ferment long time fungi can used more fiber. Khattab et al. (2013) reported *P. ostreatus* treated rice straw was decreased NDF and ADF contents when compared with control. *P. ostreatus* was succeeded to degrade lignocelluloses materials during 30 days of fermentation using solid state fermentation technique indicated by decreased hemicellulose and lignin contents (Baker et al.,

2014). The CP increase may have been an effect of increased fungal biomass (Chen et al, 1995). The increase in CP contents may be due to secretion of certain extracellular proteineous enzymes into the waste during their breakdown and its subsequent metabolism (Akinfemi et al., 2010). It may also be due to the capture of excess nitrogen during fermentation (Sallam et al., 2007). Valmaseda et al. (1991) and Gutierrez et al. (1996) ported that straw fermented by *Pleurotus* fungi reduced the cell wall contents and enhanced the soluble fraction of carbohydrates in the straw that could be as a result of enzymatic degradation.

Gas production from the fermentation of the POT treated rice stubble and untreated rice stubbles were measured at 2, 4, 6, 9, 12, 16, 24, 36, 48, 60, 72 and 96 h using *in vitro* gas production of (Ørskov and McDonald, 1979). Table 3.2 shows the results of gas production characteristics, effective degradability (ED), estimated organic matter digestibility (OMD), and metabolizable energy (ME). The production from quickly soluble fraction (a), gas production from insoluble fraction (b), and gas production rate (c) were significantly different higher ($p<0.01$) at day 25th incubation of fungal treated stubbles compared with the untreated. Moreover, potential gas production (a + b) was highest at day 25th incubated stubble. These higher values of digestibility fractions in POT fermented rice stubble indicated by the significantly higher in gas volumes from 24-96 h ($p<0.01$) at day 25th incubation by fungi treatment. The ED, OMD, and ME values also apparently highest at day 25th by POT fungi fermentation, which could be related to fungi ability according to varies former studied (Mahesh and Mohini, 2013; Nasehi et al., 2014; Yilkal' 2015; Nasehi et al., 2017). The increased of digestibility by POT fermented rice stubbles is could be influenced degradability of fungi. Increased in OMD may due to the breakdown of the CF and ADF contents of the

treated substrates and increase in its CP content (Kinfemi et al., 2009), It could also be due to breakdown of cell wall bonds during the fermentation of the substrates by the fungi (Call and Mineke, 1997; Jennings and Lyke, 1999; Akinfemi, 2012). Akinfemi et al. (2010) suggested that fungal treatment of sorghum stover resulted in improved CP and digestibility which indicated by significantly higher in insoluble fraction (b), OMD, and ME values. Bummel and Becker, (1997) suggested that gas production reflects degradable carbohydrate and the amount of gas produced depends on carbohydrates nature. Therefore, the lower levels of fiber fraction in the treated substrates increased amount of gas production (Akinfemi et al. 2010; Nasehi et al., 2017). Fungi have two types of extracellular enzymatic systems to degrade lignocellulosic materials; the hydrolytic system, which produces hydrolases that are responsible for polysaccharide degradation and a unique oxidative and extracellular ligninolytic system, which degrades lignin and opens phenyl rings (Sánchez, 2009). The *in vitro* gas production method was shown to be a reliable tool in feed evaluation because gas production was well correlated with microbial protein synthesis (Krishnamoorthy et al., 1990). Treated wheat straw with *P. Ostreatus* increased *in vitro* digestibility of OM and DM (Baker et al., 2014).Fig. 3.4 shows trend of *in vitro* gas production of rice stubbles treated and untreated POT fungi.

Table 3.1 Proximate composition and cell wall contents (%DM) of *Pleurotus ostreatus* treated rice stubbles at 20, 25, 30, 35 and 40 days compared to untreated.

Treatments	DM	Ash	OM	CP	EE	NDF	ADF	ADL	Hemicellulose	Cellulose
Control	23.90^{ab}	15.12^{d}	84.87^{a}	2.49^{c}	0.90^{a}	77.94^{a}	58.03^{a}	4.90^{a}	19.90^{a}	53.13^{a}
POT 20D	22.85^{bc}	18.79^{bc}	81.20^{bc}	3.50^{b}	0.51^{b}	69.63^{b}	51.26b	4.26^{ab}	18.36^{ab}	47.00^{b}
POT 25D	25.02^{a}	16.44^{cd}	83.55^{ab}	3.33^{b}	0.94^{a}	66.92^{c}	51.51^{b}	3.97^{bc}	15.41^{b}	47.53^{b}
POT 30D	23.12^{bc}	22.33^{a}	77.66^{d}	3.43^{b}	1.04^{a}	64.65^{c}	47.36^{b}	3.23^{d}	17.29^{ab}	44.12^{b}
POT 35D	22.82^{bc}	19.77^{ab}	80.22^{cd}	4.71^{a}	0.90^{a}	61.70^{d}	47.49^{b}	3.53^{cd}	14.21^{b}	43.95^{b}
POT 40D	22.06^{c}	20.59^{ab}	79.40^{cd}	3.72^{b}	1.02^{a}	61.29^{d}	46.98^{b}	2.23^{e}	14.31b	44.74^{b}
P-value	0.021	0.008	0.008	0.004	0.091	0.0001	0.005	0.001	0.066	0.012
SEM	0.244	0.257	0.257	0.264	0.211	0.284	0.261	0.273	0.220	0.251

$^{a, b, c,}$ Means within a column means with different superscripts differ significantly at ($p<0.05$). DM= dry matter, OM= organic matter, CP= crude protein, EE= ether extract, NDF= neutral detergent fiber, ADF= acid detergent fiber, ADL= acid detergent lignin.

Table 3.2 *In vitro* gas production characteristics, estimated organic matter digestibility (%OMD), and metabolizable energy (ME) (MJ/kgDM) treated by *Pleurotus ostreatus* (POT) fungal and untreated rice stubbles.

Treatments	a	b	c	a + b	GV24	GV 48	GV 72	GV 96	ED	OMD	ME
Control	2.29^{c}	62.18^{b}	0.036^{b}	64.48^{c}	38.47^{c}	45.92^{c}	51.90^{c}	54.51^{c}	32.24^{c}	52.02^{c}	7.44^{c}
POT 20D	7.56^{ab}	61.61^{b}	0.028^{c}	69.17^{c}	37.79^{c}	45.64^{c}	53.18^{c}	57.10^{c}	34.59^{c}	51.58^{c}	7.36^{c}
POT 25D	9.29^{a}	71.05^{a}	0.039^{a}	80.34^{a}	53.02^{a}	62.30^{a}	68.47^{a}	70.88^{a}	40.17^{a}	65.27^{a}	9.43^{a}
POT 30D	9.27^{a}	57.02^{c}	0.030^{c}	66.29^{c}	39.16^{c}	45.86^{c}	52.32^{c}	55.47^{c}	33.15^{c}	52.97^{c}	7.54^{c}
POT 35D	6.07^{b}	67.67^{a}	0.038^{a}	73.75^{b}	46.58^{b}	55.31^{b}	61.54^{b}	64.09^{b}	36.88^{b}	59.64^{b}	8.56^{b}
POT 40D	7.50^{ab}	57.89^{bc}	0.032^{c}	65.40^{c}	38.78^{c}	45.63^{c}	51.94^{c}	54.91^{c}	32.70^{c}	52.57^{c}	7.49^{c}
P-value	0.0005	0.0001	0.0003	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
SEM	0.20	0.21	0.20	0.21	0.23	0.22	0.22	0.22	0.21	0.23	0.23

$^{a, b, c,}$ Means within a column means with different superscripts differ significantly at ($p<0.05$). SEM= standard error of mean. GV = gas volume (mL); ED = Effective Degradability (%), OMD = organic matter digestibility (%), ME = metabolizable energy (MJ/kgDM).

3.5.2 Experiment 3.2

Chemical composition of untreated and PSC treated rice stubble was shown in table 3.2. Fungal treatment decreased OM content was significantly reduced ($p<0.01$) at day 30^{th} incubation and proceed decreased value as long as fermented fungi, while ash content was increased ($p<0.01$) at day 30th incubation. DM, CP, and EE contents were increased number by fungi fermentation but not significant difference. ADF, NDF, hemicellulose, and cellulose were significantly reduced by PSC fungi fermentation ($p<0.01$) at day 20^{th} incubation. ADL content was decreased ($p<0.05$) by fungi activity at day 35^{th} fermentation. These results in accordance with previous studies (Fazaeli et al., 2004; Jafari et al., 2007). In generally fungus acquires requirements from decaying of OM, especially, the lignocellulolytic components. This finding could explain the changes resulted from *Pleurotus* cultivation on rice stubble. *P. sajor-caju* is belongings to the basidomycetes which enzymes production such as lignin peroxidase, manganese peroxidase, H_2O_2 producer enzymes, arylachol oxidase and laccase (Sánchez, 2009). The fungi need carbon and energy from lignin so they require substrates like cellulose or other carbon sources for their growth and delignification (Ruggeri and Sassi, 2003). Jafari et al. (2007) reported that *P. sajor-caju* high ability to degraded cell wall constitutions that indicated by reduction the contents of ADF, NDF, ADL, and hemicellulose in rice straw fermented with *P. sajor-caju* fungi. *Pleurotus* selectively removes lignin without the loss of appreciable amounts of cellulose, and has been found extremely attractive for use in biological processes, thereby improving the digestibility of highly lignified plant residues, and are useful in the bioconversion of lignocellulosics into their products (Dhanda et al., 2005).

Table 3.3 Proximate composition and cell wall contents (%DM) of rice stubbles fermented with *Pleurotus sajor-caju* fungi at 20, 25, 30, 35 and 40 days compared to untreated.

Treatments	DM	Ash	OM	CP	EE	NDF	ADF	ADL	Hemicellulose	Cellulose
Control	23.90	15.12^{c}	84.87^{a}	2.50	0.90	77.94^{a}	58.03^{a}	4.90^{a}	19.90^{a}	53.13^{a}
PSC 20D	23.95	17.04^{bc}	82.95^{ab}	2.72	1.05	66.81^{b}	50.99^{b}	4.61^{ab}	15.81^{b}	46.37^{b}
PSC 25D	24.70	16.35^{bc}	83.64^{ab}	3.24	1.05	64.37^{b}	49.81^{b}	3.88^{ab}	14.56^{b}	45.92^{b}
PSC 30D	26.09	17.79^{b}	82.20^{b}	3.48	1.08	64.13^{b}	50.43^{b}	3.56^{abc}	13.70^{b}	46.86^{b}
PSC 35D	24.99	17.08^{bc}	82.91^{ab}	3.42	0.90	65.57^{b}	49.63^{b}	2.85^{c}	15.93^{b}	46.77^{b}
PSC 40D	21.77	21.21^{a}	78.78^{c}	2.97	0.95	57.65^{c}	42.48^{c}	3.29^{c}	15.16^{b}	39.19^{c}
P-value	0.0849	0.0093	0.0093	0.1694	0.4854	0.0002	0.0001	0.0215	0.0165	0.0001
SEM	0.213	0.255	0.255	0.190	0.132	0.280	0.287	0.243	0.247	0.283

$^{a, b, c,}$ Means within a column means with different superscripts differ significantly at ($p<0.05$), PSC = *Pleurotus sajor-caju*, DM= dry matter, OM= organic matter, CP= crude protein, EE= ether extract, NDF= neutral detergent fiber, ADF= acid detergent fiber, ADL= acid detergent lignin.

Gas production was shown in table 3.4. The production from quickly soluble fraction (a) was significantly different higher ($p<0.01$) at day 20th incubation in PSC fermented stubbles compared with the untreated, insoluble fraction (b) was significantly increased ($p<0.01$) at day 35th fermentation, gas production rate (c) fraction was not affected by PSC fungi but had effected by incubated duration. Gas production at 24, 48, 72, and 96 h incubation were apparently higher ($p<0.01$) at day 35th fermentation. ED, OMD, and ME of rice stubbles treated fungi also higher at day 35th fermentation. Present study in agreement with formers reported that fungi treated crop residues improve *in vitro* digestibility (Dhanda et al., 2005; Jafari et al., 2007). Wheat straw treated with *Pleurotus* fungi increased in vitro organic matter and dried matter digestibilities (Fazaeli et al., 2004). Kaur et al. (2012) demonstrated that *in vitro* gas production in spent straws of the *Pleurotus* species was highest ($p<0.01$) in net gas production including a, b, and c fractions. Rice husks fermentation by *Pleurotus sajor-caju* improved *in vitro* digestibility and crude protein (CP) content (Vadiveloo et al., 2009). White rot fungi produce several types of extracellular oxidative enzymes to break down lignin content in a plant cell wall. These include laccases and high redox potential ligninolytic peroxidases enzymes; the onset of their production is associated with secondary metabolism conditions in response to nutrient depletion, ligninolytic peroxidases degrades non-phenolic lignin units, whereas manganese peroxidase generates Mn^{3+}, which acts as a diffusible oxidizer on phenolic or non-phenolic lignin units via lipid peroxidation reactions; a third type of ligninolytic peroxidase that combines the catalytic properties of ligninolytic peroxidases, manganese peroxidase and plant/microbial peroxidases (Bugg et al., 2011). Fig. 3.5 shows *in vitro* gas production of PSC fungi fermented rice stubbles.

Table 3.4 *In vitro* gas production characteristics, estimated organic matter digestibility (%OMD), and metabolizable energy (ME) (MJ/kgDM) of rice stubbles fermentation by *Pleurotus sajor-caju* (PSC) fungal and untreated rice stubbles.

Treatments	a	b	c	a + b	GV24	GV 48	GV 72	GV 96	ED	OMD	ME
Control	2.29^{c}	62.18^{cd}	0.036^{ab}	64.48^{c}	38.47^{c}	45.92^{c}	51.90^{c}	54.51^{c}	32.24^{c}	52.02^{d}	7.44^{d}
PSC 20D	9.65^{a}	54.90^{e}	0.030^{b}	64.56^{c}	38.44^{c}	44.46^{c}	50.63^{c}	53.62^{c}	32.28^{c}	52.08^{d}	7.44^{d}
PSC 25D	8.12^{a}	65.73^{bc}	0.041^{a}	73.85^{b}	49.54^{b}	57.32^{b}	62.68^{b}	64.71^{b}	36.93^{b}	62.12^{b}	8.95^{b}
PSC 30D	8.33^{a}	57.95^{de}	0.030^{b}	66.29^{c}	38.15^{c}	45.08^{c}	51.80^{c}	55.14^{c}	33.14^{c}	51.89^{d}	7.40^{d}
PSC 35D	5.47^{b}	77.51^{a}	0.042^{a}	82.98^{a}	54.98^{a}	65.34^{b}	71.50^{a}	73.76^{a}	41.49^{a}	67.07^{a}	9.69^{a}
PSC 40D	5.49^{b}	68.02^{b}	0.036^{a}	73.51^{b}	45.10^{c}	54.11^{b}	60.72^{b}	63.53^{b}	36.76^{b}	58.28^{c}	8.35^{c}
P-value	0.0002	0.0001	0.0074	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
SEM	0.203	0.220	0.171	0.205	0.227	0.225	0.220	0.215	0.205	0.227	0.227

$^{a, b, c,}$ Means within a column means with different superscripts differ significantly at ($p<0.05$). SEM= standard error of mean. GV = gas volume (mL); ED = Effective Degradability (%), OMD = organic matter digestibility (%), ME = metabolizable energy (MJ/kgDM.

3.5.3 Experiment 3.3

When cultivated rice residue was inoculated with the PE fungi a decrease ($p<0.05$) in OM concentration (Table 3.5); this effect was related a greater mineralization of the organic matter, indicated by the increase ($p<0.05$) in ash concentration and greater biomass value, implied by the increase ($p<0.01$) in CP content. DM and EE contents were not affected by PE fungi fermentation ($p>0.05$). These results supported earlier studies (Mahesh and Mohini, 2013; Yilkal, 2015). Fungal treated straw contained higher CP, EE and ash contents and lower OM, CF, NFE, NDF, ADF, ADL, hemicellulose and cellulose contents than untreated straw (Yilkal, 2015). Treatment of straw incubation by fungi significant increases CP concentration (Jonathan et al., 2012; El-Rahman et al., 2014). The high CP content by fungi fermentation could be due to the production of various enzymes during the vegetative and reproductive phases with lignocellulose degrading properties then increased its cell biomass. Increasing ash content is indicated that fungi are capable of degrading fibrous fraction which caused of low cell wall contents (Sarnklong et al., 2010; Mahesh and Mohini, 2013).

The cell wall components of experimental treatments are presented in Table 3.5 The fraction of NDF, ADF and cellulose were significantly decreased upon fungal treatment ($p<0.01$) at day 20th of incubation and constant concentration until the end of testing except NDF fraction precede reduced amount by fungi fermentation. The quantity of ADL content was apparently reduced ($p<0.01$) on fungi treatment at day 25th incubation; continue decreased number along with the time fungi fermentation. Hemicellulose component was significantly decreased by fungi inoculate (($p<0.01$) at day 30th incubation and proceed reduced until the testing last. Cell wall contents were

apparently decreased by fungi fermentation according with previous studied (Mahesh and Mohini, 2013; Lynch et al., 2014; Shrivastava et al., 2014; Yilkal, 2015). The used fungal treated with rice straw which contained lesser fractions of NDF, ADF, hemicellulose, and cellulose than untreated straw (Samsudin et al., 2013). Cultivation of *Pleurotus eous* on paddy straw was higher yield than other treatments (Senthilraja, 2014). The losses of fibrous contents from the crop resedues suggested that the ability of fungi to solubilize and utilize the cell walls as carbon sources and thus changed the ratio of insoluble to soluble carbohydrates in the straw (Shrivastava et al., 2014). The change of ADL content by *Pleurotus* species fermentation of rice stubbles are shown in Figure 3.1, alteration of hemicellulose content illustrated in figure 3.2, fluctuation of cellulose in rice stubbles inoculated fugal are demonstrated in figure 3.3 These results indicated that all of fungal treatments were decreased cell wall concentration when compared with untreated treatment similar results with former studied (Akinfemi and Ogunwole, 2012; Mahesh and Mohini, 2013; Yilkal, 2015; Nasehi et al., 2017).

Gas production of the PE fungi treated and untreated rice stubbles shown in Table 3.6. Gas volumes at 24, 48, 72, 96 h incubation were significantly increased ($p<0.01$) at day 20^{th} by fungi fermentation compared with control. Quickly soluble fraction was increased ($p<0.01$) at day 20^{th} incubation, insoluble fraction apparently increased at day 25^{th} incubation, c fraction no affected by PE fungi fermentation. However, potential gas production was significantly highest ($p<0.01$) at day 30^{th} incubation by fungi. ED was highest at day 30^{th} incubation; OMD and ME values were significantly higher at day 25^{th} incubation by fungi. These results in agreement with previous studied (Mahesh and Mohini, 2013; Yilkal, 2015). The ability of fungi and their enzymes could be degraded cell wall constituents of crop residues due to high

digestibility of fiber content which influent to increased CP concentration (Akinfemi et al., 2010; Bento et al., 2014). Increased b fraction of gas production after treatment by fungi has been reported (Rodrigues et al., 2008; Okano et al., 2009; Nasehi et al., 2017). Improved (a+b) content of gas production indicates that the fermented crop residues were highly available in the rumen. Therefore, increased (a+b) fraction of gas production upon fungi can be related to reduction in cell wall constituents that implies to increased utilization of fibrous fraction (Akinfemi et al. 2010). Successful biological treatment must be based upon the use of organism which degrades lignin. The use of fungi and enzymes that metabolize lignocelluloses is a potential biological treatment to improve the nutritional value of cultivated residues by selective delignification (Malik et al., 2015). Fig. 3.6 shows *in vitro* gas production of rice stubbles fermentation by PE fungi.

Table 3.5 Proximate composition and cell wall contents (% DM) of *Pleurotus eous* treated rice stubbles at 20, 25, 30, 35 and 40 days compared to control

Treatments	DM	Ash	OM	CP	EE	NDF	ADF	ADL	Hemicellulose	Cellulose
Control	23.90	15.12^{c}	84.87^{a}	2.49^{b}	0.90	77.94^{a}	58.03^{a}	4.90^{a}	19.90^{a}	53.13^{a}
PE 20D	24.07	16.34^{bc}	83.65^{ab}	3.23^{a}	1.15	68.43^{b}	50.27^{b}	4.48^{ab}	18.15^{ab}	45.79^{b}
PE 25D	23.96	17.58^{ab}	82.41^{bc}	3.49^{a}	0.93	67.73^{b}	50.25^{b}	3.93^{bc}	17.47^{ab}	46.31^{b}
PE 30D	25.57	17.68^{ab}	82.31^{bc}	3.21^{a}	0.94	64.30^{bc}	50.46^{b}	3.14^{cd}	13.84^{bc}	47.31^{b}
PE 35D	23.99	17.79^{ab}	82.20^{bc}	3.28^{a}	0.99	64.18^{bc}	51.72^{b}	3.81^{bcd}	12.46^{cd}	47.90^{b}
PE 40D	24.99	18.66^{a}	81.33^{c}	3.40^{a}	0.93	59.52^{c}	50.82^{b}	2.95^{d}	8.69^{d}	47.87^{b}
P-value	0.77	0.04	0.04	0.009	0.46	0.001	0.001	0.008	0.006	0.001
SEM	0.084	0.228	0.228	0.255	0.135	0.273	0.272	0.256	0.258	0.271

$^{a, b, c,}$ Means within a column means with different superscripts differ significantly at ($p<0.05$), PE = *Pleurotus eous*, DM= dry matter, OM= organic matter, CP= crude protein, EE= ether extract, NDF= neutral detergent fiber, ADF= acid detergent fiber, ADL= acid detergent lignin.

Table 3.6 *In vitro* gas production characteristics, estimated organic matter digestibility (%OMD), and metabolizable energy (ME) (MJ/kgDM) of treated *Pleurotus eous* (PE) fungal and untreated rice stubbles.

Treatments	a	b	c	a + b	GV24	GV48	GV72	GV 96	ED	OMD	ME
Control	2.29^{b}	62.18^{b}	0.036	64.48^{c}	38.47^{b}	45.92^{b}	51.90^{b}	54.51^{b}	32.24^{c}	52.02^{b}	7.44^{b}
PE 20D	9.79^{a}	50.81^{c}	0.031	60.60^{c}	37.00^{b}	42.04^{b}	47.58^{b}	50.24^{b}	30.30^{c}	50.78^{b}	7.25^{b}
PE 25D	5.57^{b}	69.49^{a}	0.044	75.07^{ab}	51.28^{a}	59.38^{a}	64.44^{a}	66.20^{a}	37.54^{ab}	63.75^{a}	9.19^{a}
PE 30D	6.58^{b}	71.29^{a}	0.037	77.88^{a}	48.73^{a}	58.40^{a}	65.14^{a}	67.96^{a}	38.94^{a}	61.43^{a}	8.84^{a}
PE 35D	9.42^{a}	56.56^{bc}	0.033	65.99^{c}	40.73^{b}	47.17^{b}	53.10^{b}	55.82^{b}	33.00^{c}	54.21^{b}	7.75^{b}
PE 40D	5.79^{b}	62.33^{b}	0.032	68.12^{bc}	39.19^{b}	47.12^{b}	53.99^{b}	57.26^{b}	34.06^{bc}	52.85^{b}	7.55^{b}
P-value	0.0017	0.0001	0.1766	0.0021	0.0001	0.0002	0.0005	0.0009	0.002	0.0001	0.0001
SEM	0.792	0.892	0.462	0.785	0.874	0.860	0.837	0.816	0.786	0.875	0.875

$^{a, b, c,}$ Means within a column means with different superscripts differ significantly at ($p<0.05$). SEM= standard error of mean. GV = gas volume (mL); ED = Effective Degradability (%), OMD = organic matter digestibility (%), ME = metabolizable energy (MJ/kgDM.

3.6 Conclusions

Utilization of agricultural residues treated with white rot fungi as ruminants feed has been demonstrated in numerous studies. This study shown that all of fungal species resulted in a reduction of the cell wall components, whereas increased CP and ash contents in experimental rice stubbles; fungal fermentation enhanced *in vitro* degradation of rice stubbles. The properly fermented period as high nutritive values of fermented rice stubble depend on fungus species. From the results it could be concluded that rice stubbles treated by *Pleurotus* fungi could be successfully used to enrich rice stubbles with protein, improve cell walls degradability and organic matter digestibility. The lost fibrous contents were compensated in increased fungal biomass and their enzymes that enhanced nutritional values of plant residues treated fungal. This implies that the fungi can be included with other feedstuffs and improving the feeding value of low quality fibrous crop residues. The system so proposed for the alternation of lignocellulosics into edible fungi may be promising on conversion of plant residues to higher quality ruminant feed. Further work is needed to study the effect of using untreated and treated rice stubbles by different *Pleurotus* species fungi treatments for observation digestibility of the resultant substrates in goats. Also future studies are needed for more explained in the experiment of *in vivo* determination. From this experiment I found that POT fermented at 25 days, PSC fermented at 35 days, and PE was fermented at 30 days were the best of each type on chemical composition and organic matter digestibility.

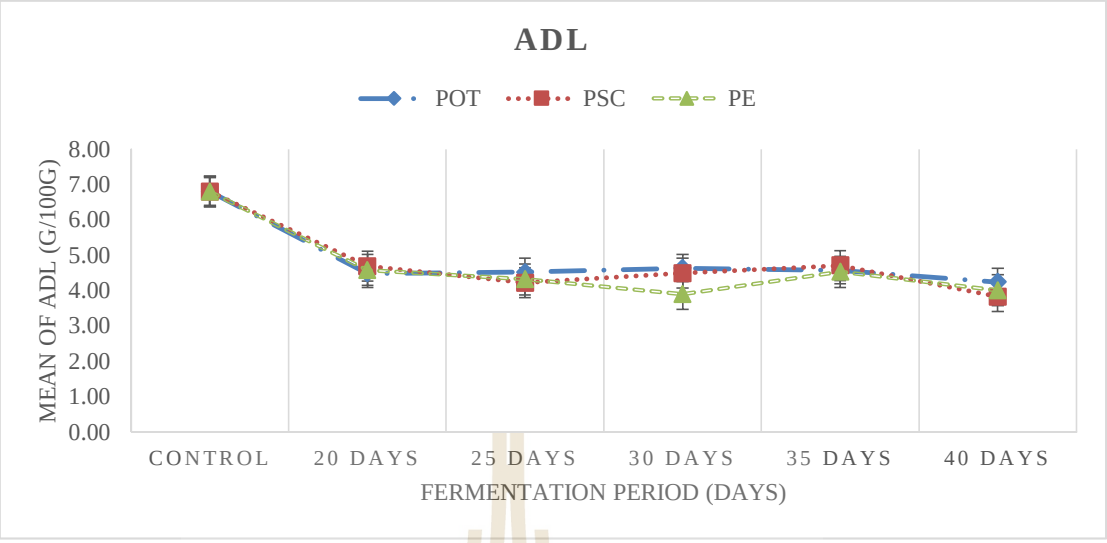

Figure 3.1 Changes in levels of acid detergent lignin (ADL) in rice stubble by*Pleurotus* species fermentation from 20 to 40 days. POT: *Pleurotus ostreatus*, PSC: *Pleurotus sajor-caju*, PE: *Pleurotus eous*.

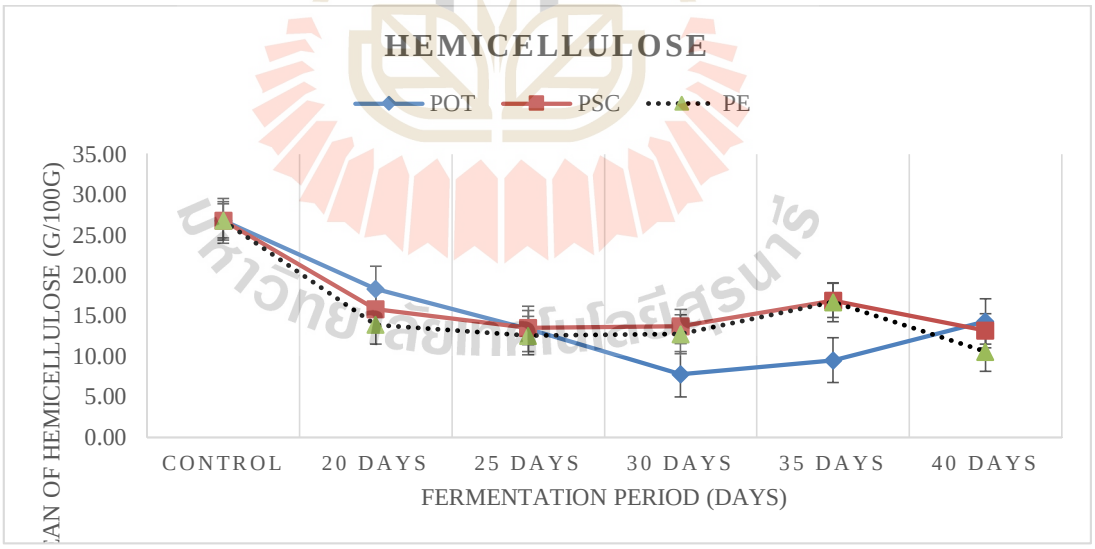

Figure 3.2 Changes in levels of hemicellulose in rice stubble fermented by *Pleurotus* species. POT: *Pleurotus ostreatus*, PSC: *Pleurotus sajor-caju*, PE: *Pleurotus eous*. Duration time to incubation: 20 to 40 days.

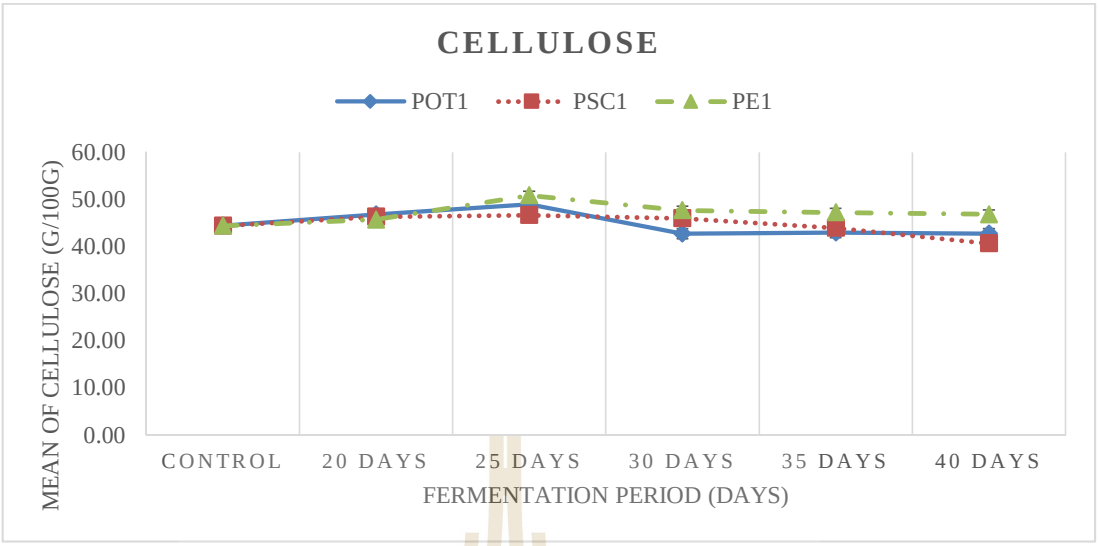

Figure 3.3 Changes in levels of cellulose in rice stubble fermented by *Pleurotus* species. *Pleurotus ostreatus*, PSC: *Pleurotus sajor-caju*, PE: *Pleurotus eous*. Duration time to incubation: 20 to 40 days.

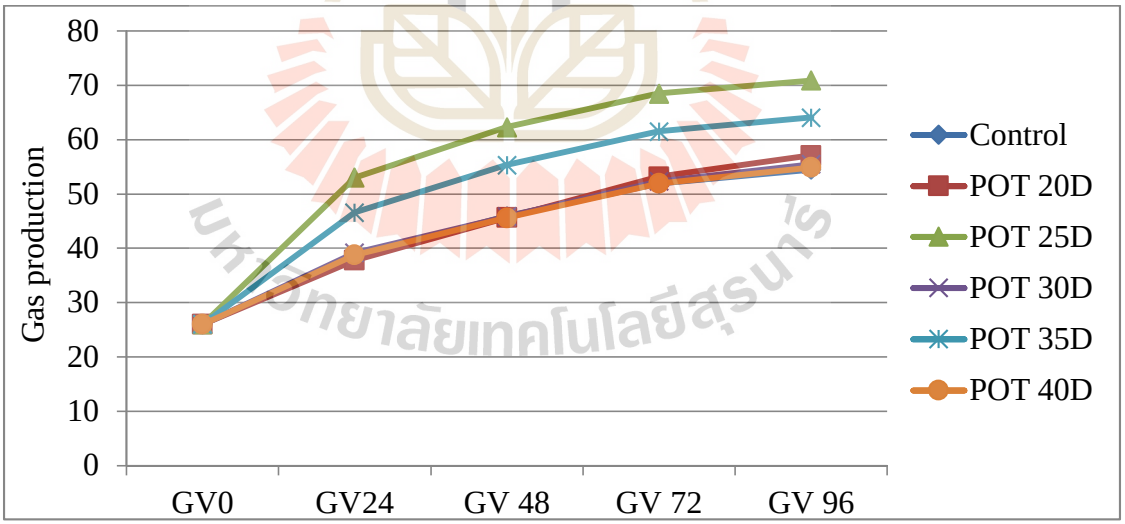

Figure 3.4 *In vitro* gas production of rice stubble treated and untreated by *Pleurotus ostreatus.*

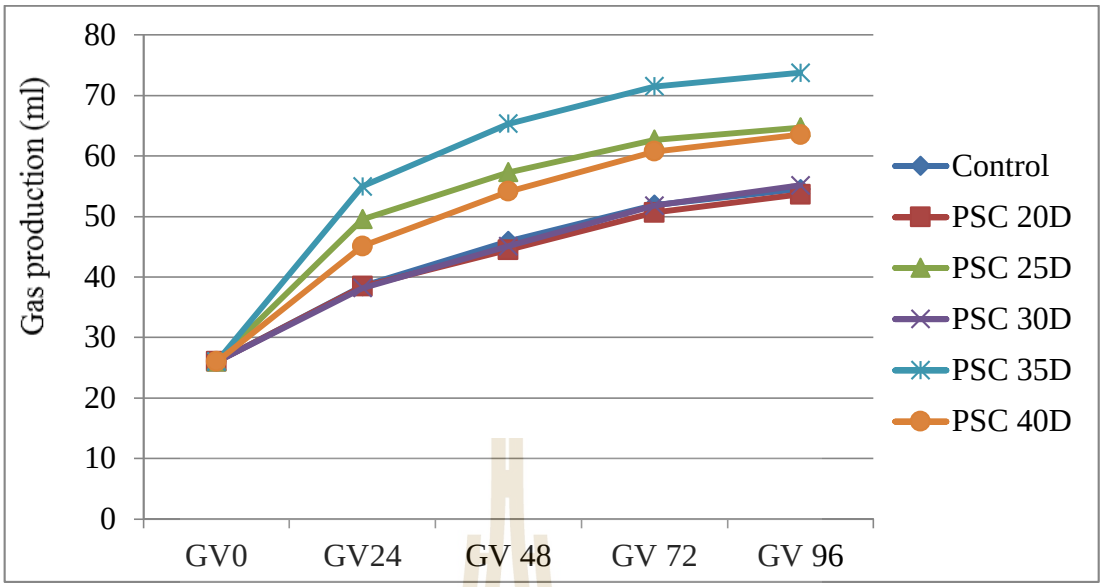

Figure 3.5 *In vitro* gas production of rice stubble fermented by *Pleurotus sajor-caju.*

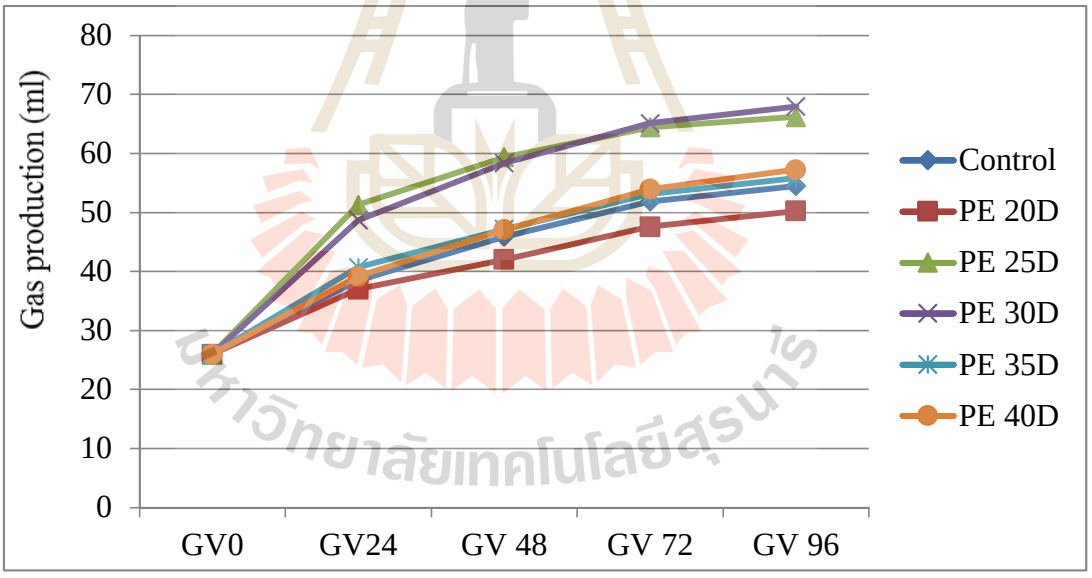

Figure 3.6 *In vitro* gas production of rice stubble fermented by *Pleurotus eous.*

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CHAPTER IV

STUDY ON UREA USE AS INHIBITOR ON WHITE-ROT FUNGI ACTIVITIES AND IMPROVE DIGESTIBILITY OF RICE STUBBLE BY *IN VITRO* GAS PRODUCTION

4.1 Abstract

Studies were conducted to evaluate culturing of the edible mushroom on the chemical composition, cell wall degradation, and *in vitro* digestibility of rice stubble was investigated *Pleurotus ostreatus* (POT), *Pleurotus sajor-caju* (PSC), as well as *Pleurotus eous* (PE) treated with two level of urea and two time for treated urea. Fungi fermentation not influence to the amount of dry matter (DM), whereas ash and crude protein (CP) content significantly increased with processing by fungi in treatments ($p<0.01$). OM, NDF, ADF, ADL, and hemicellulose were apparently decreased in rice stubble fermentation by all of fungal ($p<0.01$). Ether extract (EE) and cellulose content did not differ by processing with fungi. *In vitro* digestibility higher in all of fungal treatments, but seem to be greater in POT fungi treatment. Level of urea indicated that 2.5% more suitable for treat rice stubble and at 7 days seem to be properly treated substrates. Therefore, biological treatment can improve the nutritive value and *in vitro* digestibility of rice stubble for ruminant animals.

Key Words : Rice stubble, Urea, Fungi, Chemical composition, Digestibility.

4.2 Introduction

The large portion of agricultural residues is important feed stuff for ruminants and can be used as a potentially important source of carbohydrates and energy. However, the agricultural residues that use as animals feed are the most plenty of lignocellulosic materials (Shrivastava et al., 2011; Bento et al., 2014). Therefore, the utilization of these materials as a feed source for ruminants is limited for their complex biological structure and low protein content (Rodrigues et al. 2008; Yalchi and Hajieghrari, 2011). There are various methods could increase the nutritive value of cultivated residues through physical and chemical as well as biological processing have been studied (Rahal et al. 1997; Jafari et al., 2007; Mahesh and Mohini, 2013; Polyorach and Wanapat, 2014; Malik et al., 2015; Oladosu et al., 2016). To increasing the nutritive values of crop residues and digestibility of lignocellulosic materials, it is important to break down the linkage between cellulose, hemicellulose, and lignin bond or break down the compact nature of the tissue before animals feeding (Gomaa et al. 2012; Muhammad et al., 2014). Recently, biological delignification of cultivated residues has been considered because of its capacity to remove lignin preferentially ((Moyson and Verachtert, 1991; Akinfemi et al., 2010; Yakin et al., 2016). Nutritive value of crop residues cultured with *Pleurotus* fungi has been reported by previous researchers (Fazaeli et al., 2002; Fazaeli et al., 2004; Jafari et al., 2007; Nasehi et al., 2014; Raghuwanshi et al., 2014).

P. ostreatus, *P. sajor-caju*, and *P. eous* have been studied as an alternative to improve nutritive value of lignocellulosic substrates as ruminant feeds (Jafari et al., 2007; Akinfemi et al., 2010; Singh et al., 2011; Bento et al., 2014; Wiaee-Kwagyan et al., 2016; Nasehi et al., 2017). The aim of this experiment was to study the fungal

activities blocked by urea and *in vitro* dry matter digestibility of rice stubbles were determined during fungal growth with different species of *Pleurotus* fungi and different duration to harvesting.

4.3 Objectives

To study the effect of urea on white-rot fungus activities and urea utilization to block white-rot fungus, improvement quality of rice stubble and enhance digestibility of rice stubble *In vitro* gas production.

4.4 Materials and methods

4.4.1 Fungal species and spawn preparation

In this experiment was used three types of *Pleurotus species* and different time for fermented of each type such as, *Pleurotus ostreatus* fermented at 25 days, *Pleurotus sajor-caju* fermented at 35 days and *Pleurotus eous* fermented at 30 days. The levels of fungal were 0 and 2% of substrate according to (Survase, 2012).

4.4.2 Preparation of substrate and method of cultivation

Rice stubble was collected after harvesting of the grains in Thailand (Nakhon ratchasima). The feedstuff was chopped by chopper machine into pieces of 2-5 cm length and water was added to approximately three times the weight of the stubble and left overnight for the water to penetrate into the inner structures of the stubble and allow steam to effectively destroy the contaminated fungal spores. The stubble was weighed into plastic bag containers 200 g of rice stubble each and autoclaved, again at 121ºC for 1 h, after the first autoclaving. The autoclaved containers were cooled in an

aseptic room at 20ºC and the substrate was inoculated aseptically with 5 g of previously prepared spawn. The containers with inoculated straw were incubated in triplicate along with the control (autoclaved but un-inoculated straw) at 25-30ºC for 25 days for POT, 30 days for PE and 35days for PSC in the air-conditioned chamber.

4.4.3 Chemical analysis

Samples (control and fermented stubble) were dried immediately in an air-forced oven at 70ºC to constant weight to determine the dry matter (DM) content before being ground over a 1 mm screen using a Wiley hammer mill. Ash content was determined by combustion at 550ºC for 3 h in a muffle furnace. Ash-free neutral detergent fiber (NDF) was analyzed by a modified method of Van Soest et al. (1991) with addition of a heat stable amylase, and ash-free acid detergent fiber (ADF) and acid detergent lignin (ADL) were analyzed by the method of Goering and Van Soest (1970). The content of hemicellulose was calculated as the difference between NDF and ADF and cellulose as the difference between ADF and ADL. Nitrogen content was measured by AOAC (2002) and the crude protein (CP) content was calculated as N - 6.25. The loss of DM and other nutrients due to the incubation with fungi were calculated from the difference between the control and the fermented containers and expressed as a percentage of the total nutrient in the control.

4.4.4 Animals

Four fistulated crossbred goats (about 25 kg weighs) were used for rumen application in *In vitro* gas technique. The animals feeding twice daily with a diet

containing rice stubble (60%) and concentrate (40%) and using factorial in Completely Randomized Design (CRD).

4.4.5 *In vitro gas* technique

4.4.5.1 Reagents preparation (Menke and Steingass, 1988**)**

- **Buffer solution**

- Ammonium bicarbonate (NH_4HCO_3) 4 g
- Sodium bicarbonate ($NaHCO_3$) 35 g
- Dissolve in water and bring up to 1 L in volumetric flask.
- Increase volume of buffer solution as required.

- **Macro-mineral solution**

- Sodium hydrogen phosphate, dibasic (Na_2HPO_4) 5.7 g
- Potassium phosphate, monobasic (KH_2PO_4) 6.0 g
- Magnesium sulfate, heptahydrate ($MgSO_4 \cdot 7H_2O$) 0.6 g
- Dissolve in water and bring up to 1 L in volumetric flask.
- Increase volume of buffer solution as required.

NOTE: Buffer and Macromineral solution can be stored refrigerated for up to 3 months and at room temperature for up to 1 month.

- **Micro-mineral solution**

- Calcium chloride, dehydrate ($CaCl_2 \cdot 2H_2O$) 13.2 g
- Manganese chloride, tetrahydrate ($MnCl_2 \cdot 4H_2O$) 10.0 g
- Cobalt chloride, hexahydrate ($CoCl_2 \cdot 6H_2O$) 1.0 g
- Ferric chloride, hexahydrate ($FeCl_2 \cdot 6H_2O$) 8.0 g
- Dissolve in water and bring up to 100 mL in volumetric flask.

NOTE: Micro-mineral solution can be stored refrigerated for up to 12 months.

- **0.1% (wt/vol) Resazurin**

- Dissolve 0.1 g of resazurin 100 mL water.

- Store in dark (amber coloured) bottle at 4°C (infridge).

4.4.5.2 Substrate preparation

Substrates were dry at 55°C until dry (~48 h) and ground with mill through 1 mm screen after that weigh 0.5 g of substrate into each syringe.

4.4.5.3 Medium preparation

**This recipe is for 1 L, increase volume as required

-Weigh out 2.5 g tryptone and dissolve completely in 500 mL water

-Add 0.125 mL micromineral solution

-Add 250 mL buffer solution and 250 mL macromineral solution

-Add 1.25 mL 0.1% resazurin solution

Place container with medium in water bath (39°C) and flushed with CO_2 through solution for 45 minutes. Put in 0.313 g L-cysteine hydrochloride and 0.313 g sodium sulphide and add directly to medium and flushed with CO_2 through solution for another 15 minutes or until solution turns grey to clear. A purple/pink color indicates the presence of oxygen. Keep medium in water bath and headspace saturated with CO_2 until medium+inoculums is going to be transferred to incubation syringe. At this point rumen fluid can be collected.

4.4.6 Source of rumen fluid for *in vitro* incubations

Inoculum for the batch culture was obtained from four ruminally fistulated meat fed a diet consisting of 60% rice stubble and 40% concentrate. Rumen fluid was collected from different sites within the rumen approximately 2 h after the morning feeding, strained through 4 layers of cheesecloth into a flask and flushed with oxygen-free CO_2. Rumen fluid was transported in insulated flasks to the laboratory within less than 1 h of collection. Added rumen fluid to medium in a ratio of 1:4 (rumen fluid:medium), anaerobic buffer medium (20 mL; (Goering and Van Soest, 1970) containing tryptone, buffer, macro and micro mineral solution, resazurin and water. Forty-five milliliters of pre-warmed media and 5 mL of inoculum was added anaerobically to the 100 mL syringes by flushed with oxygen free CO_2, after that incubated at 39^{oC} for 72 h. The incubation was repeated with two runs. Blanks (rumen fluid plus anaerobic buffer medium) were also incubated using 4 replications for correction of gas production and disappearance, respectively.

4.4.7 Sample collection and processing

At pre-determined time points, headspace gas production (GP) were measured at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 24, 36, 48, 60, 72, and 96 h post incubation. Pressure values, corrected by the amount of substrate OM incubated and the gas released from negative controls, were used to generate volume using the equation of Mauricio et al. (1999) as:

$$\text{Gas volume} = 0.18 + (3.697 \times \text{gas pressure}) + (0.0824 \times \text{gas pressure}^2)$$

The kinetic parameters of GP were calculated using the equation of France et al. (2000) as:

$$A = b \times (1- e^{-c(t-L)})$$

Where A is the volume of GP at time *t*; b is the asymptotic GP (mL/g DM); *c* is the rate of GP (/h), and *L* (h) is the discrete lag time prior to gas produced.

4.4.8 Statistical Analysis

The data were analyzed by ANOVA to determine the main effects as factorial in Completely Randomized Design (CRD) design using the PROC GLM (SAS, 1998) and were curve fit program. The differences among treatments were tested for significance by the least significant difference was determined using Duncan's News Multiple Range Test (DMRT) (Steel and Torrie, 1980) and orthogonal contrast was used for trend analysis. Significant differences were based on a probability level <0.05.

4.4.9 Experimental site

The experiment was conducted at Suranaree University of Technology, Nakhon Ratchasima, Thailand.

4.4.10 Duration

The duration of the present experiment was from July to October 2016.

4.5 Results and Discussion

4.5.1 Chemical composition of treatment

The results of chemical composition are presented in Table 4.1 Dry matter (DM) content was not significant different among treatments. Ash content was apparently increased ($p<0.0001$) in all fungal fermented treatments including urea

treated only when compared with control but not affected by level and time of urea treated factors, this result supported previous studies described that spent straw fermented with WRF increased in the ash content (Langar et al., 1982; Mahesh and Mohini, 2013). Organic matter (OM) concentration was significant decreased ($p<0.0001$) by fungal fermentation and treated with urea, no affected by level urea and time of urea treated portion. The decrease in organic matter was directly related with the increase in ash content. These results according to Bento et al. (2014) reported that fermented agro-industrial residues with the white-rot fungi *P. Ostreatus* was decreased ($p<0.05$) OM and increased ash concentration ($p<0.05$) excepted coconut fiber was decreased in ash. Rajarathnam and Bano, (1989) found that straw fermented with white-rot fungi increased content of ash compared to the beginning material similar resulted of Zadražil (1997) described that wheat straw increased ash content after culture and harvesting of *P. ostreatus* mushrooms. Fazaeli and Masoodi, (2006) indicated that spent wheat straw compost from *Agaricus bisporus* mushroom production lower OM and higher ash than the initial wheat straw. Nasehi et al. (2017) suggested that agro by-products fermented with *P. florida* fungi were significantly decreased ($p<0.01$) both of dry matter and organic matter contents. However, the hypothesis of ferment process of cultivated residues with fungal respect to increase in fungal biomass cold increases the concentration of EE and CP in the residues. The unavoidable organic matter losses during biological treatments imply that an increased OM digestibility is needed to compensate for the losses.

The aim of biological method of upgrading lignocellulosics into feed for ruminants is to remove lignin from cellulose and hemicellulose in plant tissue to increase microbial digestibility. The results of this study indicated that the crude protein (CP)

contents of the fungal treated substrates was higher in *P. Ostreatus* and *P. Eous* fermented groups, *P. sajor-caju* also higher than urea treated only and control group, urea treated group was apparent significant with control group. There were significant among treatments ($p<0.0001$), level of urea also affected to the CP concentration ($p<0.0001$) but no affected by time of treated of urea. EE content was not significant different among treatments. These results in agreement with earlier studies described that Ash and CP content increased ($p<0.01$) with processing with fungi in straw (Nasehi et al. 2014; Nasehi et al. 2017). EE and ADL content of treatment did not differed by processing with fungi. The increase of CP content by fermented fungal was observed by various former studies (Streeter et al., 1982; Rajarathnam and Bano, 1989; Kakkar et al., 1990). Agriculture residues fermented by urea were increased in CP content and improved nutritive values and well as expand crop residues utilization have been reported (Wanapat et al., 2009; Gunun and Wanapat, 2012; Polyorach and Wanapat, 2014). The increase in the CP contents can be attributed to the increasing fungal protein during fermentation and extra-cellular enzymes and contained relatively high levels of nitrogen (Ball and Jackson, 1995; Nasehi et al. 2014). The bulk up in the CP concentration may be due to secretion of certain extra cellular enzymes which are proteineous in nature into the waste during their breakdown and its subsequent metabolism (Zadrazil, et al., 1996; Kadiri, 1999; Akinfemi et al., 2010). The increasing of CP content could also be due to the capture of excess nitrogen by fermentation (Sallam et al. 2007). During the growth and development of white rot fungi on lignocellulosics the digestibility of substrate changes, the substrate is decomposed, new substances and fungal biomass are built up (Zadrazil, 1997). The increase in CP content was described that fungal degradation of cellulose and hemicelluloses into monomers

could serve as a carbon source for the fungus to produce biomass and high gas production (Shrivastava et al., 2011). Jafari et al. (2007) illustrated that *Pleurotus spp*. fungal treatment significantly increased the CP content of the rice straw in agreement with Akinfemi (2010) also reported significant increased CP concentration in *P. ostreatus* fermented peanut husk compared to untreated. Increased CP content of fermented substrates was associated with increased fungal biomass (Chen et al., 1995; Mahesh and Mohini, 2013). These results indicated that the fermented substrates are good source of protein for livestock.

4.5.2 Cell-wall component of treatment

Cell wall constitution of rice stubble was decreased in all fungal treatments (Table 4.2). The content of neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), hemicellulos, and cellulose were significantly decreased ($p<0.05$) in all fungal fermented treatments when compared to control and treated urea only. The level of urea and time for urea treated also affected to ADF, hemicellulos as well as cellulose content according to previous studies (Fazaeli and Talebian Masoodi, 2006; Lynch et al., 2014; Raghuwanshi et al., 2014). Nasehi et al. (2014) reported that treating wheat straw with fungi decreased ($p<0.01$) the amount of NDF and ADF in treatments. Jennings and Lysek, (1996) suggested that decreasing of NDF content of wheat and barley straw by fungal treatment might be due to the natural habitats of the white-rote fungi that largely depend on organic carbon for their energy requirement form of structural material such as lignocellulosic. These fungi could solubilize and utilize the cell wall as carbon source and thus chang-

Table 4.1 Effect of level urea, fermented duration, and types of fungal on chemical composition of rice stubble.

Items	Level	Time	DM	Ash	OM	CP	EE
Treatments							
RS			23.62	15.13	84.87	2.50	0.90
USR	2.5	7	23.72	20.69	79.31	4.06	0.90
	2.5	14	22.82	22.41	77.59	5.27	0.94
	5	7	25.79	20.59	79.41	8.95	1.08
	5	14	23.96	23.73	76.27	8.58	0.99
POT	2.5	7	22.85	22.86	77.14	9.51	0.51
	2.5	14	22.07	21.17	78.83	10.46	1.05
	5	7	24.99	21.03	78.97	16.19	0.94
	5	14	25.13	21.13	78.87	18.29	1.02
PSC	2.5	7	24.77	19.76	80.24	9.66	1.05
	2.5	14	23.95	20.86	79.14	10.81	0.93
	5	7	21.78	19.86	80.14	14.26	0.91
	5	14	23.99	20.31	79.69	13.10	0.95
PE	2.5	7	23.09	20.74	79.26	9.81	1.15
	2.5	14	24.71	21.53	78.47	11.36	1.04
	5	7	24.07	21.24	78.76	15.91	0.90
	5	14	24.99	20.83	79.17	14.93	0.93
SEM			0.24	0.20	0.20	0.16	0.03
Comparison							
A			0.72	0.0001	0.0001	0.0001	0.45
B			0.06	0.64	0.64	0.0003	0.74
C			0.87	0.80	0.80	0.05	0.38
Interaction							
AxB			0.01	0.56	0.56	0.0001	0.14
AxC			0.17	0.02	0.02	0.20	0.12
BxC			0.5	0.63	0.63	0.02	0.53
AxBxC			0.37	0.40	0.40	0.06	0.25

[abc] Means in the same row with different superscript differ ($p<0.05$); DM= Dry matter, CP = Crude protein, EE = ether extract, OM = Organic matter; untreated rice stubble (Control), urea treated rice stubble (URS), urea treated rice stubble fermented with *Pleurotus ostreatus* (POT), urea treated rice stubble fermented with *Pleurotus sajor-caju* (PSC), urea treated rice stubble fermented with *Pleurotus eous* (PE), A= factor from roughage source, B= factor from level of urea, C factor from period time for urea fermentation.

ed the ratio of insoluble to soluble carbohydrates in the straw, and consequently, the losses of NDF content from the straw (Fazaeli et al., 2004). Reduction in the fiber fraction of fungal-treated substrate has been reported (Nasehi et al., 2017). The lower amount of fibrous contents could be the result of decreased OM in the fungal fermented processing. Akinfemi et al. (2010) described that the degradation of the cell wall component of the substrates produced by extra cellular enzymes of fungus. Fungi have two kind of extra cellular enzymatic systems: the hydrolytic system which produces hydrolyses that are responsible for polysaccharide degradation and a unique oxidative and extracellular ligninolytic system, which degrades lignin and opens phenyl rings, laccases or lininolytic peroxidases produced by white-rot fungi oxidize the linin polymer have been explained (Sanchez, 2009). White-rot fungi lignocellulolytic microorganisms are able to decompose and metabolize all plant cell constituents (cellulose, hemicellulose, and lignin) by their enzymes (Malik et al., 2015). The decreasing of CF and CF fractions (NDF, ADF and ADL) in the treated sorghum stover may be the result of cellulase enzymes secreted by cellulolytic fungi (Akinfemi et al., 2010). Isikhuemhen and Nerude, (1999) explained that white-rot fungi produce extracellular lignin modifying enzymes, the best characterized of which are laccase, lignin peroxidase and manganese peroxidases. Lignin loss varied in fungus cultured straw, which increased progressively with period of incubation (Tripathi et al., 2008).

Most white-rot fungi during bio-processing of plant material degrade lignin and cellulose simultaneously (Okano et al., 2005). Breaking down a considerable amount of lignin by fungus it’s causable to changes the components of lignin result in improvements in digestibility.

Table 4.2 Effect of level urea, fermented duration, and types of fungal on fibrous contents ($p<0.05$).

Items	Level	Time	NDF	ADF	ADL	Hemi	cellu
Treatments							
RS			77.94	58.04	4.90	19.90	53.14
USR	2.5	7	74.36	59.73	4.07	12.14	55.66
	2.5	14	75.41	63.42	4.34	9.49	59.08
	5	7	77.84	59.45	4.26	18.39	55.19
	5	14	73.19	53.97	4.78	19.22	49.19
POT	2.5	7	61.96	57.08	3.53	4.89	53.55
	2.5	14	62.07	56.33	3.15	5.74	53.18
	5	7	69.35	55.50	3.20	13.85	52.62
	5	14	61.81	49.66	3.10	12.15	46.56
PSC	2.5	7	65.53	58.90	3.41	6.63	55.49
	2.5	14	66.87	54.47	3.20	12.40	51.38
	5	7	60.25	56.77	3.08	3.48	53.68
	5	14	68.60	55.40	3.09	13.20	52.32
PE	2.5	7	66.50	55.69	3.32	10.81	52.37
	2.5	14	63.84	52.91	3.50	10.93	49.41
	5	7	56.51	53.19	3.18	8.29	50.01
	5	14	68.33	54.36	3.17	13.97	51.19
SEM			0.37	0.33	0.04	0.44	0.33
Comparison							
A			0.0001	0.0001	0.0001	0.0001	0.0001
B			0.8900	0.0003	0.09	0.0001	0.001
C			0.1400	0.0030	0.31	0.006	0.002
Interaction							
AxB			0.01	0.01	0.01	0.0001	0.004
AxC			0.00	0.15	0.07	0.001	0.200
BxC			0.13	0.13	0.13	0.100	0.090
AxBxC			0.0001	0.0001	0.27	0.260	0.001

[abc] Means in the same row with different superscript differ ($p<0.05$); NDF = Neutral detergent fiber, ADF = Acid detergent fiber, ADL = Acid detergent lignin, Hemi = Hemicellulos, Cellu = Cellulose; untreated rice stubble (Control), urea treated rice stubble (URS), urea treated rice stubble fermented with *Pleurotus ostreatus* (POT), urea treated rice stubble fermented with *Pleurotus sajor-caju* (PSC), urea treated rice stubble fermented with *Pleurotus eous* (PE), A= factor from roughage source, B= factor from level of urea, C factor from period time for urea fermentation.

4.5.3 *In vitro* gas production

The *in vitro* digestibilities of rice stubble incubated with different types of *Pleurotus spp.* and treated by urea are presented in Table 4.3 The results of gas production characteristics demonstrated that greater content of readily soluble fraction of OM (a) and potential degradability (a+b) fractions of DM digestibility increased ($p<0.01$) with processing by fungi in all types of fungal include URS treatment when compared with control, High level of urea also improved a fraction but no influence to a + b fraction. The b fraction of OM degradability significantly increased with processing by PE fungi in rice stubble ($p<0.01$), but was not apparently different by POT and PSC fungal as well as URS when compared with control including level of urea and time to treated urea. The degradation rate of insoluble fraction (c) was significantly greater by rice stubble treated with urea, but apparently reduced in all of fungal treatments. Lower level of urea and early treated urea were higher volume of c fraction. This study validated earlier report that *in vitro* gas production technique can be used to evaluate the potential value of feedstuffs. *P. ostreatus* can improve the digestibility of several agro-industrial residues of low nutritive value (Bento et al., 2014) in agreement with former studies reported that *Pleurotus* species fungi can increase in vitro digestibility (Zadražil, 1997; Jafari et al., 2007).

The reason for such improvement in the degradability might be due to the breaking down of cell wall bonds during the fermentation of straw with the fungi (Fazaeli et al. 2004; Shrivastava et al., 2011). Biologically treated roughages with ligninolytic fungi have higher digestibility for most of the nutrients both cell walls and cell soluble (Malik et al., 2015). The use fungal treatment of rice stubble not only

Table 4.3 Effect of level urea, fermented duration, and types of fungal on the organic matter degradation parameters of gas production of rice stubble.

Items	Level	Time	a	b	c	a+b
Treatments						
RS			-5.61	91.43	0.04	85.82
USR	2.5	7	-0.63	108.22	0.05	107.59
	2.5	14	-0.21	112.95	0.03	112.75
	5	7	-1.26	112.46	0.04	111.20
	5	14	0.44	82.29	0.03	82.73
POT	2.5	7	1.23	104.92	0.04	106.15
	2.5	14	-3.10	115.08	0.03	111.98
	5	7	2.06	102.82	0.03	104.88
	5	14	7.96	94.12	0.02	102.08
PSC	2.5	7	1.14	104.58	0.03	105.72
	2.5	14	-3.73	114.21	0.04	110.48
	5	7	7.06	88.41	0.02	95.46
	5	14	2.29	98.53	0.03	100.82
PE	2.5	7	0.71	112.67	0.03	113.38
	2.5	14	0.77	107.44	0.03	108.20
	5	7	9.11	84.12	0.02	93.23
	5	14	7.67	152.82	0.01	160.49
SEM			0.71	2.22	0.00	2.29
Comparison						
A			0.0001	0.00	0.0001	0.0001
B			0.0001	0.01	0.0002	0.34
C			0.37	0.02	0.0004	0.05
Interaction						
AxB			0.01	0.02	0.17	0.01
AxC			0.16	0.0001	0.01	0.0002
BxC			0.22	0.42	0.85	0.25
AxBxC			0.18	0.0001	0.30	0.00

[abc] Means in the same row with different superscript differ ($p<0.05$); a: the volume of gas production from soluble fraction (ml/gDM), b: the volume of gas production from insoluble but potentially degradable fraction (ml/g DM), a + b: potential degradability, c: rate of degradation of fraction b (/h) from the fraction b; untreated rice stubble (Control), urea treated rice stubble (URS), urea treated rice stubble fermented with *Pleurotus ostreatus* (POT), urea treated rice stubble fermented with *Pleurotus sajor-caju* (PSC), urea treated rice stubble fermented with *Pleurotus eous* (PE).

improved the CP contents but also enhanced digestibility; fungal treated crop residues have a good potential as feed resources for ruminant animals and could be used in combination with other feedstuffs.

Gas volume after 96 h incubation upon fungal treatments and urea treated rice stubble was increased ($p<0.01$) when compared with control (Table 4.3). Gas production from the fermentation of the treated and untreated rice stubbles were measured at 96 h shown greater volumes in all of fungal and URS treatment excepted PE fungi was not significantly different with control group at 24 and 48 h. Using 2.5% of urea was higher gas volume than 5% of urea addition and time to treated urea suitable at 7 days ($p<0.05$). *In vitro* gas production tests are routinely used in feed research as gas volumes are related to both the extent and rate of substrate degradation (Blümmel et al., 1997). This result in accordance with Nasehi et al. (2017) suggested that after 96 h incubation upon fungal treatment, gas produced from all crop residues was increased ($p<0.01$). Although several species of higher fungi possess ligninolytic activity, *Pleurotus* species is the most studied fungi since they improved the digestibility and nutritional quality of straws (Streeter et al., 1982; Kakkar et al., 1990; Mahesh and Mohini, 2013). The degradation of lignin, a complex polymer is important because using lignin degrading fungi is to make as much as possible the digestibility of the substrates degraded (Kuforiji and Fasidi, 2004; Adenipekun and Fasidi, 2005). There are many factors influencing the amount of gas produced during fermentation, such as the nature or level of fiber and potency of the rumen liquor used for incubation (Babayemi 2007). High nutritional value straw was greater than for the low-nutritional value straw and at the end of the 96 h of incubation (Peripolli et al., 2017). Fibrous constituents affected *in vitro* gas

production negatively; therefore, the lower levels of fiber fraction in the treated substrates increased amount of gas production (Akinfemi et al. 2010).

The results of the efficiency digestibility (ED) showed higher values estimated in all the fungi cultured substrates comprehend urea treated stubble when compared with control group and highest in PE treatment ($p=0.0001$), there were no significantly different among treatments by the level of urea and treated duration. The value of organic matter digestibility (OMD) was higher in POT and PSC fungal treatments ($p<0.01$) but there were not apparently significant among treatments of PE fungi, URS, and control groups, not affected by duration of treated urea but level of urea influence to OMD. The metabolizable energy (ME) was higher in URS and all of fermented fungal ($p=0.0001$) excepted PE fungi was not significantly different when compared with control, ME value also affected by the level of urea and times. Rice straw treated with urea increased ED have been informed in varieties former studies (Fadel Elseed, et al., 2003; Sarnklong et al., 2010; Malik et al., 2015; Foiklang et al., 2016). Nasehi et al. (2014) illustrated that straws cultured with *Pleurotus florida* increased the degradability parameters of the DM in both of wheat and barley Straw. The high volume obtained for OMD in this study in accordance with previous studies (Jafari et al., 2007; Akinfemi et al., 2010; Mahesh and Mohini, 2013) indicated that the microbes in the rumen and animal have high nutrient uptake. ME was significantly increase ($p<0.05$) in rice straw, wheat straw, barley straw, soybean straw, pea straw, and rice husk fermentation by *P. florida* (Nasehi et al., 2017).

A number of factors could be responsible for ME improvement in crop residues treated with fungi, such as high gas production in the treated substrate, reduction in cell wall components and an increase in CP content of urea treated rice st-

Table 4.4 Effect of level urea, fermented duration, and types of fungal on gas volume and *in vitro* digestibility characteristics.

Items	Level	Time	96	ED	OMD	ME
Treatments						
RS			82.53	42.91	60.90	8.78
USR	2.5	7	106.86	53.80	66.87	12.65
	2.5	14	105.07	56.37	67.48	9.73
	5	7	109.36	55.60	62.01	11.91
	5	14	79.47	41.37	59.49	8.51
POT	2.5	7	104.31	53.08	79.27	11.49
	2.5	14	107.90	55.99	73.99	10.70
	5	7	100.06	52.44	55.16	10.24
	5	14	79.87	51.04	61.04	7.37
PSC	2.5	7	100.84	52.86	64.35	10.26
	2.5	14	106.54	55.24	69.99	10.60
	5	7	85.52	47.73	59.68	8.30
	5	14	93.41	50.41	62.48	9.22
PE	2.5	7	106.83	56.69	70.31	10.15
	2.5	14	100.21	54.10	65.31	9.39
	5	7	83.64	46.61	54.67	8.33
	5	14	85.55	66.91	51.42	6.76
SEM			1.35	0.88	0.83	0.11
Compearision						
A			0.0001	0.0001	0.0003	0.0001
B			0.0001	0.01	0.0001	0.0001
C			0.01	0.21	0.90	0.0001
Interaction						
AxB			0.004	0.09	0.0001	0.0001
AxC			0.0007	0.001	0.12	0.0001
BxC			0.01	0.84	0.47	0.03
AxBxC			0.001	0.0001	0.14	0.03

[abc] Means in the same row with different superscript differ ($p<0.05$); GV: gas volume (mL), ED: efficiency digestibility (%),OMD: organic matter digestibility (%), ME: metabolizable energy (MJ/kgDM); untreated rice stubble (Control), urea treated rice stubble (URS), urea treated rice stubble fermented with *Pleurotus ostreatus* (POT).

ubble fermented with *Pleurotus sajor-caju* (PSC), urea treated rice stubble fermented with *Pleurotus eous* (PE). Treated substrate (Sallam et al. 2007; Nasehi et al., 2017).

Moreover, Zadrazil (2000) explained that WRF attack unaltered lignin polymers causing cleavage of interlignol bonds and aromatic ring cleavage, which ultimately results in an increase in *in vitro* digestibility. They mainly degrade polysaccharides by hydrolytic enzymes like cellulases and xylanases, and lignin by oxidative ligninolytic enzymes such as lignin peroxidase, manganese peroxidase and laccase (Mahesh and Mohini, 2013).

4.6 Conclusions

It can be concluded that practical use of fungal treated rice stubbles, blocked by urea as roughage feed for ruminants could be possible, contained considerable amount of crud protein and may be used as a ruminant feed. This study suggests that rice stubble is suitable substrate for growing of all the *Pleurotus* species tested and rice stubble fermentation by fungi can improve its nutritive value for ruminant. Although all of fungal species demonstrate high capability improving the nutritive value and digestibility of rice stubble, however *Pleurotus ostreatus* fungi seem to be more potent for upgrading of rice stubble was indicated as greater crud protein content, compose lower lignin, and higher *in vitro* digestibility. Therefore, the conversion of lignocellulosics into edible fungi as animal feed may be the first economical technology for biological upgrading of cultivated residues.

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CHAPTER V

EFFECT OF UREA TREATED RICE STUBBLE FERMENTED FUNGI ON NUTRIENT DIGESTIBILITY RUMEN FERMENTATION AND GROWTH PERFORMANCE IN GROWING GOATS

5.1 Abstract

Cultivated residues are often referred to as lignocellulosics as they are high in cellulose which is bound with a biopolymer lignin. Biological treatment of such crop residues using fungi can break the ligno-cellulose complexes, liberating free cellulose and thus improving their feeding value for ruminants. The present research aims to investigate the effect of rice stubble treated by urea and fermented with fungi on digestibility, rumen fermentation, and growth performance in meat growing goats. Eighteen crossbred Thai native x Anglo-Nubain meat goats (average BW 20.4±4kg) were randomly assigned to feed on one of treatment diets according to completely randomized design. The goats were divided into three groups of six goats each to receive untreated rice stubble (control), urea treated rice stubble (URS), and rice stubble fermented with fungi (POT) and treated by urea (URSF). All animals were offered *ad-libitum* daily throughout 90 days. The result shows a significant ($p<0.05$) increase in dry matter intake (DMI) and nutrient intakes for goats fed URS and URSF diets; the goats fed with URSF had significantly higher digestibility, ammonia

nitrogen (NH_3-N) concentration, C2:C3 ratio for 2 h post feeding in the rumen, nitrogen (N) balance, blood urea nitrogen (BUN), and average daily gain (ADG) as well as body weight gain; while pH value, total volatile fatty acid (VFA), and bacteria population there were not significantly difference among diet treatments. The results of this study indicated that using fungi treatment of rice stubble resulted in improved apparent feed intake and nutrient digestibility, crud protein utilization, hence increased the growth and feed efficiency of growing goats. Therefore, the treated rice stubble or cultivated residues has a good potential as feed for ruminants. Moreover it's expected to be an impractical, cost-effective and environmental-friendly.

Key Words : Rice stubble, Urea, Fungi, Utilization, Goat

5.2 Introduction

In most developing countries particularly Southeast Asia, the main problem in ruminant production system is feed shortage, seasonal availability of forages, especially in dry season. Therefore, rice straw including rice stubble (rice stubble is a part of rice straw which high lignin content and low nutritive value) is available in plenty from the rice cultivated field has used as main part of feeding ingredients for the ruminants. However use of rice straw or stubble as an animal feed is limited by several factors such as the low nutritional quality due to high fiber content and lignification process, low nutritive value, low voluntary intake, slow rate of digestion and low content of available energy, protein, minerals and vitamins (Van Soest, 2006; Kholif et al., 2014; Malik et al., 2015). Lignin and cellulose commonly form lignosellulose bounds, a very strong bound, the higher lignin content resulting in poor

digestibility as lignification increase (Mustabi et al., 2013). Ester bonding and covalent bonds between lignin and polysaccharides contended in plant cell walls which prevents enzymes to degradation, hence lignin are the primary limiting factors in rice straw digestibility in ruminant animals (Hatfield, 1989; Jung, 1989). Therefore use rice straw or rice stubble as animal feed must be processed before using. There are various processing to enhanced rice straw utilization such as physical, chemical, or biological treatments have been tried (Mahesh and Mohini, 2013; Mustabi et al., 2013; Kholif et al., 2014; Malik et al., 2015).

One of the main processing methods to improve the nutritive value of stubbles or lignocellulosic residues could be the application of biotechnology. Fungal treatment as a biological method has been recently considered promising manner for enhancing the nutritive value of straw (Jafari et al., 2007; Akinfemi et al., 2010; Kholif et al., 2014; Malik et al., 2015). The potential of biological treatments has been explained by the ability of certain microbes to disrupt plant cell wall by partial breakdown of the lignin-carbohydrate complex thus improving their utilization in the rumen by increasing the digestibility of lignocellulosic materials, it is important to break down the linkage between cellulose, hemicellulose, and lignin or break down the compact nature of the tissue (Keller et al., 2003; Mahesh and Mohini, 2013; Kholif et al., 2014; Malik et al., 2015). White rod fungi (WRF) have the natural ability in upgrading lignocellulosics have been reported by various studies (Jafari et al., 2007; Akinfemi et al., 2010; Mahesh and Mohini, 2013; Mustabi et al., 2013; Kholif et al., 2014). Although several species of higher fungi possess ligninolytic activity, *Pleurotus* sp. is the most studied fungi since they improved the digestibility (Kundu et al., 2005; Mahesh and Mohini, 2013).

The aim of present study was to evaluate utilization of spent rice stubble treated by urea and fungal fermented in the diet of meat goats, to observed voluntary feed intake, nutrient digestibility, rumen fermentation parameters, and growth rate of growing goats.

5.3 Objectives

This study was designed to investigate the effect of urea treated rice stubble fermented with fungi compared with rice stubble and rice stubble treated urea on nutrient digestibility, rumen fermentation and growth performance in meat goats.

5.4 Materials and Methods

5.4.1 Animals, treatments, and experimental design

Eighteen meat goats crossbred Thai native x Anglo nubain an average body weight (BW) of 20.4 kg were allocated in Completed randomized design (CRD). The goats were housed in individual pens and allowed 2 weeks to adapt to the experimental conditions. Animals were received dietary treatments as followed:

Control = Rice stubble (rice stubble untreated with urea)

URS = Urea treated rice stubble

USRF = Urea treated rice stubble fermented fungi

TMR was offered twice daily ad libitum; approximately at 0700 and 1700 h. Diets were allowed to have 5% left over. Feed ingredients of the experimental diets are shown in Table 5.1. Water was available at all times.

Animals were individually housed and intensively cared according to procedures of goat farm at Suranaree University of Technology.

5.4.2 Data collection, sampling and chemical analysis

5.4.2.1 Feed sampling

Feed was sampled daily during the collection period and was composition prior to analyses. During the last seven days of 30^{th}, 60^{th} and 90^{th} days of fermentation. Feed samples were collected every day and divided into two parts, the first part be analyzed for DM, while the second part was kept and pooled at the end of each period for chemical analysis. Samples were dried 60°C and ground (1mm screen) and then analyzed for DM, ash, EE and CP content (AOAC, 2002), NDF, ADF (Van Soest et al., 1991).

5.4.2.2 Fecal and Urine samplings

Fecal samples were total collected and weighed during the last 7 days of day 30^{th}, 60^{th} and 90^{th}. The fecal samples were collected about 5% of total fresh weight and divided into two parts, the first part being analyzed for DM, the second part kept for chemical analysis at the end of each period.

Urine samples were collected the same time with fecal sampling, recording total collection and urine samples were collected 10% of total of the day acidified with 50% H_2SO_4 then mixed together of each goat after that were stored at -20°C until analyzed NH_3-N by the method of (Bremner and Keeney, 1965).

5.4.2.3 Blood sampling

Blood sample (about 10 ml) was collected from a jugular vein (at the same time as rumen fluid sampling) into tubes containing 12 mg of EDTA, and plasma was separated by centrifugation at 500×g for 10 min and stored at -20°C until analysis of blood urea-N (BUN) according to the method of Crocker (1967).

Table 5.1 Ingredients and chemical composition of the experimental diets in total mixed ratio (TMR) (% DM).

Items	Control	URS	URSF	Control	URS	URSF
Ingredients, %DM						
Soybean meal	-	-	-	3.0	3.0	3.0
Rice bran	-	-	-	5.0	5.0	5.0
Cassava hay	-	-	-	47.0	48.5	49.3
Rice stubble	-	-	-	35.0	35.0	35.0
Urea	-	-	-	3.0	1.5	0.7
Molasses	-	-	-	5.0	5.0	5.0
Salt	-	-	-	0.4	0.4	0.4
Sulfur	-	-	-	0.2	0.2	0.2
DCP	-	-	-	0.5	0.5	0.5
Limestone	-	-	-	0.2	0.2	0.2
Mineral premix	-	-	-	0.7	0.7	0.7

Control = Rice stubble, URS= urea treated rice stubble, URSF= urea treated rice stubble fermented fungi, DCP= Dicalcium phosphate

5.4.2.4 Rumen fluid sampling

Rumen fluid samples were collected by stomach tube at 0, 2 and 4 h-post morning feeding in the day 30th, 60th and 90th. Approximately 500 ml of rumen fluid was taken using stomach tube at each time at the end of each period. Rumen fluid was immediately measured for pH using a portable pH meter. Rumen

fluid samples were then filtered through four layers of cheesecloth. Samples were

Table 5.1 Ingredients and chemical composition of the experimental diets in total mixed ratio (TMR) (% DM) (con.)

Items	Control	URS	URSF	Control	URS	URSF
Chemical composition, % DM						
DM	96.00	23.90	23.00	46.25	48.78	41.95
	……..%DM……..					
Ash	15.12	20.89	22.86	8.19	7.82	15.51
OM	84.87	79.31	77.14	91.81	92.18	84.49
CP	2.49	4.06	9.51	12.21	12.03	12.32
EE	0.9	0.8	0.8	1.48	1.59	1.56
NDF	77.94	76.82	65.92	35.39	35.41	32.45
ADF	58.03	59.74	59.08	24.40	25.38	24.05
ADL	4.90	4.02	3.50	1.66	0.32	0.28

Control = Rice stubble, URS= urea treated rice stubble, URSF= urea treated rice stubble fermented fungi, DM= Dry matter, CP = Crude protein, EE = ether extract, OM = Organic matter, NDF = Neutral detergent fiber, ADF = Acid detergent fiber.

divided into three portions; first portion was used for NH_3-N analysis where 10 ml of 50%H_2SO_4 solution was added to 100 ml of rumen fluid. The mixture was centrifuged at 16,000 x *g* for 15 minutes and supernatant was stored at -20 °C prior to NH_3-N measurement (Bremner and Keeney, 1965) and VFA analysis (HPLC; model RF-10AXmugiL; Shimadzu; Japan) according to Zinn and Owens (1986). Second portion

was fixed with 10% formalin solution in normal saline (0.9% NaCl, Galyean, 1989) and stored at 4 °C. The total direct counts of bacteria and protozoa numbers were counted using the methods of Galyean (1989) based on the use of a haemacytometer. The third portion was collected for microbial analyzed by roll tube technic according to Hungate (1969).

5.4.3 Data Statistical Analysis

All data obtained from the experiment were statistically subjected to ANOVA and analyzed as a Complete Randomized Design (CRD) design using the PROC GLM (SAS, 1998). Significant differences between treatments were determined using Duncan's News Multiple Range Test (DMRT) (Steel and Torrie, 1980) and orthogonal contrast was used for trend analysis. The following statistical model was used:

$Y_{ijk} = \mu + \tau_i + \varepsilon_{ij}$

Where Y_{ij} = represents of observation from animals

μ = overall mean

τ_i = Effect of treatment (i = 1-3)

ε_{ij} = Error of the term

5.5 Results and discussions

5.5.1 Chemical composition of feeds

The chemical compositions of feed ingredients are shown in Table 5.2. DM of experimental diets was adequate for protein requirement for growing and maintenance. TMR contained DM 46.25, 48.78 and 41.95%. For crude protein content

was similar among treatment 12.21, 12.03 and 12.32%, neutral detergent fiber was decrease on treatment 3 and acid detergent fiber was 24.4, 25.38 and 24.05% respectively.

5.5.2 Feed intake and digestibility

Feed intake, nutrient intake and nutrient digestibility data are presented in table 5.3. Feed intake of total mixed ration were significantly different ($p<0.05$) among control and treated treatments (618.49, 658.47 and 687.35 gDM/d respectively). When compared by percentage of body weight and gram per kilo gram body weight 0.75 (g/kg $BW^{0.75}$) found control and URS were not significant ($p>0.05$) but when compared to URSF was significantly difference ($p<0.05$), URSF was higher 2.75, 2.76 and 3.10% for percentage of body weight and 59.91, 61.08 and 67.31 g/kg$BW^{0.75}$ respectively. It might be that rice stubble was spongier more than URS and USRF, Wanapat et al. (2009) reported that 5.5% urea treated rice straw increased DM intake in dairy cows when compared with RS. More reason the improvement of intake of rice stubble treated could be due to the physical character (the softness of the treated rice stubble structure) and the chemical changes (the biodegradation of its cell wall) in the straw during the biological fermentation (Arora et al., 1994; Zadrazil et al., 1995).

5.5.3 Average daily gain and feed conversion ratio

URSF was increased average daily gain (ADG) apparent significantly ($p<0.05$) when compared to control and URS. But control and URS were not significant different 43.33, 48.41 and 79.36 g/d. In the case feed conversion ratio

(FCR) was lower number in goats fed URSF treatment but not distinctive different among treatments. The results indicated that used biological treatment of rice stubble with fungi was improved the efficiency of feed utilization in growing goats. It may be explained by more feed intake in gram per day and percentage of body weight affected to this group increased weight gain. Moreover, the nitrogen intake in goats fed URSF was higher than other groups. Similar with Jeerasak et al. (2009) studied level of protein on growth performance found ADG was 63 to 92 g/day of 12-14% CP. Moreover, Khotsakdee et al. (2010) reported that goats fed low fat (3%), high fat (6%) and high fat with yeast has average daily gain value equal 41.67, 66.67 and 80.56 g/d, respectively. Omer et al. (2012) had presented that biologically treated corn stalks can completely replace clover hay in the ration of growing sheep which was evident by a favorable increase in DM intake, and an improvement in the digestibility of all nutrients with higher ADG.

5.5.4 Nutrient intake

Rice stubble which is a part of rice straw is a by-product of the rice grain industry. It has limited nutritive value (low crude protein, palatability digestibility and high oxalates) that may cause of restricted feed intake. Feed intake can be used as an indicator of the palatability of the diets. To improve the utilization of crop residues for ruminants is to overcome the barriers to rumen microbial fermentation of lignocellulosics. In this study the results of nutrient intakes of growing goats fed rice stubble are presented in Table 5.2 Nutrient intakes were affected by fermented stubble diets. The feed in take gram dry matter intake per day found that URS and URSF was not significantly different but when compared with control was

highly significant different. Feed in take in this study was 618.49, 658.47 and 687.35 gDM/day similar with Moreira et al. (2016) who studied ammoniated babassu palm hay in anglo-nubian goat diets found feed intake range from 570 to 947 gDM/d. The OMI and NDFI were highest level in goats fed URS dietary treatment, there were significantly distinctive difference ($p<0.05$) when compared with control group but not significant different with goats fed URSF. These data support earlier works were descripted rice straw treated with urea improved DMI, OMI, and fibrous substrates (Mould et al., 1982; Trach et al., 2001; Qingxiang 2002; Wanapat et al., 2013; Polyorach and Wanapat. 2014). The increase of stubble intake in the present study may thus be explained by virtue of its increased degradability in the rumen and an increase in the outflow of stubble cell walls into the abomasum as reported by Trach et al. (2001); Wanapat et al. (2013). The higher intakes of treated treatments compared with untreated treatment may be because treated treatment is more palatable than Untreated. In the same way, particle passage is expected to decrease with increasing NDF intake. Van Soest (1965) opined that feed intake is limited by the amount of fiber in the diet when cell wall content lies between 50 and 60% of forage dry matter. Also the voluntary intake is expected to be inversely related to the fiber content of the forage because further intake is limited as the slower digesting fraction becomes large in relation to the volume of the digestive tract. Methods of treating straw may be classified broadly into mechanical, physical, chemical and biological categories. Several studies have reported the physical, chemical characterization and utilization of rice straw as ruminant feed also improve its nutritive value, enhance feed intake and digestibility (Trach et al., 2001; Vadiveloo, 2003; Wanapat et al., 2009; Malik et al., 2015).

CPI per day was apparent ($p<0.05$) highest level in goats fed URSF formula. This result according with previous studies were reported utilization of fungal treated crop residues increased feed intake as well as nutrients utilization (Mahesh, 2012; Omer et al., 2012; Shrivastava et al., 2012; Mahesh and Mohini, 2013). Furthermore, Langar et al. (1980) reported spent straw remaining after edible mushroom harvesting, generally contains an increased CP, cell-wall soluble, total and acid insoluble ash and reduced cell wall components which might be more useful than the original straw for feeding ruminants. Various studies reported fungal treated cultivated remainders improved nutritive values; Increased CP content of fermented substrates was associated with increased fungal biomass (Chen et al., 1995; Tripathi et al., 2008; Akinfemi et al., 2009; Akinfemi 2010; Kholif et al., 2014). Walli et al. (1988) observed that the N intake, its digestion and retention in cross-bred calves fed fungal treated wheat straw supplemented with groundnut cake was higher than urea treated straw fed group. The fermented maize straw with *P. ostreatus* increased voluntary daily intake as well as gain in body weight of Pelibuey sheep (Díaz-Godínez and Sánchez. 2002). White-rot fungi (WRF) are capable of degrading lignin without affecting much of cellulose and hemicelluloses (Zadražil and Brunnert, 1982). WRF attack unaltered lignin polymers causing cleavage of interlignol bonds and aromatic ring cleavage, which ultimately results in an increase in in vitro digestibility (Zadražil et al., 1999). They mainly degrade poly-saccharides by hydrolytic enzymes like cellulases and xylanases, and lignin by oxidative ligninolytic enzymes such as lignin peroxidase, manganese peroxidase and laccase. Akinfemi and Ogunwole, (2012) reported the use of three different edible mushrooms: *Pleurotus ostreatus*, *Pleurotus pulmonarius*, and *Pleurotus tuberregium* treated with rice straw to examine nutritive

values, were studied through analysis of their proximate composition, mineral composition, crude fiber fractions and *in vitro* digestibility.

Table 5.2 Effect of URS and URSF on feed intake, nutrient intake, apparent digestibility, metabolizable energy and microbial crude protein of growing meat goats.

Items	Control	URS	URSF	SEM	P-value
Feed intake					
gDM/d	618.49^{b}	658.47^{a}	687.35^{a}	6.62	0.003
%BW	3.02^{b}	3.00^{b}	3.63^{a}	0.05	0.030
g/kgBW$^{0.75}$	64.27^{b}	64.93^{b}	75.76^{a}	1.00	0.018
Nutrient intake gDM/d					
OMI	567.86^{b}	607.00^{a}	580.77^{ab}	5.77	0.041
CPI	75.51^{b}	79.24^{b}	84.74^{a}	0.81	0.001
EEI	9.21^{b}	10.47^{a}	10.74^{a}	0.10	0.0001
NDFI	218.90^{b}	233.22^{a}	223.04^{ab}	2.22	0.050
ADFI	150.94^{b}	167.12^{a}	165.31^{a}	1.61	0.002

Untreated rice stubble (Control), Rice stubble treated urea (URS), Rice stubble fermented fungi and treated with urea (URSF), OMI= Organic matter intake, CPI= crude protein intake, EEI= ether extract intake, NDFI= neutral detergent fiber intake, ADFI= acid detergent fiber intake, and SEM = Standard error of the mean.

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It was observed that treatment of rice straw with different edible mushrooms improved the potential feeding value. Therefore, the product of fungal treatment has a good potential as feed resources for ruminants. The use of fermented rice straw and elephant grass with WRF can be improved feed intake in goats (Mustabi et al., 2013).

The value of EEI and ADFI were similar value between goat fed stubble fermented with urea and stubble fermented with fugal and treated with urea groups.

Both were significantly different ($p<0.05$) levels with control group. These results similar with former studies reported biologically treated corn stalks fed with growing

Table 5.2 Effect of URS and URSF on feed intake, nutrient intake, apparent digestibility, metabolizable energy and microbial crude protein of growing meat goats. (Con.)

Items	Control	URS	URSF	SEM	P-value
Apparent Digestibility, % of intake					
DDM	58.69^{b}	62.68^{ab}	68.88^{a}	1.24	0.014
DOM	62.61^{b}	65.93^{b}	71.49^{a}	1.02	0.010
DCP	57.58^{b}	63.59^{ab}	67.12^{a}	1.51	0.060
DEE	65.08^{b}	69.13^{ab}	72.54^{a}	0.79	0.006
DNDF	46.87b	53.64ab	65.29a	2.31	0.017
DADF	44.91	51.07	57.19	2.05	0.081
ME (Mcal/kgDM)	1.78^{b}	1.98^{a}	2.02^{a}	0.03	0.017
MCP (kg/d)	0.84^{b}	0.93^{a}	0.95^{a}	0.02	0.016

^{abc} Means in the same row with different superscript differ ($p<0.05$); Untreated rice stubble (Control), Rice stubble treated urea (URS), Rice stubble fermented fungi and treated with urea (URSF) ME = Metabolizable energy, 1 kg DOMI = 3.8 Mcal ME/kgDM (Kearl, 1982), MCP = Microbial crude protein (kg/d) = 0.13*kgDOMI;, SEM = Standard error of the mean.

Sheep which was evident by a favorable increase in DM intake, and an improvement in the digestibility of all nutrients with higher ADG (Omer et al., 2012). These reports clearly indicate that majority of the fungal treated can be improved more palatable feeds (Kamra and Zadražil, 1988; Mahesh and Mohini, 2013). When rice straw was treated with urea, it resulted in improving the nutritional quality of the straw in terms

of nitrogen content, palatability and digestibility. During the treatment process, ammonia generated from urea, in the presence of water formed (Polyorach and Wanapat, 2014; Oladosu et al., 2016). Break down of rice stubble as lignin bond may reduce the time of passage in the rumen and improve feed for ruminants. Both of urea treated and biological treatment can be employed for improving the feeding value of low quality fibrous cultivated residues.

5.5.5 Nutrient digestibility

Digestibility of ADF was not obviously different among treatment ($p>0.05$). DM and OM digestibility of URSF was highest and significant different when compared with control and URS ($p<0.05$), but control and URS were not significant different between treatment (Table 5.2). Digestibility of the straw is dependent on the deploy merisation of its structural carbohydrates. Enzymatic degradation of these macromolecules in the straw will result in degradation and increase in digestibility and availability of carbohydrates (Fazaeli et al., 2004; Mahes and Mohini, 2013). Karunanandaa et al. (1995) studied the effect of incubation of rice straw for 30 days with white-rot fungi, found that *Pleurotu sajor-caju* enhanced dry matter digestibility in animal, in both leaves and stems of rice. Fazaeli et al. (2002) observed fungal treated wheat straw fed cattle can increased DM and OM digestibility and palm leaves treated with *pleurotus florida* for sheep (Kabirifard et al., 2007). White-rot fungi are capable of degrading lignin without affecting much of cellulose and hemicelluloses thus causing decayed residue to turn white, WRF attack unaltered lignin polymers causing cleavage of interlignol bonds and aromatic ring cleavage, and increased digestibility (Zadražil and Brunnert, 1982; Zadražil et al., 1999). They

mainly degrade poly-saccharides by hydrolytic enzymes like cellulases and xylanases, and lignin by oxidative ligninolytic enzymes such as lignin peroxidase, manganese peroxidase and laccase (Mahes and Mohini, 2013). The examine in late lactating Holstein cows fed with fungal treated straw upto 30% of the total mixed ration in improved the nutrients digestibility (Fazaeli et al., 2004). White rot fungi use enzymatic mechanisms to break down lignin, alter lignocellulose structures, and improve the nutritive value of low quality feeds (Tripathi et al., 2008; Tuyen et al., 2013; Yilkal, 2015).

Digestibility of CP, EE and NDF were not significant different between control and URS. As compared to URSF was significant different ($p<0.05$). Digestibility of CP was 57.68, 83.59 and 67.12% in treatment 1, 2 and 3 respectively. EE digestibility was 65.08, 69.64 and 72.54% and NDF digestibility was 46.87, 53.64 and 65.29%, respectively. The explanation might be the URSF was already fermented of fiber during the fungi fermentation. So the fiber fraction in URSF is easily digested by microbes in the rumen leading to increased fiber digestion after feeding (Yuangklang et al., 2004). To improve digestibility and protein enrichment of the low quality fodder are known to be the aim of the bioconversion process when the product is destined for ruminant nutrition that is proposed process of biological upgrading of lignocellulosics into animal feed should be characterized by marked lignin decomposition and liberation of nutrients from the lignocellulose-matrix with contemporary accumulation of digestible substances along with enriching the final product with microbial protein (Kamra and Zadražil, 1988; Zadražil et al., 1999; Villas-Bôas et al., 2002; Mahes and Mohini, 2013). However, a high quality standardized products of the acculturative remainder along with controlled conditions,

which the fungus, its enzymes, physical structure of substrate, physiological factors of fermentation and culture as well as nutritional conditions play an important role in controlling lignin degradation and digestibility of fermented substrate since cheap and safe to animal and environment (Zadražil, 1986; Zadražil et al., 1999). By the way using urea to treat crop residues have been reported to increase feed intake as well as nutrients digestibility in ruminants (Wanapat et al., 2009; Gunun and Wanapat, 2012; Wanapat et al., 2013; Cherdthong et al., 2014; Oladosu et al., 2016). The increases in apparent digestibility of the treated straw were reasonable due to increased rumen degradability resulted from increased susceptibility of structural carbohydrates of straw cell walls to rumen fermentation as well as more energy being made available for better growth of rumen microbes which degrade straw (Silva and Ørskov, 1988; Wanapat et al., 2013).

5.5.6 Metabolizable energy and microbial crude protein

Metabolizable energy (ME, Mcal/kgDM) is derived from the calculation of digestible organic matter intake. It is an import to know that the more fermented organic matter in the rumen is associated with the high amount of metabolizable energy in the diet. The microbial crude protein (MCP, kg/d) value is also derived from the digestible organic matter intake in the rumen. The present experiment showed that metabolizable energy and microbial crude protein were significantly ($p<0.01$) increased in the goats fed URS and URSF (ME: 1.78, 1.98 and 2.02 Mcal/kgDM respectively and MCP: 0.84, 0.93 and 0.95 kg/d respectively). It may be explained that fungi break down lignin in rice stubble straw structure and easy degrade in the rumen which enhancing organic matter digested, led to increase

metabolizable energy and microbial crude protein values. Such improvement of intake of the treated stubble could be due to the physical character (the softness of the treated straw structure) and the chemical changes (the biodegradation of its cell wall) in the straw during the biological fermentation as reported (Arora et al., 1994; Zadrazil et al., 1999; Fazaeli et al., 2002). Feed intake in ruminants is dominated by two factors, the digestibility of the fed forage and the intake capacity of the animal (McOueen and Robinson, 1996).

5.5.7 Body weight change

Initial and overall weights score not apparent difference among treatments. Nevertheless, body weight gain was significant different ($p<0.01$) among treatments (Table 5.3). Stubble fermented with fungi and treated by urea resulted in a higher than stubble treated urea only, and control (untreated) group results as (5.00, 3.68, and 2.78) respectively. When the rice stubble fermented with fungi and treated by urea was higher level of intake and digested protein it could improve the metabolism and biological values of the protein and amino acid balance, this result supported previous studies (Silva et al. 2002; Fazaeli et al., 2002; Tripathi et al., 2008; Mahesh and Mohini, 2013; Yilkal, 2015). Ramirez-Bribiesca et al. (2010) reported that used corn straw fermented with *P. ostreatus* for 15 days increased crude protein 39.5% and soluble protein 165%, soluble carbohydrates 621%, ash 188.32% and decreased neutral detergent fiber 14.5% similar results with our first study. This result supported by Díaz-Godínez and Sánchez, (2002) who reported that when spent maize straw of *P. ostreatus* increased voluntary daily intake as well as gain in body weight of Pelibuey sheep. In cattle consuming fungal treated wheat straw diet was observed

by (Fazaeli et al., 2002); the influence of *P. ostreatus* spent corn straw on the performance of feedlot Pelibuey lambs was increased weight gain (Ramirez-Bribiesca et al., 2010); A significantly increased DM intake and growth rates in West African dwarf lambs fed with biologically treated maize cobs replacing wheat offal in guinea grass based diets (Akinfemi and Ladipo 2011). Fazaeli et al. (2004) reported that inclusion of fungal treated straw up to 30% of the total mixed ration in late lactating Holstein cows increased fat in milk yield by 13% and daily average body weight gain by 2.7 times. The tendency to higher body weight change in fermentation of the fungi rice stubble and treated with urea (Fig. 5.1).

Table 5.3 Effect of URS and URSF on body weight gains (kg).

Items	**Control**	**URS**	**URSF**	**SEM**	**P-value**
Body weight					
Initial weight, kg	20.47	21.95	18.92	0.40	0.22
Final weight, kg	23.20	25.00	23.92	0.39	0.22
Weigh change, kg	2.73^{c}	3.05^{b}	5.00^{a}	0.25	0.01
ADG, g/d	43.33^{b}	48.41^{b}	79.36^{a}	4.33	0.02
FCR, kg	19.59	19.96	12.40	1.89	0.23

^{abc} Means in the same row with different superscript differ ($p<0.05$). Untreated rice stubble (Control), Rice stubble treated urea (URS), Rice stubble fermented fungi and treated with urea (URSF), ADG= Average daily gain, FCR= Feed conversion ratio SEM = Standard error of the mean.

5.5.8 Rumen fermentation characteristics

The mean pH in the rumen fluid (Table 5.4) ranged from 6.34 to 7.09 and there was affected by the diets ($p>0.05$) for all duration after feeding. This result similar ranging with previous studied which reported that feeding rice straw treated with *Pleurotus ostreatus* for lactating goats (Akinfemi and OgunwOle, 2012; Gomaa et al., 2012; Kholif et al., 2014).

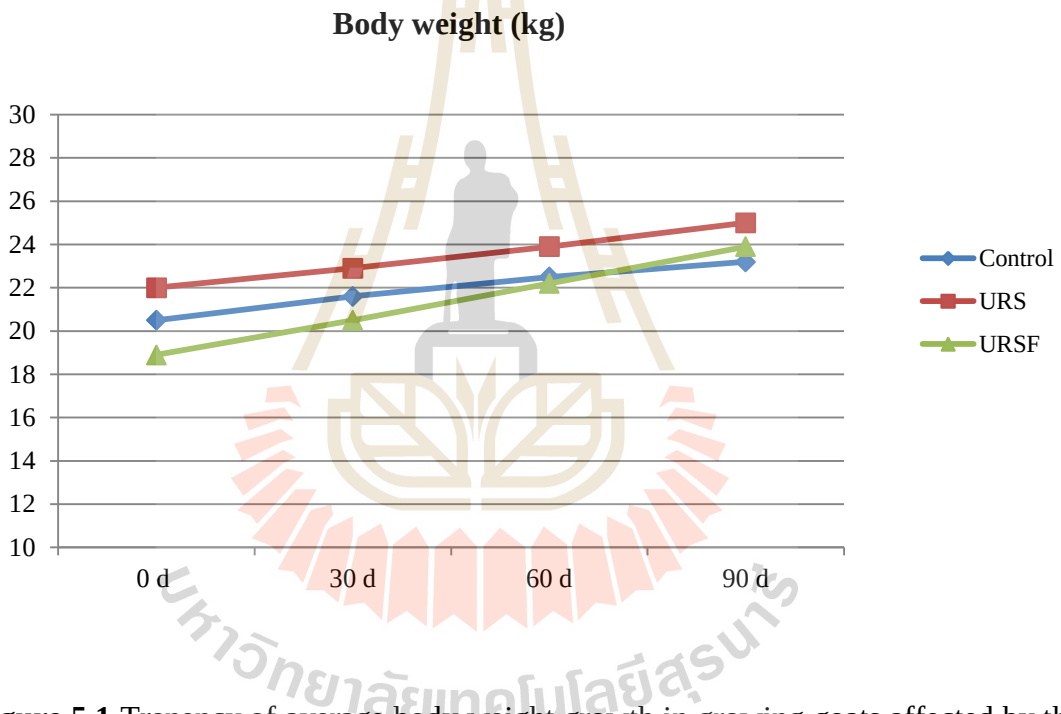

Figure 5.1 Trenency of average body weight growth in growing goats affected by the using of fungi to ferment and urea treated from 0-90 days.

The mean concentration (mg %) of ammonia-N in the rumen fluid was affected by the diets ($p=0.002$), goats consuming URSF diet was higher for 2, 4 h post feeding in the morning including mean value. The ammonia-N concentration increased rapidly after 2 h, increasing ammonia-N concentration in the rumen might

be explained by the treatment higher CP intake and digestibility which caused by fugal break down lignin bond for growing up its cell; therefore, URSF treatment high as microbes mass. Ruminal NH_3-N concentration in this study ranged from 15.50 to 19.8 mg/dl. Which these results were similar to the values obtained by Khejornsart and Wanapat (2010) and Gunun and Wanapat (2012) who studied effect of physical form of urea-treated rice straw found NH_3-N concentration range from 12.4 to 22.8 mg/dl. Moreover Gomaa et al., 2012 have been reported to increased ammonia-N concentration in rumen 3 h after feeding.

The mean total VFA concentration (mM/L) in the rumen fluid (Table 5.4) was not affected by the diet treatments. The concentration of Acetic acid (molar%) was affected by the diet ($p<0.05$) for 2 h after feeding, and the higher concentration was found in goats fed URSF when compared with control group but not ($p>0.05$) by the diet compared to URS treatment. Propionic acid and Butyric acid concentration were not significant different among treatments in any duration post feeding. C2:C3 ratio was also higher ($p<0.05$) for URSF treatment for 2 h post feeding but not significant different for 0 and 4 h after feeding. Volatile fatty acids concentrations in this experiment were similar ranges with previous studied (Aderinboye et al., 2012). Probable changes in the integrity of cell walls are also supported by the VFA concentrations. The tendency to higher acetate production in fermentation of the fungi wheat straw substrates corresponds to an improvement of fiber degradation (Rodrigues et al., 2007).

5.5.9 Rumen micro-organism population

Table 5.5 illustrated the result on ruminal microorganism population affected by urea treated and fungal fermented rice stubble in growing goats. The mean number of total bacteria and protozoa in the ruminal fluid was no affected by diet treatments of all duration post feeding. These results might be described as physical and chemical of fibrous have been affected by fungal fermented process, hence no affected to microbe population in the rumen. The higher digestibility in the fugal treated straw was likely to have been caused by its reduced contents of cell wall; especially lignin has been implicated in rations (Akinfemi and OgunwOle, 2012; Mahesh and Mohini, 2013).

Table 5.4 Effect of URS and URSF on rumen fermentation in growing goats.

Items	Control	URS	URSF	SEM	P-value
pH					
0 h	7.09	6.82	6.94	0.05	0.120
2 h	6.78	6.59	6.58	0.06	0.290
4 h	6.34	6.53	6.52	0.05	0.250
Mean	6.74	6.65	6.68	0.03	0.450
NH_3-N, mg%					
0 h	13.00	14.21	16.15	0.53	0.080
2 h	16.31^{b}	17.38^{b}	20.72^{a}	0.42	0.002
4 h	17.19^{ab}	16.12^{b}	18.01^{a}	0.22	0.010
Mean	15.50^{b}	15.90^{b}	18.29^{a}	0.29	0.002
Total VFAs mM/l					
0 h	75.36	62.92	75.20	4.16	0.400
2 h	92.66	92.02	84.60	3.23	0.540
4 h	98.79	110.57	90.81	5.34	0.340
Mean	88.93	88.50	83.54	1.06	0.600

^{abc} Means in the same row with different superscript differ ($p<0.05$); h= hour.

Untreated rice stubble (Control), Rice stubble treated urea (URS), Rice stubble fermented fungi and treated with urea (URSF); SEM = Standard error of the mean.

Table 5.4 Effect of URS and URSF on rumen fermentation in growing goats (con).

Items	Control	URS	URSF	SEM	P-value
Acetic acid, molar%					
h0	58.99	59.41	59.25	0.93	0.98
h2	58.85^{b}	61.34^{ab}	66.14^{a}	0.07	0.02
h4	58.94	58.84	59.94	0.75	0.80
Mean	58.93	59.87	61.78	0.46	0.07
Propionic acid, molar %					
0 h	30.82	28.64	30.40	0.95	0.62
2 h	27.78	28.00	24.05	0.79	0.10
4 h	28.34	27.13	27.75	0.85	0.85
Mean	28.98	27.92	27.40	0.36	0.23
Butyric acid, molar %					
0 h	10.19	11.95	10.35	0.49	0.30
2 h	13.36	10.65	9.80	0.64	0.09
4 h	12.72	14.03	12.31	0.53	0.40
Mean	12.09	12.21	10.82	0.28	0.12
C2:C3 ratio					
0 h	1.97	2.13	1.97	0.10	0.75
2 h	2.16^{b}	2.22^{b}	2.81^{a}	0.11	0.04
4 h	2.14	2.23	2.18	0.10	0.94
Mean	2.09	2.20	2.32	0.04	0.14

^{abc} Means in the same row with different superscript differ ($p<0.05$); h= hour.

Untreated rice stubble (Control), Rice stubble treated urea (URS), Rice stubble fermented fungi and treated with urea (URSF); SEM = Standard error of the mean.

Feeding of URSF diet to growing goats increased Amylolytic bacteria in the ruminal fluid evidently different ($p<0.05$) for 0 h after feeding compared with both of control and URS groups, for 4 h post feeding also higher significant different ($p<0.05$) when compared to URS treatment but not apparently different with control group. There were not significant different among treatments for 2 h and average. Proteolytic bacteria was distinctive higher population ($p<0.05$) in goats fed URSF for 0 and 4 h post feeding, but no significant different among treatments for 2 h and average times. Cellulolitic bacteria population was not affected by the treatment diets.

Table 5.5 Effect of URS and URSF on microbial population in the rumen of growing goats.

Items	**Control**	**URS**	**URSF**	**SEM**	**P-value**
Bacteria (log_{10})					
0 h post feeding	6.92	6.90	6.90	0.03	0.97
2 h post feeding	6.91	6.96	7.00	0.02	0.30
4 h post feeding	7.02	6.99	7.00	0.01	0.62
Mean	6.95	6.95	6.97	0.01	0.83
Protozoa (log_{10})					
0 h post feeding	6.02	6.05	5.77	0.07	0.26
2 h post feeding	5.86	6.05	5.95	0.08	0.62
4 h post feeding	5.95	6.24	6.10	0.07	0.28
Mean	5.94	6.11	5.94	0.03	0.45

[abc] Means in the same row with different superscript differ ($p<0.05$); h= hour.

Untreated rice stubble (Control), Rice stubble treated urea (URS), Rice stubble fermented fungi and treated with urea (URSF); SEM = Standard error of the mean.

5.5.10 Nitrogen balance

The effect of dietary treatments on nitrogen (N) utilization is presented in Table 5.6 Present results expressed nitrogen retention as the cumulative nitrogen balances. N intake, N absorbed, and N balance, were higher significant different ($p<0.01$) level in goats fed URSF treatment compared with both of control and URS treatment diets, while N in urine and N in feces were not significant different level among treatments ($p>0.05$). Moreover, N absorption, % of intake and N balance, % of

Table 5.5 Effect of URS and URSF on microbial population in the rumen of growing goats (Con).

Items	Control	URS	URSF	SEM	P-value
Amylolytic Bacteria (log_{10} cfu/ml)					
0 h post feeding	8.58^{b}	8.63^{b}	8.73^{a}	0.01	0.0001
2 h post feeding	8.60	8.69	8.64	0.03	0.46
4 h post feeding	8.55^{ab}	8.50^{b}	8.61^{a}	0.01	0.02
Mean	8.58	8.61	8.66	0.01	0.06
Proteolytic Bacteria (log_{10} cfu/ml)					
0 h post feeding	8.24^{b}	8.22^{b}	8.37^{a}	0.02	0.03
2 h post feeding	8.28^{b}	8.40^{ab}	8.64^{a}	0.05	0.03
4 h post feeding	8.17	8.17	8.27	0.05	0.66
Mean	8.35	8.27	8.31	0.03	0.44
Cellulolitic Bacteria (log_{10} cfu/ml)					
0 h post feeding	9.17	9.15	9.20	0.03	0.70
2 h post feeding	9.24	9.18	9.26	0.04	0.24
4 h post feeding	9.23	9.26	9.26	0.03	0.87
Mean	9.21	9.20	9.27	0.02	0.43

^{abc} Means in the same row with different superscript differ ($p<0.05$); h= hour. Untreated rice stubble (Control), Rice stubble treated urea (URS), Rice stubble fermented fungi and treated with urea (URSF); SEM = Standard error of the mean.

Intake were higher in URSF group apparently different level compared with control group but not significant different volume when compared with URS treatment group. Therefore, animals fed with URSF was improved retention of nitrogen according to previous studies were reported utilization of fungal treated crop residues increased nutrients utilization as well N balance (Mahesh 2012; Omer et al., 2012; Shrivastava et al., 2012; Mahesh and Mohini 2013).

Table 5.6 Effect of URS and URSF on nitrogen utilization of growing goats.

Items	Control	URS	URSF	SEM	P-value
N in intake, g/d	12.08^{b}	12.67^{b}	13.55^{a}	0.13	0.0012
N in feces, g/d	5.13	4.62	4.46	0.19	0.1237
N in Urine, g/d	1.63	1.12	1.09	0.13	0.1879
N absorbed, g/d	6.95^{c}	8.05^{b}	9.09^{a}	0.23	0.0021
N balance, g/d	5.32^{b}	6.39^{b}	8.00^{a}	0.25	0.0008
N absorption, % of intake	57.58^{b}	63.59^{ab}	67.12^{a}	1.51	0.0599
N balance, % of intake	44.04^{b}	50.49^{ab}	59.08^{a}	1.68	0.0081

^{abc} Means in the same row with different superscript differ ($p<0.05$); N= nitrogen.Untreated rice stubble (Control), Rice stubble treated urea (URS), Rice stubble fermented fungi and treated with urea (URSF); SEM = Standard error of the mean.

5.5.11 Blood urea nitrogen

Table 5.7 was showed that blood urea nitrogen (BUN) concentration in goats fed URSF was higher than goats fed URS and control at 0 h or before feeding and significantly different ($p<0.05$) 8.64, 11.11, and 14.70 mg% respectively. After feeding 2 h and 4 h found BUN in goat fed URS was increased higher than control

($p<0.05$), but when compared to URSF was not significantly different. There has been known that URSF was easily degraded in the rumen, then it will use by the microbes together with carbon skeleton for their growth. Thus, there was less ammonia nitrogen to absorb through the rumen wall for urea synthesis by urea cycle (Van Soest, 1994). The mean BUN in this study was range from 11.80-19.12 mg%. It was similar with the optimal level in normal goats which has been reported by Lloyd. (1982) the range of BUN start from 11.2 to 27.7 mg%. Preston et al. (1965) reported that the concentration of BUN is correlated to the level of ammonia production in the rumen. Furthermore, Wanapat et al. (2008) suggested that concentrations of blood urea N are highly correlated to the concentration of NH_3 production in the rumen.

Table 5.7 Effect of URS and URSF on blood urea nitrogen of growing goats.

Items	**Control**	**URS**	**URSF**	**SEM**	**P-value**
BUN mg%					
0 h post feeding	8.64^{b}	11.11^{b}	14.70^{a}	0.65	0.009
2 h post feeding	13.32^{b}	16.65^{ab}	20.83^{a}	0.84	0.012
4 h post feeding	13.44^{b}	16.95^{ab}	21.83^{a}	0.92	0.011
Mean	11.80^{b}	14.90^{b}	19.12^{a}	0.78	0.009

^{abc} Means in the same row with different superscript differ ($p<0.05$); BUN= blood urea nitrogen; Untreated rice stubble (Control), Rice stubble treated urea (URS), Rice stubble fermented fungi and treated with urea (URSF); SEM = Standard error of the mean.

5.6 Conclusion

Ruminant production has had and will continue to play a very important role in developing country. A large proportion of the rice straw product was used as ruminant feed. However, its major problems of crop residues as low quality of nutritive value and high lignin composition which are the major sources of available feed for ruminant. It is concluded that fungi can be used for improving utilization of rice stubble as animal feed, that was indicator by apparently increased of OM intake, nutrient intakes, nutrients digestibility, N utilization, and ADG as well as body weight gain of growing goats. The results might be affected from fermented processing by fungal in degrading lignin or fibrous contents of rice stubble before feeding which improve its nutritional value. The use of biological treatments can be employed for improving the feeding value of low quality fibrous crop residues. Using fungal treated rice stubble has a good potential as feed resources for ruminant animals and could be used in combination with other feedstuffs. Moreover biological treatments are expected to be a practical, cost-effective, and environmental-friendly approach for heightening the nutritive value and digestibility of rice stubble.

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CHAPTER VI

OVERALL CONCLUSION AND IMPLICATION

6.1 Conclusion

Rice cultivation generates large amount of crop residues which is a main source of roughage for ruminants in most developing countries especially southeast Asia. Carbohydrates in plant cell walls are highly fermentable and could be used as a source for ruminant nutrition. The constituent of lignin in cell walls obstructs the utilization of these carbohydrates and should thus be removed. The present study was conducted to enhancing the efficient utilization of rice stubble fermented by white-rot fungi and treated with urea as goat diets. The study was divided into 3 experiments. The first experiment was subdivided into 3 parts including *in vitro* studies. The experiment was conducted to evaluate the effect of *Pleurotus* species pretreatment of lignocellulose on a reduction in lignin and the biodegradation of rice stubble in dry season in different period time for fermented. The second experiment was conducted to investigate study urea utilization for block white-rot activities and improve digestibility of rice stubble by *in vitro* gas production and the last experiment was studied the effect of urea treated rice stubble fermented fungi on nutrient digestibility rumen fermentation and growth performance in meat goats.

In experiment 1 the fermentation of fungal with rice stubbles to observed chemical composition and *in vitro* digestibility. The results showed that treated rice stubbles with *Pleurotus* species fungi increased CP content, in contrast decreased

fibrous contents. All of fungal treatments enhanced *in vitro* degradation of rice stubbles. From the results it could be concluded that the stubbles treated by fungi could be successfully used to enrich rice stubbles with protein, improve nutrients digestibility and nutritive value of rations those containing fungi treated.

Experiment 2 indicated that practical use of urea to treat stubbles fermentation by fungi can be operated silages as sustainable both of physical and chemical characteristic in feedstuffs. The properly level of urea to stop fungi activities as 2.5% and duration time as 7 days. This study shows that rice stubble is suitable substrate for growing of all the *Pleurotus* species tested and rice stubble fermentation by fungi can improve its nutritive value for ruminant. Although all of fungal species demonstrate high capability improving the nutritive value and digestibility of rice stubble, however *Pleurotus Ostreatus* fungi seem to be more potent for upgrading of rice stubble was indicated as greater crud protein content, compose lower lignin, and higher *in vitro* digestibility. Therefore, the conversion of lignocellulosics into edible fungi as animal feed may be the first economical technology for biological upgrading of cultivated residues.

In experiment 3 fungi treatment and treated urea was apparently increased of OM intake, nutrient intakes, nutrients digestibility, N utilization, and ADG as well as body weight gain of growing goats. The results implied that rice stubbles fermented with *Pleurotus* fungi could be increased in degrading lignin or fibrous contents of rice stubble before feeding which improve its nutritional value. The use of biological treatments can be employed for improving the feeding value of low quality fibrous crop residues. Using fungal treated rice stubble has a good potential as feed resources for ruminant animals and could be used in combination with other feedstuffs.

This thesis describes the capability of fungal treatment to increase utilization of

lignocellulosic materials. Fungal treatment resulted in increased CP content and *in vitro* rumen degradability, thus increased cell wall accessibility. Moreover, increases the feeding value of stubbles, improves its quality, and betters animal performance. The same theory applies for agricultural residues fermentation in which fungal treatment results in increased accessibility of fibrous compounds by their enzymes. The crucial disadvantages of biotechnological treatment are chosen by considering cost effectiveness, relatively simple and environmentally-friendly. Future studies should focus on optimize chemical or method to keep fungal treatment after harvesting.

6.2 Implications

Overall, based on experimental data, each fungi was effected on period time for fermentation so in experiment 1 POT was highest in digestibility at 25 days of incubated, PSC was 35days and PE was 30 days are suggested in term of increase protein content and digestibility.

In second experiment POT fermented at 25 days with 2.5% urea is recommended on digestibility and in third experiment URSF was suggested for growing meat goats.

The studies in this thesis have been performed to get more information and implication such as using difference substrate for fermentation, study type of rumen microbial by PCR technique

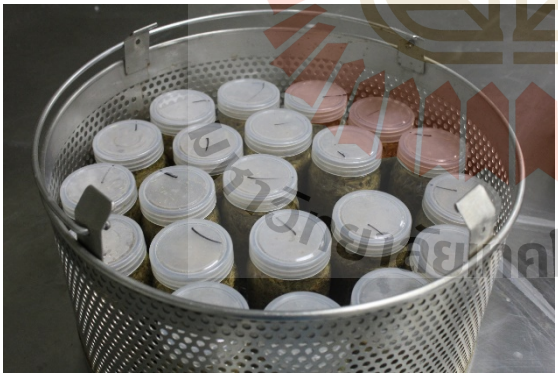
APPENDIX

A. the spawn of white-rot fungi

B. Jam bottle use for fermentation

C. Added water into substrate

D. Cover by plastic lid



E. Autoclave

F. Waiting for cold


G. inoculate step



H. Incubated in the air-conditioned chamber

I. After fermented

CURRICULUM VITAE

Mr. Thansamay Vorlaphim was born on the 31st of January 1985 in CHampasak province, Laos. He graduated Bachelor of Science from Faculty of Agriculture, National University of Laos in 2009. After graduation, he was studied master degree at Faculty of Natural Resources, Rajamangala University of Technology-Isan, Sakon Nakhon Campus, Thailand. In 2013, he obtained the scholarship from One Research One Graduate program (OROG) to presence a Doctor degree at school of Animal Production Technology, Institute of Agricultural Technology, Suranaree University of Technology, under the supervision of Associate Professor Dr. Pramote Paengkoum. . He conducted the research in the topic of Enhancing the efficient utilization of rice stubble fermented by white-rot fungi and treated with urea as for goat diets. In 2016, Associate Professor Dr. Pramote Paengkoum had supported and recommended him to undertake research at the Animal Nutrition Group, Department Animal Sciences, Wageningen University, the Netherland, for 3 months for training in *in vitro* gas production techniques with Prof. Wouter Hendricks and Dr. John Cone.