MINISTRY OF EDUCATION AND TRAINING

MINISTRY OF AGRICUTURE AND RURAL DEVELOPMENT

NATIONAL INSTITUTE OF ANIMAL SCIENCE

PHAM NGOC THACH

RESEARCH ON USING FIBER-DEGRADING PROBIOTICS IN RATION REARING CATTLE

Major: Animal production

Code number: 9.62.01.05

SUMMARY OF THESIS

HANOI - 2020

THIS THESIS IS COMPLETED AT:

NATIONAL INSTITUTE OF ANIMAL SCIENCE

Supervisors:

1. Ph.D. PHAM KIM CUONG

2. Assoc.Ph.D. MAI VAN SANH

Opponent 1: Assoc.Ph.D. Bui Quang Tuan

Opponent 2: Assoc.Ph.D. Nguyen Hung Quang

Opponent 3: Assoc.Ph.D. Duong Van Hop

The thesis will be defended at the State Thesis Judging Council meeting at:

National Institute of Animal Sciences, Thuy Phuong, Bac Tu Liem, Ha Noi

Time : … h of day ….. month …… year 20....

Thesis can be found at:

1. Library of National Institute of Animal Sciences
2. National Library Hanoi

PUBLISHED SCIENTIFIC PAPER
RELATED THESIS

-------&&-------

1. **Pham Ngoc Thach, Pham Kim Cuong, Mai Van Sanh, Le Van Hung, Chu Manh Thang và Nguyen Thien Truong Giang. (2020).** Effects of adding fibrolytic Enzimes on *In Vitro* gas production of several rich celllose forage for ruminant. Journal of Animal Husbandry Sciences and Technics, No 259 – September 2020. pp 24 – 34.
2. **Pham Ngoc Thach, Pham Kim Cuong, Mai Van Sanh, Le Van Hung, Chu Manh Thang và Nguyen Thien Truong Giang. (2020).** The effects of adding fibrolytic enzymes on rumen degradation (*in sacco*) of several rich cellulose forage for ruminant. Journal of Animal Husbandry Sciences and Technics, No 260 – October 2020. pp 53 – 62

INTRODUCTION

1. The necessity of the study

Roughage feed is a major source of energy for ruminants, one of the main contents in roughage feed is cellulose, which is the most abundant biofilm-forming agent on earth (Paloheimo *et al.,* 2010). Many types of roughage are orginal botanic such as forage, crop by-products (straw, corn stalks, sugar cane leaves, etc.) and some industrial-agricultural by-products are usually of low qulity due to low digestibility and limited energy supply for the animal. This requires finding a way to optimize the use of forage in livestock production. One of the options mentioned is to use exogenous enzymes to aid and promote digestion (Avellaneda *et al.*, 2009). Exogenous enzymes used of ruminants are derived from fungi (mostly *Trichoderma longibrachiatum, Aspergillus niger* and *A. oryzae*) and from bacteria (*Bacillus spp., Penicillium funiculosum*) with cellulosolytic activity and high hemicellulo, combined in liquid or powder form and then added to the complete feed, hay, silage, concentrate, supplement or premix to increase nutrient degradability in the cell wall (Beauchemin *et al.,* 2004).

In Vietnam, there are few research projects on producing microbiological for digesting fiber-rich feed for animal, especially ruminant cattle. Stemming from the above reasons, the topic uses bio-products with high activity domestically produced on the basis of using safe microorganisms (mycelium, bacteria) to biosynthesize the cellulase enzyme system from Museum of microbiological strain that supplement for cow diets to improve the resolving ability of rich-fiber foods to improve feed efficiency, reduce product costs and contribute to increase economic efficiency for the animal production.

2. Objecties

– Determine the appropriate supplemental dose of fiber-degradable probiotics in order to improve the digestibility of rich-fiber feed ingredients.

– Evaluate the efficiency of using fiber-degradable probiotics in ration rearing beef and dairy.

3. The scientific and practical significance of the thesis

– The results of the thesis have proven that the addition of a fiber-degradable probiotic to the cow's diet has a positive effect on the rate and characteristics of *in vitro* gas production, rate and degradation characteristics *in sacco*, the nutrient digestibility of rich-fiber feed, while increasing body weight, reducing feed consumption for Sind crossbreeds and increasing productivity and reducing milk loss coefficient for crossbred cows ¾HF.

– The results of the topic are also references in specialized research and teaching.

– Supplementing with probiotics Best^{F}Rumen1 (A) and Best^{F}Rumen2 (C) has brought good results in beef and dairy farming, so the results of the topic are easy to apply practically to muscles beef and dairy farms to increase productivity and economic efficiency.

4. New contributions of the thesis

– The results of the thesis have added data on the chemical composition and nutritional value of ruminant feed;

– The thesis is the first scientific work that has evaluated the effects of the addition of a fiber-degrading probiotic to the diet on the speed and characteristics of *in vitro* gas gasproduction, the ratio and degradation characteristics *in sacco*, digestibility of organic matter, energy value (ME) and short-chain fatty acid content of some high-fiber foods. On the other hand, it also contributes to an effective suggestion of whether the supplementation of Best^{F}Rumen1 (A) and Best^{F}Rumen2 (C) preparations to the ability to degrade some high-fiber feed for crossbred sind and dairy cows ¾HF is raised in Vietnam.

– These new points have very high scientific and practical significance in teaching materials and scientific research as well as in current cattle breeding practices.

5. The layout of the thesis

The whole thesis consists of 161 pages, 4 chapters, 39 tables, 24 figures, references to 289 domestic and foreign documents, 2 scientific research works related to the thesis are published and the appendix.

CHAPTER I: OVERVIEW DOCUMENT

1.1. CHARACTERISTICS OF RICH FIBER FEED FOR RUMINANT

1.1.1. Structure of the plant cell wall

Lignocellulose is the main structural component of woody plants and other plants such as grass, rice, maize... The main components of lignocellulose are cellulose, hemicellulose and lignin. Furthermore, the composition of the same tree or different plants is different based on the age, stage of growth and development of the plant and other conditions. Cellulose, hemicellulose and lignin in the plant cell wall account for 35-50% respectively; (20-35%) and (10-15%) (Burton *và cs.*, 2010).

1.1.2. Xenlulo

Cellulose is an insoluble β-glucan linear molecule consisting of >15,000 D-anhydroglucopyranose radicals linked together by bonding with the-bridge (1-4). Plant cellulose is hydrolyzed by many cellulose: the endoglucanas randomly hydrolyze cellulose chains and produce cellulose isomers; exoglucanase produces cellobiose by hydrolysis of the cellulose chains in their final bond and the β-glucosidases that release glucose from the cellobiose (Khandelwal và Windle, 2013).

1.1.3. Hemicellulose

Hemicellulose is a heterogeneous polysaccharide group characterized by β (1-4) bonds in equatorial configurations, which include xyloglucans and glucuronoxylans, glucuronoarabinoxylans, glucomannans and galactoglucomannans. Hemicellulose is considered to be an important part of ruminant nutrition (Paloheimo *và cs.,* 2010).

1.1.4. Lignin

Lignin is a branched polymer formed by four alcohols (coniferyl, hydroxyconiferyl, coumaryl, and sinapyl alcohol) from the plant's phenylpropanoid, giving rise to different forms of lignin. Lignin has a high molecular weight, hardens the cell walls of plants, thus limiting the availability of structural carbohydrates to rumen microorganisms. Hence, this limits the lignin digestibility and overall availability of nutrients in the forage. (Moore và Jung. 2001).

1.2. FIBER DIGESTION IN RUMINANT

1.2.1. Summary of rumen function

The prominent feature of the digestive apparatus in ruminants is large bulging chambers, where there are favorable environmental conditions for microorganisms (bacteria, fungi, protozoa, mycoplasma, viruses and entities. Mycoplasma) ferment carbohydrates and other organic matter of the food. Main products of the fermentation here are volatile fatty acids, CH_4, CO_2 and ATP which are essential energy carriers for microorganisms to grow and develop.

1.2.2. Ruminal fermentation

Ruminal fermentation is a complex and involves the interaction of physical, biological and chemical processes, depending on the host, type of feed and microbial rumen.The final products of feed fermentation include: microorganisms, volatile fatty acids (VFA), CO_2, CH_4 and NH_3.The energy-carrying molecule (ATP) produced during fermentation is hydrolyzed, providing energy for the synthesis of microbial cells from metabolites and from substrates in gastric juice. grass (such as NH_3, amino acids, volatile fatty acids, CO_2, S, vitamins ...).

1.2.3. The process of digestion into plant cells of rumen microorganisms

1.2.3.1. Rumen microorganisms are involved in plant cell wall degradation

Plant cell walls are degradation by certain types of bacteria, fungi and protozoa, bacteria and fungi contribute about 80% of the degradation activity and 20% are from protozoa. Fibrobacterial strains such as *Fibrobacter succinogenes*, flavefaciens *Ruminococcus* and *Ruminococcus albus* are major organisms involved in the breakdown of plant cell walls in the rumen. Rumen fungi produce enzymes and break down substrates on a larger scale than rumen bacteria (Dijkstra và Tamminga, 1995). Protozoa activity in the rumen contributes significantly to the digestion of polymers into plant cell walls.

1.2.3.2. Production of complete enzyme system

The degradation of plant cell walls in the rumen is the result of a complex, coordinated interaction between groups of microorganisms producing different enzymes and food in the rumen. The hydrolysis of solid structured substrates requires a variety of rumen microorganisms because they can produce many enzymes and each has its own characteristics. The diversity of enzymes in the rumen comes not only from the diversity of the microbiota, but also from the diversity of the fibrolytic enzymes produced by each type of microorganism. (Yanke *và cs.*, 1995).

1.2.3.3. Microorganisms adhere to and penetrate into feed particles

Rumen bacteria digest food through the action of enzymes produced by them. Adhesion to feed particles is the most effective way for the bacteria to prolong their stay in the rumen and allow the enzymes they secreted to come into contact with the surface of feed particles to help efficiently digestion forage and grain grains in the rumen (McAllister và Cheng, 1996).

1.2.3.4. Interaction between microorganisms in rumen

The enzyme activity interaction is evidence of the activity of the rumen microbiota to digest the plant cell walls effectively in increasing the synthesis of xylanase, cellulase, increasing the rate and level of digestion cellulose (Teunissen *và cs.*, 1992).

1.3. USE OF PROBIOTIC FOR RUMINANT

1.3.1. Microbial strains are used as probiotics

The main microorganisms used as probiotics for ruminant animals include microorganisms derived from *Lactobacillus*, *Streptococcus*, *Entrococcus*, *Bacillus*, *Clostrididium*, *Bifidobacterium species*, *Propionibacterium*, *E.coli* Nissle 1917, *Megasphaera elsdenii* và *Prevotella bryantii*. Yeast and fungal probiotics such as Saccharomyces and Asperillus give better results in adult ruminants (Seo *và cs.*, 2010).

1.3.2. Mechanism of action of probiotics

Some of the proposed mechanisms of action of probiotics when added to animal diets are: (i) Production of a variety of antimicrobial agents and metabolic inhibitors such as organic acids, bacteria, diacetyl, antibiotic and H_2O_2 (Rolfe, 2002); (ii) Compete with pathogens for adhesion or nutrient sources (Guillot, 2003) because they are immobilized on the intestinal wall and resist being pushed out during peristalsis, as well as occupying a suitable position, reducing the potential of harmful organisms; (iii) Produces nutrients (such as amino acids, vitamins) or other growth factors that stimulate other microorganisms in the digestive tract and host; (iv) Immune regulation of the host (Isolauri *và cs.*, 2001); (v) Producing and stimulating enzymes; (vi) Metabolism and detoxification of compounds or substrates detrimental to the host.

1.3.3. Effects of probiotics on performance and health of ruminants

1.3.3.1. Nutritional feed intake

The animal more feed intake per day, the greater their chances of increasing their daily production. Probiotic supplementation has been found to increase feed intake and have a positive effect on ruminant performance. The reason for the increased feed intake and production capacity is that the probiotic supplement in the diet enhances the activity of cellulose-degrading bacteria and positively affects rumen pH, thereby improving rumen pH for fiber digestion and dry matter intake (Desnoyers *và cs.*, 2009).

1.3.3.2. Nutritional digestion

Improved ruminant production is closely related to improved nutrient digestibility. Ruminant probiotics are mainly selected to improve the digestibility of various foods in ruminants through increased pH, digestion of fiber and microbial protein synthesis rumen (Uyeno *và cs.*, 2015).

1.3.3.3. Improved growth rate

Probiotics can help increase the body weight of ruminants. Probiotic strains used alone or in combination have improved microbiological ecosystem, nutrient synthesis and absorption, stabilizing pH and lactate in ruminant animals thereby increasing live weight gain cattle (Musa *và cs.*, 2009).

1.3.3.4. Milk yield and quality

In dairy cattle, supplementation with probiotics produces beneficial effects on milk yield, fat and protein content in milk. Increased milk yield, solids none fat and milk protein ratios in dairy cows are related to numbers of bacteria that degrade cellulose, break down fiber and change volatile fatty acids in rumen (Poppy *và cs.*, 2012)

1.3.3.5. Meat yield and quality

The addition of probiotics has also been shown to increase the carcass weight and water hydration capacity, while also reducing cooking loss and meat firmness. The change in body fat is due to a change related to the total volatile fatty acid concentration which increases lipid synthesis and greater fat distribution in different body tissues. (Ceslovas *và cs.*, 2005).

1.3.3.6. Reduce the load of pathogens and enhance the immune response

Supplementing with probiotics has also been linked to beneficial immune system effects such as improved resistance to disease and reduced risk of allergies, stimulation of immune responses, and enhanced immune defense to more effective production and disease resistance (Ceslovas *và cộng sự.,* 2005).

1.3.3.7. Stabilize rumen pH, prevent and treat a number of metabolic diseases

a/ Stabilize rumen pH: Yeast has also been shown to modulate rumen pH and limit the risk of rumen acidosis through interaction with lactate production and utilization bacteria, reducing lactate levels in ruminants in the case of rumen acidosis by competing with S. bovis for starch fermentation or by stimulating lactate-using bacterial populations in ruminants (Lynch và Martin, 2002).

b/ Prevent and treat acidosis: Probiotics are effective in preventing and treating rumen acidosis effectively. This is said to be a probiotic supplement capable of regulating rumen bacterial activity, increasing cellulose hydrolysis and inhibiting lactic acid-producing bacteria, making rumen pH stable (Lettat *và cs.*, 2012).

c/ Reduce stress for young calf: Probiotics can reduce stress problems in calves, and other studies using LAB probiotics, have also been shown to reduce the incidence of diarrhea in calves (Jatkauskas và Vrotniakiene, 2010).

1.3.3.8. Establishment of bacteria that produce fiber-degradation enzymes in the rumen

Research with lamb by Chaucheyras-Durand and Fonty (2001; 2002) showed that in lambs supplemented with *S. cerevisiae* strain daily there was a higher rate of bacteria producing fibrosis enzyme and this microbial population was fine was more stable than the control sheep.

1.3.3.9. Improves fiber degradation in rumen

Most of the forage in ruminant diets are of low quality and by improving their digestibility, the use of fiber-degradable probiotics often improves the digestive function of the ruminant as well as in ruminant animals before weaning (Kumar và Sirohi, 2013)

1.4.3. The origin of bio-products of the thesis topic

Powdered probiotic products are researched, produced and tested by the National Institute of Animal Science and Institute of Microbiology and Biotechnology (Hanoi National University) for project *"Using probiotics to treat rich xellulose materials for animal feed "*.

– Probiotic named Best^{F}Rumen① (referred to as A): Is a product produced from the fermentation process of useful mycelium *A.oryzae* with the concentration of cellulase, amylase and xylanase reaching>1100 UI/g and ß-glucanase reaching >200 UI/g.

– Probiotic named Best^{F}Rumen② (referred to as C): Is a product produced from the fermentation process of useful mycelium *A.oryzae* and microbial strain *Lactobacillus*, *Bacillus* và *Saccharomyces* with concentration of enzyme cellulase, amilase and xylanase >1100 UI/g and ß-glucanase >200 UI/g and concentration of beneficial microorganisms >10^{9}CFU / g.

CHAPTER II: CONTENT AND METHOD OF RESEARCH

2.1. SUBJECTS, TIME AND LOCATION OF THE STUDY

2.1.1. Research subjects

a/ Probiotic: Powder products (i) named **Best^{F}Rumen➀** (referred to as A) và (ii) named **Best^{F}Rumen➁** (referred to as C). Activation of the probiotic (see item 1.4.3 page 6).

b/ Roughage: rice straw, Pangola hay, elephant grass 45 day of age, maize stalk.

c/ Animal: mature crossed breeding bull Sind, average weight of 200 kg, installed cannula, crosed breeding Sind and ¾ Holstein Frisian cow.

2.1.2. Location research

Department of Animal Nutrition and Feed, Animal Experiments and Domestic Animal Conservation Center (National Institute of Animal Sciences - NIAS), beef farms in Eaka (Đăk Lăk) and Bavi cattle and forage reseach centre.

2.1.3. Duration time of study: from 2013 to 2019

2.2. CONTENT OF RESEARCH

– Effects of supplementation probiotic on rate and characteristics of *in vitro* gas production of several fiber rich feeds for ruminant.

– Study the effect of probiotics supplementation on the ability to resolve some fiber-rich foods by *in sacco* method and change rumen microbiota

– Study the effect of probiotics supplements on the ability to digest feed by *in vivo* methods

– Study the effect of adding probiotics to the basic diet of Sind crossbred on the feed intake, weight gain, feed use efficiency and economic efficiency.

– Study on the effects of adding probiotics to the diet of dairy crossbred HF cows on the feed intake, yield and quality of milk, feed use efficiency and economic efficiency.

2.3. METHODOLOGY

2.3.1. Effects of supplementation probiotic on rate and characteristics of *in vitro* gas production of several fiber rich feeds for ruminant

* Materials and feeds

a/ Probiotics: (i) named **Best^{F}Rumen①** (referred to as A) and (ii) named **Best^{F}Rumen②** (referred to as C). The microbiological composition and activity of the products (see item 1.4.3 page 6).

b/ Roughage: rice straw; Pangola hay; elephant grass and maize stalk.

c/ Animal: Sind crossbred bulls, average live weight of 200 kg, rumen fistula to put canul.

* Prepare the experiment

a/ Chemical composition analysis

Feeds are sampled according to (TCVN 4325-2007) and analyzed by chemical composition according to the following standards: dry matter (TCVN 4325-2007), crude protein (TCVN 4328-2001), crude fiber (TCVN 4326-2007), lipid (TCVN 4331-2007), total mineral (TCVN 4327-2007), separately NDF, ADF and ADL were analyzed according to Goering and Van Soest (1970) at the Department of Animal Products and Animal Feed Analysis (NIAS).

b/ In vitro gas production: following the procedures of Menke and Steingass (1988). Adding to the probiotic sample (A) at the rate of 9‰; 11%; 13‰ and (C) according to the ratio 11‰, 13‰; 15‰ (on DM of feed), inject into a syringe 30ml of buffer 2 mixture and rumen fluid with samples and products. Put the cylinder in the incubator 39^{0}C and read gas at the times of 3, 6, 9, 12, 24, 48, 72 and 96 hours..

* Data recording and processing

– The amount of gas generated during *in vitro* fermentation of experimental feed was recorded at 3, 6, 9, 12, 24, 48, 72 and 96 hours.

– Characteristics of gas production when in vitro accumulation for 96 hours is calculated according to the equation of Orskov and McDonald (1979): $P = a + b (1 - e^{-ct})$

2.3.2. Study the effect of probiotics supplementation on the ability to resolve some fiber-rich foods by *in sacco* method and change rumen microbiota

* Materials

a/ Probiotic: (i) **Best^{F}Rumen①** (A) and (ii) **Best^{F}Rumen②** (C). The microbiological composition and activity of the products (see item 1.4.3 page 6).

b/ Roughage: rice straw; Pangola hay; elephant grass and maize stalk

The experiment was performed on 3 Sind crossbred bulls, average weight 200 kg/head. Cows are cut open for rumen detection and canul placed. Cows were kept in captivity and raised on a baseline diet (elephant grass 20 kg and 1 kg mixed feed) at a level maintained by the standards of Kearl (1982). Feeding design is presented in Table 2.1.

Table 2.1. Basal diet rearing cattle for in sacco (DM basic)

Feeds	Diets		
	I (control)	II	III
Rice straw*	*	*	*
Concentrates	*	*	*
Mineral block	adlib.	adlib.	adlib.
Probiotic A (g/head/day)**	-	30	40
Probiotic C (g/head/day)**	-	40	50

*Note: (i) * Pangola hay, Elephant grass and maize stalk desiged semilarly to rice straw; (iii) ** probiotic A stage 1 and probiotic C stage 2*

The experiment was following the procedures according to the plastic bag technique of Orskov et al. (1980). Rumen incubation: 4; 8; 16; 24; 48; 72 and 96 hours.

* Data recording and processing

– The ratio of in sacco solids resolution of the experimental feed was recorded at the time 4; 8; 16; 24; 48; 72 and 96 hours.

– Use the NEWAY program to calculate the dry solubility characteristics of *in sacco* according to the equation of Orskov and Mc Donal (1979): $P = a + b\,(1 - e^{-ct})$

– *Chemical composition analysis:* similar to item 2.3.1 page 7.

Study the effect of probiotics supplementation on changes in rumen microbiota

The rumen fluid in sacco was collected at 0 and 4 hours after feeding. Determination of total number of bacteria, unicellular and fungal populations according to Galyean (1989) by erythrocyte count chamber (Boeco, Hamburg, Germany).

2.3.3. Study the effect of probiotics supplements on the ability to digest feed by *in vivo* methods

Used of 15 Sind crossbred bulls with avarage age of 15 months, average weight of 200 kg for this study. Experimental design is shown in Table 2.4.

Table 2.4. In vivo experimental design

Items	Diet		
	ĐC0	*A40*	*C50*

Items	Diet		
	ĐC0	*A40*	*C50*
No animal (head)	5	5	5
Feeds*****	adlib.	adlib.	adlib.
Probiotic A (g/head/day)	-	40	-
Probiotic C (g/head/day)	-	-	50
Water	free	free	free
Mineral block	free	free	free

Note: (i) feeding standards based on Kearl (1982) for tropical cattle; (ii) ĐC0: control; (iii) A40 supplemented 40g Best^{F}Rumen①*; (iv) C50 supplemented 50g* Best^{F}Rumen②*; (v)* ***** *Order each type of feed in each period: rơm khô; cỏ Voi; thân cây ngô; cỏ khô Pangola; TMR.*

Thus, this experiment was conducted 5 times, each time using one type of food. *In vivo* digestibility of feed samples was determined by total fecal collection technique of Cochran and Galyean (1994).

** Data recording:* (i) Feed intake: weigh and record individual daily intake and residue; (ii) *In vivo* digestibility of diet: calculated from nutrient intake and excretion in the indicate as (%) of intake according to methods of Cochran and Galyean (1994), Burns and Pond (1994).

– *Chemical composition analysis:* similar to item 2.3.1 page 7.

2.3.4. Study the effect of adding probiotics to the basic diet of Sind crossbred on the feed intake, weight gain, feed use efficiency and economic efficiency

Conducted on 15 Sind crossbred male calves with an average age of 15-18 months, average weight 190 kg. Cattle were randomly assigned complete randomization block design (CRBD) with equal body weight (5 heads/lot). Diets in batches were similar in energy and protein. Experimental design is presented in Table 2.5.

Table 2.5. Experimental design

Items	ĐC0	A40	C50
No animal (head)	5	5	5
Initial period (day)	15	15	15
Elephant grass	adlib.	adlib.	adlib.
Concentrate*****	*	*	*
Probiotic A (g/head/day)		*40*	
Probiotic B (g/head/day)			*50*
Mineral block	free	free	free
Water	free	free	free

Ghi chú: (i) ĐC0: control; (ii) A40 supplemented 40g Best^{F}Rumen①*; (iii) C50 supplemented 50g* Best^{F}Rumen②*;* ***** *Concentrate (dried cassava: 25%; Rice bran: 50%; Maize: 9%; Soya bean: 16%) supplements ensure balance according to standards of Kearl (1982).*

Experimental cows were raised individually and fed 2 times/day (8am and 4pm). Feeding method: The probiotic is mixed well into the refined feed and fed first, then the roughage is fed later to ensure the animal finishing eaten probiotic.

* *Chemical composition analysis:* similar to item 2.3.1 page 7.
* *Data recording:* (i) Amount of food received; (ii) Cumulative volume; (iii) Absolute weight gain: kg/head/day; (iv) Increase relative volume: (%); (v) Consume feed for weight gain; (vi) Efficiency of feed: kg feed/kg increases weight; (vii) Preliminary calculation of the effectiveness of rearing.

2.3.5. Study on the effects of adding probiotics to the diet of dairy crossbred HF cows on the feed intake, yield and quality of milk, feed use efficiency and economic efficiency

The experiment was conducted on 15 ¾HF cows that are exploiting the third calving milk with average yield of 15 kg, milk 3-4 months, average weight 435 kg. Cattle were arranged in random blocks, completely uniform in milk yield. The diets of the batches were similar in terms of ME and protein. Experimental design is presented in Table 2.7.

Table 2.7. Experimental design

Items	ĐC0	A40	C50
No animal (head)	5	5	5
Initial period (day)	15	15	15
Experiment period (day)	90	90	90
Ration	NRC 2002	NRC 2002	NRC 2002
Probiotic A (g/head/day)		*40*	
Probiotic B (g/head/day)			*50*
Mineral block	free	free	free
Water	free	free	free

Ghi chú: ĐC0 (control); A40 supplemented 40g Best^{F}Rumen①*; (iii) C50 supplemented 50g* Best^{F}Rumen②*; Feed intake was adjusted through monitoring milk yield*

The experimental cows were individually raised and fed according to the milking cow nurturing protocol. Experimental cows were fed twice a day and fed 2 times/day (8am and 4pm). The probiotic was mixed evenly with the concentrate then pre-fed, elephant grass and Pangola hay were milled (5-7 cm) by a lawn mill and then mixed with silage before feeding.

* *Chemical composition analysis:* similar to item 2.3.1 page 7.
* *Data recording:* (i) Amount of food received; (ii) Milk yield: converted to standard milk (FCM) by the formula: FCM = actual milk production × [0.4 + (0.15 × actual milk fat ratio)]; (iii) Milk quality; (iv) Milk reduction coefficient; (v) Change of live weight; (vi) Feed efficiency: kg feed/kg FCM; (vii) Preliminary calculation of the effectiveness of rearing.

DATA PROCESSING FOR ALL EXPERIMENT

Data processed by the method of analysis of variance (ANOVA) on the software Minitab 16.0 (USA). General model: $Y_{ij} = \mu + A_i + \varepsilon_{ij}$. Of which: Y_{ij} *is the dependent variable,* μ *is the overall average,* A_i *effects of ration,* ε_{ij} *is the* *random error*. If the ANOVA shows differences, the Tukey mean pair comparison method will be used to determine the differences between treatments.

CHAPTER 3: RESULTS AND DISCUSSION

3.1. THE EFFECTS OF PROBIOTIC ADDITIONAL SUPPLEMENT ON THE SPEED AND CHARACTERISTICS OF *IN VITRO* GAS PRODUCTION SEVERAL RICH FIBER FEEDS FOR RUMINANT

3.1.1. The chemical composition of feeds

Table 3.1. Chemical composition of feeds

Type of feed	DM	CP	EE	NFE	CF	NDF	ADF	Ash
		(%) DM						
Rice straw	88,7	5,6	1,5	44,8	34,9	73,1	40,7	13,3
Pangola hay	87,7	7,0	2,6	48,2	36,2	78,2	42,2	6,1
Elephant grass	19,9	9,2	2,3	43,6	34,0	63,2	37,7	10,9
Maize stalk	18,0	9,9	2,4	59,3	22,8	61,4	30,4	5,7

Note: DM:Dry matter; CP:Crude protein; NFE:Nitrogen Free Extract: EE: Esters Extract; CF:crude fiber; NDF:Neutral Detergent Fiber; ADF:Acid detergent fiber; Ash:Total mineral

Data in table show that, the chemical composition such as DM, CP, EE, NFE, CF, NDF, ADF and Ash from diffrent type of feeds is big variation, example, elephant grass has lowest DM (19,9%) but the CP content was higher than its of rice straw and pangola hay, however, the CF, NDF và ADF is 34,0; 63,2 và 37,7% respectively, lower than its of rice straw and Pangola hay... In general differents of chemical parametters were due to different type of feeds, in addition that depend on weather and type of land of other region.

3.1.2. Gas accumulation and *in vitro* gas production characteristics of rice straw

At 24^h after incubation, the amount of gas generated was significantly different (P<0.05) between samples supplemented with probiotic with the control samples. When probiotic A was added to the rice straw, the gas generated was highest at 11‰ (21.1ml) supplementation and the lowest at 13‰ (17.1ml) supplementation, much higher than the control level (15.1ml). Also at this incubation period, when adding probiotic C to the rice straw, the amount of gas generated also had a significant difference (P<0.05), the amount of gas produced was the highest at 13‰ supplementation (20,3 ml), the lowest at 15‰ supplementation level (17 ml) but still higher than the control (15.1 ml).

The gas produce potential in the samples supplemented with probiotic A was the highest at level of 11‰ (29.6 ml), while supplementing with probiotic C, the gas potential was highest at level of 13‰ (29.3 ml) (P<0.05). Addition of probiotic A at level of 11‰ and probiotic B at 13‰ for higher OMD and VFA values (37.0; 36.3% and 0.46; 0.45 mmol), respectively is different from the remaining and control doses (P<0.05).

3.1.3. Gas accumulation and *in vitro* gas production characteristics of Pangola hay

Tại 24^h, lượng khí tích lũy trong mẫu đối chứng thấp hơn đáng kể (P <0,05) so với mẫu bổ sung, đạt cao nhất ở mức bổ sung ở mức 11 ‰ (25,5ml) và 13 ‰ (24,4ml) đối với men vi sinh A và C tương ứng trong khi mẫu đối chứng đạt 15,6ml. Khả năng tiêu hóa chất hữu cơ và axit béo bay hơi của cỏ khô Pangola trong thí nghiệm cũng cho thấy rằng việc bổ sung probiotic A ở mức 11 ‰ và C ở mức 13 ‰ cho giá trị OMD và VFA lần lượt là (41,1; 40,1% và 0,56; 0,54 mmol) đạt mức cao nhất so với các liều bổ sung còn lại và đối chứng (P <0,05).

3.1.4. Gas accumulation and *in vitro* gas production characteristics of elephant grass

The results showed that, when probiotic A was added to elephant grass, the gas were highest at level of 11‰ (31 ml) and lowest at 13‰ (23.9 ml) but still higher than the control (23.7ml). Similarly, when probiotic C was added to elephant grass, gas production was highest at 13‰ (30 ml). Regarding the digestibility of organic matter and volatile fatty acid of elephant grass, it was shown that when probiotic A was added at level of 11‰ and probiotic B was at 13‰, the values of OMD and VFA respectively is (47.3; 46.4% and 0.68; 0.66 mmol) reaching the highest level compared to the remaining additional doses and control (P<0.05).

3.1.5. Gas accumulation and *in vitro* gas production characteristics of maize stalk

The data obtained showed that at 24 hours after incubation, when probiotic A was added to the maize stalk, the gas produced was highest at level of 11‰ (31 ml) and lowest at 13‰ (25.3 ml) but still higher than control (22.8ml). Similarly, when probiotic C was added to corn stalks, gas production was highest at the supplementation level of 13‰ (30.1 ml) and higher than the control (22.8 ml). The parameters of digestibility of organic matter and total volatile fatty acids of maize grass hay showed that when probiotic A was added at level 11‰ and probiotic B was at 13‰, the values of OMD and VFA correspondingly (47.3; 46.5% and 0.68; 0.66 mmol) reached the highest level compared to the remaining additional doses and control (P<0.05).

General discussion of experiment 1

The results obtained from our study indicate that probiotic supplementation enhanced rumen fermentation rate by increasing the amount of gas produced, parameters of fermentation biogas characteristics and digestibility of organic matter. Lactic acid bacteria strains and to varying degrees may have altered rumen fermentation. The role of rumen microorganisms, including bacteria and protozoa, in digesting the soluble and insoluble portions of feed is well known. In this study, the improvement in gas production characteristics can be explained by the fact that supplementing with a fiber degraded enzyme inoculant improved the activity of the microbial population, significantly increasing the amount of volatile fatty acids in rumen fluid. The fibrous structural components of plant cell wall are the main source of carbohydrates for ruminant animals, they are fermented by rumen microorganisms to produce VFA, which is then absorbed through the rumen wall, contributing a main source of energy for the host animal (Candyrine et al., 2017). Improved substrate digestion through probiotic supplementation contributed to higher VFA production. In the current study, the improvement in total cellulose-degradable bacteria and bacteria could be attributed to the interaction of a complementary fibrolytic enzyme probiotic containing metabolites of the useful mycelium *A. oryzae* with *Lactobacillus, Bacillus* and *Saccharomyces* bacteria with rumen bacteria.

3.2. THE EFFECTS OF PROBIOTIC ADDITIONAL SUPPLEMENT ON *IN SACCO* DEGRADATION OF SEVERAL RICH FIBER FEEDS AND RUMEN MICROOGANISATION CHANGE

Based on the results obtained from content 1, we chose to supplement preparation A (Best^{F}Rumen①) at 9‰ mức and 11‰; preparations C (Best^{F}Rumen②) supplemented with 11 ‰ and 13‰ levels were used for this content study to select appropriate supplement levels.

3.2.1. Rate and degradability characteristics dry matter *in sacco* of rice straw

The results showed that the rate of dry matter degradation of rice straw at different times was different between diets. The speed of degradation increases strongly in the time from 4 – 48^{h} and from 72 – 96^{h} but increases but slowly. At the time of 24^{h} of incubation, the DM degradation ratio of the rice straw in the supplement diets of 40g probiotic A and C at level of 50g were 45.80 and 44.36%, respectively while control diet was 34.04% ($P<0.05$) and significantly higher than the other supplementation levels. In general, the DM degradation rate of rice straw in the diet supplemented with probiotics was 30% higher than the control diet, especially at the 24h incubation period.

The degradation efficiency of the diets was different: the highest was in the supplementary diet of 50g of probiotic C (34.37%) followed by the supplementary diet of 40g of probiotic A (34.18%) and lowest in the control diet (26.68%).

3.2.2. Rate and degradability characteristics dry matter *in sacco* of Pangola hay

The results showed that, at 24^{h} of incubation, the probiotic A supplementation at level of 30; 40g and probiotic C at level of 40; 50g is 39.47; 53,23 and 46,26; 47.06% while this value in the control was 36.69% ($P <0.05$). In general, the DM degadation rate of Pangola hay in the supplementation of fiber-degradable probiotics was 28-45% higher than the control diet, especially in the 24^{h} period.

The degradation efficiency of Pangola hay DM was highest in the supplemental to diet of 50g probiotic C (32.58%), followed by the supplementary diet of 40g of probiotic A (29.84%) and the lowest was in the diet control (24.21%) ($P <0.05$).

3.2.3. Rate and degradability characteristics dry matter *in sacco* of elephant grass

At 24^{h} incubation, the DM degradation of elephant grass in supplementation probiotic A at level of 30; 40g and probiotic C level 40; 50g was 61.71; 62,65 and

57.94; 59.49%, respectively meanwhile it was 56.59% in the control ($P<0.05$). In general, the rate of dry matter degradation of elephant grass in the dietary supplement of probiotics was 10% higher than that of the control diet, especially at the 24^h period.

The degradation efficiency of the diets was significantly different between the diets ($P<0.05$), the highest in the supplemental diet of 50g of probiotic C (46.51%) followed by the supplementary diet of 40g of probiotic A (46.32%) and lowest in the control diet (43.32%).

3.2.4. Rate and degradability characteristics dry matter *in sacco* of maize stalk

The results showed that, at 24^h of incubation, the supplementation probiotic A at level of 30; 40g and probiotic C at level 40; 50g is 62.63; 62.64 and 62.03; 63.71% while this value in the control was 58.87% ($P<0.05$). In general, the DM degradation rate of maize stalks in probiotic supplementation was 5-8% higher than that in the control diet, especially in the 24^h.

The degradation efficiency of the diets was different and ranged from 45.75 to 51.15%, highest in the supplement diet of 50g of probiotic C and lowest in the control diet (45.75%) ($P<0.05$).

3.2.5. Effect of probiotics addition to the basal diet on total rumen microorganisms

The effects of the addition of probiotic A with dosage (30 and 40g) and probiotic C dose (40 and 50g) to the basal diet were rice straw; Pangola hay; elephant grass and maize stalks to total rumen microorganisms showed that the mean values of microorganisms, protozoa (protozoa) and fungi were counted in the rumen of cattle fed the basal diets. is straw; Pangola hay; Elephant grass; The probiotic supplemented maize stem was higher than that of the control animal and there was a significant difference ($P<0.05$). For example, the number of microorganisms counted in the animal fed the 30 and 40 g dietary supplements of probiotic A ranged from 4.7×10^{10} - 4.8×10^{10} and 5.9×10^{10} - $6{,}0\times10^{10}$ respectively, for dietary supplementation of 40 and 50 g of probiotic C this value ranged from 4.1×10^{10} - 4.3×10^{10} and 4.6×10^{10} - 4.8×10^{10}, respectively, while in the control animal were 3.6×10^{10} - 3.8×10^{10} depending on diets.

Regarding the average value of protozoa counted in rumen fluid, it showed an increasing trend when supplementing probiotics, at the same time there was variation between different diets. For example, the highest mean protozoa in rumen fluid were in the group of animal supplemented with 40g of probiotic A ranging from 4.3×10^5 to 4.5×10^5 followed by 50g supplementation probiotic C ranged from 4.1×10^5 to 4.3×10^5, the lowest seen in control animals ranged from 3.7×10^5 to 3.9×10^5. The results for the number of fungal cells tended to be similar, which was highest in the group of cows that received 40g of probiotic A and followed by 50g of probiotic C, ranging from 2.4×10^4 -$3{,}5\times10^4$ and và $2{,}9\times10^4$ –

$3{,}1\times10^4$. The lowest value found in control cows ranged from $1{,}1\times10^4 - 1{,}3\times10^4$ depending on the diet.

General discussion of experiment 2

As the enzyme concentration increases, the rate of degradation of dry matter tends to increase. However, when the enzyme concentration reaches a certain threshold, the substrate concentration becomes a limiting factor for the reaction rate. This is perfectly plausible because the rate of dry matter degradation depends not only on the nature of the feed, but also on the concentration of enzymes present in the rumen. According to Colombatto et al., (2003) the best activation of enzymes is to remove the feed structural barriers that slow down the entry of microorganisms for digestion and thus speed up degradation. Results obtained from our study indicate that probiotic supplementation improved solids degradation of high-fiber feed, and there was a difference in the dry degradation rate value of the diets that supplement levels of different bio-products. Research by El-Waziry and Ibrahim, (2007) showed that, supplementing with exogenous enzymes from fungi and microorganisms increased the ability to break down fibers due to increased fibrolytic fermentation of rumen microorganisms thanks to the enzyme activity of the supplement. Supplementation with a fiber-degradable probiotic provided more substrate for the lactic fermenting bacteria group and promoted the growth of bacteria using cellulolytic and lactate (Chaucheyras-Durand et al., 2008), increasing the pH in the rumen (Mohamed et al., 2009; Paryad and Rashidi, 2009) and thereby increases the digestibility of fibers and fiber components (NDF and ADF).

In this study, the improvement in total cellulose-degrading bacteria and bacteria could be attributed to the interaction of an additional fibrinolytic enzyme probiotic containing metabolites of the useful mycelium *A.oryzae.* and *Lactobacillus, Bacillus* and *Saccharomyces* bacteria with rumen bacteria. According to Jiao et al. (2017) probiotic supplementation created favorable conditions for the activity of cellulosic bacteria and increased fiber digestibility. This is consistent with current research, in which an increase in the degradation of high-fiber foods simultaneously improves the rate of physical digestibility of the feed when supplementing with probiotics.

3.3. STUDY THE EFFECT OF PROBIOTICS SUPPLEMENTS ON THE ABILITY TO DIGEST FEED BY IN VIVO METHODS

3.3.1. Chemical composition of feeds

The obtained data showed that the straw with DM content, crude protein, crude lipid, crude fiber, NDF, ADF and total minerals were respectively as follows: 88.70%; 5.64%; 1.45%; 44.76%; 34.88%; 73.11%; 40.65% and 13.28%. For Pangola hay, the chemical compositions of DM, CF, NDF and ADF are 87.66% respectively; 36.21%; 78.19% and 42.23%. The chemical components of elephant grass in DM, crude protein, crude lipid, crude fiber, NDF, ADF and total mineral respectively: 19.98%; 9.19%; 2.34%; 34.04%; 43.04%; 63.22%; 37.65% and 10.86%. For corn stalks, dry matter, crude protein, crude lipid, crude fiber, NDF, ADF and total minerals respectively: 18.00%; 9.89%; 2.39%; 22.80%; 61.38%; 30.40% and 5.67%. Overall, the complete complete feed (TMR) had the highest crude protein content of 16.32%. However, due to the different types of feed, the fluctuation of the chemical composition and nutritional values is natural.

3.3.2. Feed intake and digestibility of nutrients of various feeds

The experiment was conducted 5 times, each time using one type of feed. Feed for cattle at the maintenance level consisted of the: (i) control; (ii) supplementation of probiotic A at dose of 40g/head/day and (iii) supplementation of probiotic C at dose of 50g/head/day

3.3.2.1. Feed intake

Research data shows that the intake of DM and organic matter differs depending on the type of feed, however, the general trend when supplementing with probiotics A and C results in the amount of DM intake increased compared to the control ($P<0.05$). Specifically, the DM intake of rice straw: 4.13 and 4.04 compared with 3.83 kg; Pangola hay: 4.15 and 3.93 compared to 3.81 kg; elephant grass: 4.49 and 4.49 versus 3.91 kg; corn stalks: 3.95 and 4.09 versus 3.90 kg; TMR: 4.11 and 4.20 versus 3.89 kg.

Organic matter intake also tended to be similar to DM obtained from experimental animals compared to controls, for example, organic matter intake: 3.59 and 3.52 vs 3.34 kg; Pangola hay: 3.84 and 3.68 vs. 3.62 kg; elephant grass: 4.0 and 4.01 vs. 3.38 kg; corn stalks: 3.75 and 3.74 vs. 3.61 kg; TMR: 3.63 and 3.72 vs. 3.38 kg.

3.3.2.2. The rate of digestion of nutrients

The DM digestibility rate of the rice straw in the animal fed control diet was 45.50% while the rice straw animal fed the supplement diets A and C were 48.99 and 49.49%, respectively ($P <0.05$) similarly, for Pagola hay, elephant grass, maize stalks and complete complete feed (TMR) were 39.53 vs. 45.26 and 44.65%,; 57.03 vs. 63.43 and 62.75%; 46.98 vs. 49.24 and 48.96%; 64.99 vs. 67.92 and 65.74%, respectively ($P <0.05$).

Protein digestibility was higher when supplementing probiotics A and C compared to the control ($P<0.05$). Specifically, rice straw: 42.65 and 42.83 vs. 36.06%; Pangola hay: 50.77 and 47.21 vs. 33.31%; elephant grass: 71.11 and 70.97 vs. 65.75%; corn stalks: 65.06 and 64.94 vs. 62.18%; TMR: 84.72 and 83.61 vs. 67.79%. For crude fiber, the digestibility of this indicator of rice straw, Pangola hay, elephant grass, maize stalks and TMR when supplemented with inoculants A and C also increased significantly ($P <0, 05$) compared to the control is 62.40 and 62.66 vs. 60.10%; 58.30 and 50.46 vs. 36.57%; 67.51 and 66.02 vs. 62.92%; 39.53 and 39.63 compared with 34.91%; 61.86 and 60.63 vs. 53.68%.

General discussion of experiment 3

The results on the DM and organic matter intake of the feeds in this study varied differently, which can be explained by the difference in chemical composition and nutritive value of feeds. However, the general trend when adding a bio-product A and C, the amount of feed and organic matter intake increased compared to the control (P <0.05). In this study, adding probiotics A (containing strains of *Aspergillus oryzae*) and probiotic C (containing strains *Aspergillus oryzae*, *Lactobacillus*, *Bacillus* and *Saccharomyces*) to the diet significantly increased digestibility major nutrients are found in feeds. Specifically, the digestibility of DM of the rice straw of animal fed on control diet was 45.50% while its value of anmals fed on the supplement diets A and C were 48.99 and 49.49%, respectively (P<0.05) similarly, for maize stalks, elephant grass, Pagola hay and TMR is 46.98 vs. 49.24 and 48.96%; 57.03 vs. 63.43 and 62.75%; 39.53 vs. 45.26 and 44.65%; 64.99 vs. 67.92 and 65.74%, respectively (P<0.05). The reason for the increase in feed intake is that supplementing with probiotics in the diet enhances the activity of cellulose-degrading bacteria in the rumen and positively affects rumen pH, thereby improving the process fiber degradation and dry matter intake (Desnoyers et al., 2009).

3.4. STUDY THE EFFECT OF ADDING PROBIOTICS TO THE BASIC DIET OF SIND CROSSBRED ON THE FEED INTAKE, WEIGHT GAIN, FEED USE EFFICIENCY AND ECONOMIC EFFICIENCY

3.4.1. Chemical composition and nutritive value of the feeds

The results showed that the metabolic energy (MJ/kg DM) values of elephant grass and concentrate in the experiment were 8.69 and 11.6 MJ/kg DM, respectively. About the MJME/kg DM of elephant grass and forage in this experiment was not much different from the results of research on two similar foods previously in Vietnam. According to Dinh Van Muoi. (2012) ME value of the feed depends on many factors, among them the chemical composition of the feed and the digestibility of nutrients contained in that feed.

3.4.2. Effects of dietary supplement of probiotics on intake of cattle

Table 3.14. Effect of probiotic supplementation on feed intake (Mean ± SD)

Items	Diet		
	ĐC (0)	A40	C50
Dry matter intake			
kg/head/day	$6,40^{c} \pm 0,91$	$7,04^{a} \pm 0,47$	$6,72^{b} \pm 0,46$
kg/100 kg BW	$2,57^{c} \pm 0,11$	$2,70^{a} \pm 0,18$	$2,61^{b} \pm 0,14$
g/BW^{0,75}	$102,04^{c} \pm 17,64$	$108,63^{a} \pm 19,32$	$104,48^{b} \pm 20,11$
Nutritional intake			
ME, *MJ/head/day*	$61,47^{c} \pm 8,97$	$67,28^{a} \pm 4,05$	$63,62^{b} \pm 4,08$
CP, *kg/head/day*	$0,81^{c} \pm 0,12$	$0,89^{a} \pm 0,05$	$0,84^{b} \pm 0,05$
NDF, *kg/head/day*	$3,60^{b} \pm 0,49$	$4,01^{a} \pm 0,32$	$3,90^{a} \pm 0,32$
ADF, *kg/head/day*	$2,05^{b} \pm 0,28$	$2,29^{a} \pm 0,19$	$2,22^{a} \pm 0,18$

Note: ĐC0 (control); A40 supplemented 40g Best^F^Rumen①*; (iii) C50 supplemented 50g* Best^F^Rumen②*; BW: body weight;*

Table 3.14 shows that the amount of dry matter intake ranged from 6.40 - 7.04 kg/head/day, the lowest was in the control diet group (ĐC0), the highest in the animal fed on dietary supplementation (A40) (P <0.05). The amount of dry matter ingested per 100 kg of body weight ranged from 2.57 to 2.70 kg, the highest was found in ration cattle (A40) followed by diet (C50) and lowest was in the animal control group (ĐC0) is 2.70; 2.61 and 2.57 kg (P <0.05).

Regarding nutrients intake such as (ME-MJ/head/day), crude protein (kg/head/day), NDF (kg/head/day) and ADF (kg/head/day) was (61.47 - 67.28 MJ); (0.81 - 0.89 kg); (3.60 - 4.01 kg) and (2.05 - 2.29 kg), respectively depending on the diet and there was a difference (P <0.05) between the group of animals that ate the diet (A40) and (C50) compared with animal fed on the control diet (ĐC0).

3.4.3. Effects of dietary supplement of probiotics on live weight changes

Table 3.15. Live weight change of cattle (Mean ± SD)

Items	Diet		
	ĐC (0)	A40	C50
Initial BW ban đầu (kg)	224,6 ± 10,6	222,4 ± 8,3	225,0 ± 12,5
BW after 3 months (kg)	273,8^{b} ± 7,7	298,2^{a} ± 8,2	290,4^{a} ± 10,5
ADG (kg/head/day)	***0,586^{c} ± 0,050***	***0,902^{a} ± 0,033***	***0,779^{b} ± 0,037***

Note: ĐC0 (control); A40 supplemented 40g Best^F^Rumen①*; (iii) C50 supplemented 50g* Best^F^Rumen②*; BW: body weight; ADG: average daily gain*

The results showed that, the average live weight increase in the whole experimental period, the average daily gain increase of the group of cattle that ate the experimental diet supplemented with probiotic (0.779 - 0.992 kg/head/day), meanwhile cattle ate the control diet (ĐC0) was only 0.586 kg/head/day and there was a difference (P <0.05). This result is also shown by the relative growth of cow groups eating different diets from high to low order of (A40), (C50) and (ĐC0) respectively 7.3; 6.3 and 4.9%.

3.4.4. Feed efficiency

Table 3.16. Feed efficiency of experimental cattle (Mean ± SD)

Items	Diet		
	ĐC (0)	A40	C50
DM intake (kg/head/day)	6,40^{c} ± 0,91	7,04^{a} ± 0,47	6,72^{b} ± 0,46
ADG (kg/head/day)	0,586^{c} ± 0,050	0,902^{a} ± 0,033	0,779^{b} ± 0,037
FE (kg DM/kg tăng KL)	10,92^{c} ± 1,02	7,80^{a} ± 1,13	8,63^{b} ± 1,08
ME intake (MJ/head/day)	61,47^{c} ± 8,97	67,28^{a} ± 4,05	63,62^{b} ± 4,08
EE (g live gain/MJME)	9,53^{c} ± 1,15	13,41^{a} ± 1,21	12,24^{b} ± 1,18

Note: ĐC0 (control); A40 supplemented 40g Best[F]Rumen①*; (iii) C50 supplemented 50g* Best[F]Rumen②*; ADG: average daily gain; FE: Feed efficiency; ME: metabolism energy; EE: Energy efficiency*

The results showed that feed consumption for 1 kg live weight gain ranged from 7.80 - 10.92 kg DM/kg live weight gain, the lowest in the group of cows fed dietary supplements A (A40) followed by the group of cows on the diet supplemented with probiotics C (C50) and highest in the group of cows on the control diet (ĐC0), respectively low-high, 7.80; 8.63 and 10.92 kg and there are differences (P <0.05).

The efficiency of feed energy use of the experiment ranged from 9.53 - 13.41g of weight gain/MJ metabolic energy that was highest in the group of animal fed with diet A followed by is the group of animal on the dietary supplement with probiotics C and the lowest in the group of animal control diet is 13.41; 12,24 and 9,53 (g gain/MJME) and there was difference (P <0.05).

3.4.5. Preliminary calculation of feeding efficiency of experiment

Based on the materials prices for the concentrate, animal bought and sold at the beginning and the end of the experiment, the feeding effect was carried out preliminary calculations. The results showed that, the profit earned in the group of animal raised by dietary supplements A and C were 1,226,000 VND and 1,003,000 VND/head/month, respectively while the group of animal fed the control diet also reached 713,000 VND/head/month.

General discussion of experiment 4

Regarding the feed intake of Sind crossbred cattle raised in the experimental diet, ranged from 6.40 - 7.04 kg dry matter/head/day (Table 3.14), specifically the amount of experimental group (A40) and (C50) were 7.04 and 6.72 kg/ animal/day, respectively, while it was 6.40 kg/head/day in the control group (ĐC0) and there was difference (P<0.05). This is consistent with research results of Kumar and Sirohi, (2013); Præsteng et al. (2013) is a fibrolytic enzyme commonly used to improve the digestive function of adult ruminants. According to Kearl (1982) cows 200-300 kg, weight gain 0.75 kg/head/day need 5.4 –7.4 kg dry matter/head/day.

The results of cattle live weight gain in this experiment (0.586 - 902 kg/head/day) are higher than the previous study results of Vu Van Noi et al. (1999) and higher than the results of Clarke et al. (1996), the increase in weight in fattened Sind crossbred cows was 0.60 - 0.66 kg in discarded cows. Thus, supplementation of probiotics has enhanced the ability to break down fiber in the diet, thereby increasing the digestibility and absorption of nutrients of the diet. The higher mass gain in ruminants is due to increased cellulose degradation activity thereby improving fiber breakdown thereby reducing the activity of ammonia-producing microorganisms resulting in the available protein being absorbed receptor in the intestinal tract is greater (Kowalski et al., 2009).

Feed consumption for 1 kg of live weight gain of cattle in this study ranged from 7.80 - 10.92 kg DM/kg corresponding to the group of cows fed diets supplemented with probiotics (40g) and C (50g). Thus, this indicator of the group of animal on diets supplemented with probiotics is within the recommended range of ARC (1980); NRC (1984); INRA (1989); and AFRC (1993) ranged from 7.1 to 8.8 kg DM/kg live weight gain. According to Kearl (1982) cattle 200-300 kg, weight gain of 0.75 kg/day needs 5.4-7.4 kg of DM/head/day. Feed energy efficiency of the dietary supplements of probiotics (A40) and (C50) in this experiment were 13.41 - 12.24 g gain/MJ ME, respectively. Thus, this index is within the range of energy efficiency values calculated from the feeding standard of Kearl (1982); NRC (1984) and AFRC (1993) from 11.45 - 12.58g weight gain/MJ exchanged energy.

3.5. STUDY ON THE EFFECTS OF ADDING PROBIOTICS TO THE DIET OF DAIRY CROSSBRED HF COWS ON THE FEED INTAKE, YIELD AND QUALITY OF MILK, FEED USE EFFICIENCY AND ECONOMIC EFFICIENCY

3.5.1. Thành phần hóa học và giá trị dinh dưỡng của thức ăn thí nghiệm

Table 3.18. Chemical composition and nutritional value of the feed

Type of feed	DM (%)	CP	CF	NDF	ADF	Ash	ME (Kcal)
				% DM			
Elephant grass	12,8	6,95	37,19	69,31	40,11	11,88	2061
Pangola hay	91,68	5,14	39,15	80,88	43,67	4,28	1312
Corn silage	22,07	6,72	33,81	68,88	44,83	5,7	2500
Cassava root	27,7	3,25	2,61	19,57	4,05	2,53	2884
Concentrate C40	91,25	16,8	4,92	19,4	6,3	8,13	2740

Analysis of the chemical composition and nutritional value of the experimental dairy feed showed that, except for mixed bran and fresh cassava, the raw feed ingredients used in combination with the feeding diet fluctuated in the chemical ingredients. This difference in results may be due to the different sources of feed ingredients, different climatic and soil conditions in each region.

3.5.1. Effects of dietary supplement of probiotics on feed intake

Table 3.19. Effect of probiotic supplementation on feed intake of dairycattle (Mean ± SD)

Items	Diet		
	ĐC (0)	A40	C50
Dry matter intake			
Kg/head/day	$13,3^{b} \pm 1,82$	$13,6^{a} \pm 1,36$	$13,5^{a} \pm 1,54$
% Body weight	2,92	3,02	2,99
Nutritional intake			
CPI (kg/head/day)	$1,4^{b} \pm 0,46$	$1,5^{a} \pm 0,48$	$1,5^{a} \pm 0,53$

Items	Diet		
	ĐC (0)	A40	C50
NDFI (kg/head/day)	6,4^b ± 1,57	6,6^a ± 1,60	6,5^a ± 1,49
ADFI (kg/head/day)	3,4^b ± 0,53	3,6^a ± 0,85	3,5^a ± 0,74
OMI (kg/head/day)	12,5^b ± 0,82	12,8^a ± 0,85	12,7^a ± 0,84

Note: ĐC0 (control); A40 supplemented 40g Best^{F}Rumen①*; (iii) C50 supplemented 50g* Best^{F}Rumen②*; CPI: protein intake; NDF: NDF intake; ADF: ADF intake; OMI: organic matter intake*

The results in Table 3.19 show that the amount of dry matter intake whole period ranged from 13.3 to 13.6 kg/head/day and there was no difference in the amount of dry matter intake by cow in the experimental diets A40 and C50, however, had a clear difference (P<0.05) in the amount of feed intake of the group of cows fed the experimental diet compared to control diet (ĐC0). Thus, the palatability of the ration is acceptable, and the amount of food received depends on the type of feed and the structure of the diet.

Regarding the intake of nutrients such as CPI, NDFI, ADFI and OMI in cows fed the experimental diets A40 and C50, fluctuated (1.5 kg/head/day); (6.5 - 6.6 kg/head/day), (3.5 - 3.6 kg/head/day) and (12.7 - 12.8 kg/head/day) were higher than that of cows in control diet (ĐC0) (1.4 kg/head/day; 6.4 kg/animal/day; 3.4 kg / head/day and 12.5 kg/head/day) (P<0.05). This shows that the addition of probiotics has enhanced the ability to break down fiber in the diet, improving the digestibility rate, thereby increasing the amount of food intake.

3.5.2. Effects of dietary supplements of probiotics on live weight change

The results of changes in the live weight of cow in the experiment showed that the average increase in weight during the experiment ranged from 8.1 to 10.2 kg. In general, the weight gain of the experimental cows is low, the reason the dairy cows have 3th calved so the weight of the dairy cow has reached maturity and the weight gain of all 3 groups of cows is low.

3.5.3. Effects of dietary supplements of probiotics on milk yield

Table 3.21. Milk yield of experimental dairy cattle (Mean ± SD)

Items	Diet		
	ĐC (0)	A40	C50
Initial milk yield (kg FCM/day)	14,8 ± 0,98	15,6 ± 1,81	15,3 ± 1,75
Final milk yield (kg FCM/day)	11,77^b ± 1,12	13,56^a ± 1,12	13,01^a ± 1,03
Average milk yield (kg FCM/day)	13,23^b ± 1,66	14,53^a ± 1,40	14,11^a ± 1,47
MRC (%)	20,47^a ± 7,58	13,08^b ± 7,18	14,97^b ± 6,72

Note: ĐC0 (control); A40 supplemented 40g Best^{F}Rumen①*; (iii) C50 supplemented 50g* Best^{F}Rumen②*; FCM: Fat corrected milk; MRC: Milk reduction coefficient in cows*

In terms of standard milk yield (FCM) at the beginning of the period ranged from 14.8 to 15.6 kg, by the end of the experiment the highest milk yield was in the group of cows that ate the experimental diet (A40), followed by the group of cows that ate the experimental diet (C50) and lowest in the control group (ĐC0) were 13,56; 13.01 and 11.77 kg head, respectively and there was a difference (P <0.05) between the group of cow raised by the experimental diet compared to the control cow.

The average milk yield of the experimental cows for the whole period ranged from 13.23 to 14.53 kg/head and there was a difference between the experimental diets and the control cows (P<0.05). Thus, supplementation of probiotics has increased milk yield from 6.7 to 9.8% while maintaining milk stability.

The milk loss coefficient of experimental cows showed that: lowest in the group of cows fed the experimental diet (A40) followed by the group of cows fed diet 3 (C50) and highest in the control group (ĐC0) is 13.08; 14.97 and 20.47%, respectively.

3.5.4. Effects of dietary supplement of probiotics on milk quality

The results showed that the DM content ranged from 12.20 to 1.48%, highest in the group of cows fed the A40 diet and lowest in the group of cows fed the control diet. Similar to the DM content, the highest milk protein content was 3.37% in the group of cows fed the A40 diet and the lowest was 3.19% in the group of cows fed the control diet (ĐC0). The milk fat content was also different (P<0.05) between the group of cows fed the diets supplemented with probiotics compared to the control group (4.33 and 4.21 vs 4.10%). For fat-free solids (SNF), there was also a difference (P<0.05) between the group of cows fed the probiotic diet versus the control group (7.87 and 7.61 compared with 7.38%). This shows that the addition of probiotics and dairy feed not only increase milk yield but also improve milk quality, which is reflected in the milk fat value of the dairy cow group. inoculant supplementation compared with milk fat of the control diet group (4.33 and 4.21 vs 4.10%). The indicators of non-fat solids and milk density also showed that the cow group received the supplementary diet with higher value than the group of cows fed the control diet.

3.5.4. Feed efficiency

Table 3.23. Feed efficiency of experimental daity cattle (Mean ± SD)

Items	Diet		
	ĐC (0)	A40	C50
DM intake (kg/con/ngày)	$13{,}3^{b} \pm 1{,}82$	$13{,}6^{a} \pm 1{,}36$	$13{,}5^{a} \pm 1{,}54$
Average milk yield (kg FCM/day)	$13{,}23^{b} \pm 1{,}66$	$14{,}53^{a} \pm 1{,}40$	$14{,}11^{a} \pm 1{,}47$
FE (kgDM/kg FCM)	1,01	0,94	0,96

Note: ĐC0 (control); A40 supplemented 40g Best[F]Rumen①*; (iii) C50 supplemented 50g* Best[F]Rumen②*; FE: Feed efficiency*

Table 3.23 shows that feed consumption (kgDM/kg of FCM) ranges from 0.94 to 1.01 kg, the lowest in dairy cows fed the diet A40 (0.96 kg DM) followed and cows fed the C50 diet (0.99 kg) and the highest was in the cows fed the diet

ĐC0 (1.01 kg DM/kg of FCM).

3.5.5. Preliminary calculation of feeding efficiency of experiment

On the basis of materials prices for feed and milk sales at the beginning and end of the experiment, preliminary calculation of economic efficiency is carried out. The results showed that income in the group of cows raised by dietary supplements probiotic A (Best^{F}Rumen①) and C (Best^{F}Rumen②) was 1,722,000 and 1,558,500 VND/ head/month, respectively) meanwhile the group of cows that ate the control diet only reached 1,153,500 VND/head/month. The difference in income compared to the control is from 405,000 to 568,500 VND/head

General discussion of experiment 5

The amount of DM intake in this study ranged from 13.3 to 13.6 kg/head/day. The amount of DM intake per 100 kg of body weight varied from 2.92 to 3.02 kg, according to NRC (2001), the amount of DM intake of cattle ranged from 2.8 to 3 2%. Regarding nutrient intake, CP (1.5 kg/head/day), NDF (6.5 - 6.6 kg/head/day), ADF (3.5 - 3.6 kg/head/day) and OMI (12.7 - 12.8 kg/head/day) were both higher in the cow fed the experimental diet (A40) and (C50) compared to the cows fed the control diet (ĐC0). This suggests that the addition of probiotics enhances the ability of the dietary fiber to break down through the effect of faster the breakdown of the silage sub-particles in the rumen and thus through the rumen faster, higher digestibility (Moseley and Jones, 1984; Jamot and Grenet, 1991), thereby increasing feed intake. According to Poppy et al. (2012) adding *S. cerevisiae* strain to dairy cow diets, the amount of DM intake by cows at the beginning and the end of the period for milk increased by 0.62 and 0.78 kg/day, respectively. Increasing feed intake along with improved microbial digestibility can be thought to be the mechanism of action of probiotics improving the performance of animals.

The average milk yield for the whole period of experimental cows in this study ranged from 13.74 to 15.36 kg/head and there was a difference between the experimental diets and the control cows ($P<0.05$), the addition of probiotics A40 and C50 increased milk yield from 6.7 to 9.8%. This result is consistent with the results of big data analysis by Poppy et al. (2012) showed that bio-products containing *S. cerevisiae* used as supplement to the diet increased milk yield by 1.18 kg/day.

Regarding milk quality, the milk fat content in this study also differed ($P <0.05$) between the group of cows fed the diets supplemented with probiotics A40 and C50 compared with the control group (4.33 and 4.21 vs. 4.10%). According to research by Dutta et al., (2009), direct supplementation of two strains of *Enterococcus faecium* and *Saccharomyces cerevisiae* increases the percentage of fat in cow's milk due to increased production of volatile fatty acids. According to Poppy et al. (2012) adding *S. cerevisiae* strain to dairy cow diets increased standard milk fat by 1.61 kg/day, milk protein by 0.03 kg/day and standard milk calibrated energy by 1.65 kg/day. Increases in milk yield, fat-free solids and milk protein ratios in dairy cows are associated with numbers of bacteria that break down cellulose, break down fiber, and change volatile fatty acids in rumen (Martin and Nisbet, 1990).

Feed consumption (kgDM/kg FCM) ranged from 0.96 - 1.05 kg, the lowest found in the group of dairy cows fed the A40 diet (0.96 kg VCK) and the cow fed the C50 diet (0.99 kg) and was highest in the control diet (1.05 kg VCK / kg FCM milk). Research by Weiss et al. (2008) showed that, when adding *Propionibacterium* P169 and *Saccharomyces cerevisiae* strains to the diet, there was no significant difference in cow's milk yield compared to the control, but reduced feed consumption and increased efficiency by 4.4%. use of energy.

CHAPTER IV: CONCLUSION AND RECOMMENDATION

4.1. Conclusions

– Supplementing with probiotic A (Best^{F}Rumen①) at levels of 11 ‰ and 13 ‰; or probiotic C (Best^{F}Rumen②) level 13 ‰ and 15 increased *in vitro* gas production, organic matter digestibility and VFA compared with other levels of addition.

– Supplement of probiotic A level of 40g; or probiotic C at 50g level increased the ratio of degradation dry matter *in sacco*, the number of bacteria, protozoa (protozoa) and fungi (fungi) in the rumen of cows eating the basal diets of straw; Elephant grass; Pangola hay; maize stalks compared to the other level supplement.

– Supplement of probiotic A level of 40g; or probiotic C level of 50 g on straw base diets; maize stalks; elephant grass; Pangola hay or TMR increased feed intake and organic matter intake; increased digestibility *in vivo* of Crude Protein, Crude Fat, NDF, ADF, Crude Fiber, Organic matter compared to the other level or without supplement.

– Supplementing probiotics A of 40g; or probiotic C of 50g in Sind crossbreeding diets increased weight from 586.0 kg/head/day (without supplement) to 0.779 - 0.902 kg ($P < 0.05$), reduced feed consumption (kgVCK/kg live weight gain) from 10.92 kg (without supplement) to 7.80 - 8.63 kg. Increased energy efficiency of the ration from 9.53 to 12.24 - 13.41 g weight gain/MJ ME

– Supplementing probiotics A of 40g; or a probiotic C of 50g to the ¾ HF cow feeding diets (i) increased milk yield from 13.23 kg to 14.53 kg FCM milk; (ii) reducing feed consumption (kg VCK/kg of standard milk) from 1.01 kg (without supplementation) to 0.94 - 0.96 kg VCK/kg of FCM milk (iii), reducing the coefficient milk loss from 20.47% (without supplement) to 13.08 and 14.97%; (iv) improving milk quality index.

4.2. Recommendation

Use a fiber degradation enzyme product A (Best^{F}Rumen①) at a dose of 40g/head/day or a preparation C (Best^{F}Rumen②) at a dose of 50g/head/day to the diets for rearing beef crossbred cattle and HF dairy cows.