การเสริมมันเส้นหมักยีสต์ทดแทนอาหารข้นต่อนิเวศวิทยารูเมน และอัตราการเจริญเติบโตในโคบราห์มันลูกผสม

Supplementation of Yeast Fermented Cassava Chip (YFCC) Replace Concentrate on Rumen Ecology and Average Daily Gain in Crossbred

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บทคัดย่อ

การวิจัยครั้งนี้มีวัตถุประสงค์เพื่อศึกษาผลของการเสริมมันเส้นหมักยีสต์ทดแทนอาหารข้นต่อนิเวศวิทยารูเมนและอัตราการเจริญเติบโตในโคบราห์มันลูกผสม โดยใช้ลูกโคบราห์มันเพศเมียอายุ 1 ปี น้ำหนักตัว 250±10 กก. จำนวน 6 ตัว แบ่งออกเป็น 2 กลุ่ม แบบอิสระต่อกัน (Group T-test) ดังนี้กลุ่มที่ 1 ได้รับอาหารข้นโปรตีน 12 เปอร์เซ็นต์ (T1) และกลุ่มที่ 2 ได้รับมันเส้นหมักยีสต์ CCFY (T2) โคทดลองทุกตัวเลี้ยงในคอกเดี่ยวได้รับอาหารทดสอบในปริมาณ 1.5 เปอร์เซ็นต์ของน้ำหนักตัว ร่วมกับฟางข้าวเป็นอาหารหยาบให้กินเต็มที่และมีน้ำสะอาดและแร่ธาตุอย่างพอเพียง ผลการศึกษาพบว่าการเสริมมันเส้นหมักยีสต์ทดแทนอาหารข้นไม่มีผลแตกต่างทางสถิติต่อปริมาณการกินได้ทั้งหมด โดยสัตว์ทดลองกลุ่มที่ 1 มีปริมาณการกินได้เฉลี่ย 9.1 กก./ตัว/วัน และกลุ่มที่ 2 มีปริมาณการกินได้ 8.7 กก./ตัว/วัน (2.9 และ 2.8 เปอร์เซ็นต์ ของ

คำสำคัญ: มันเส้นหมักยีสต์ อาหารข้น กระบวนการหมักในรูเมน โคบราห์มันลูกผสม

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น้ำหนักตัวตามลำดับ) จุลินทรีย์กลุ่มแบคทีเรียและเชื้อรา ไม่ลดจำนวนประชากรของกลุ่มโปรโตซัวในกระเพาะรูเมน จากการศึกษาครั้งนี้จะเห็นได้ว่าการเสริมมันเส้นหมักยีสต์ช่วยเพิ่มประสิทธิภาพกระบวนการหมัก เพิ่มประชากรของจุลินทรีย์ในกระเพาะรูเมน แต่อัตราการเจริญเติบโตได้เพิ่มขึ้น ดังนั้นมันเส้นหมักยีสต์ เมื่อพิจารณาจากตาราง Table1 ต้นทุนต่อหน่วยการผลิตราคา 7.50 บาท/กิโลกรัม (T2) จะมีต้นทุนที่ต่ำกว่า จึงสามารถใช้เป็นแนวทางในการผลิตอาหารต้นทุนต่ำได้ ในโคบราห์มันลูกผสม

Abstract

Six, female one year old crossbred Brahman cattle with initial body weight of 250±10 kg were randomly divided into two groups and received concentrate at 12 %CP (T1), and cassava chip fermented yeast (CCFY) (T2). The animals were offered the treatment concentrate at 1.5 %BW and rice straw was fed *ad libitum*. Means were compared using Group T-test. All animals were kept in individual pens and free access to clean water and mineral blok. The results have revealed that supplementation of CCFY replacement of concentrate on total feed intake was non-significantly different average at 9.1 kg/hd/day (T1) and 8.7 kg./hd/day (T2) or (2.9 and 2.8 %BW, respectively). While average daily gain (ADG) was non-significantly different average at 550 g/day (T1) and 542 g/day (T2), respectively. In addition, the ruminal pH, ammonia-nitrogen and blood urea nitrogen concentration were non-significantly different ($p>0.05$) which in optimum levels. Supplementation of CCFY (T2) could improve population of bacteria and fungal zoospore, but not decreased populations of Holotrich and Entodiniomorph protozoa in the rumen. The results indicated that supplementation of cassava chip fermented yeast (CCFY) as alternative replacement concentrate at 12 %CP could improve ruminal fermentation efficiency, microorganism populations and no negative effects on average daily gain in crossbred Brahman cattle.

Keywords: Yeast fermented cassava chip (YFCC), concentrate, rumen fermentation, crossbred Brahman cattle.

Introduction

Cassava (*Manihot esculenta*, Crantz) production in tropical areas has a potential use in ruminant livestock nutrition and feeding. Cassava root contains high levels of energy and has been used as a source of readily fermentable energy in ruminant rations (Wanapat, 2003; Promkot et al., 2013; Surapong and Cherdthong, 2020). One strategy for using high degradable carbohydrates is to use in combination with readily available non-nitrogen (NPN) sources such as urea. Urea is commonly used as N source when highly soluble carbohydrates are fed and maintained (Wohlt et al., 1978). However, efficient utilization of protein and non-protein nitrogen (NPN) in ruminants depends upon knowledge of the basic principles underlying ruminal microbial N metabolism (Fernandez et al., 1997). Moreover, ruminal pH has great impact on rumen fermentation efficiency (Wanapat, 2003; Wanapat et al., 2013).

In addition, supplementing diets with yeast (*Saccharomyces cerevisiae*) increases milk production of dairy cows and weight gain of growing cattle (Brossard et al., 2006; Nagarajan et al., 2020). Production responses attributed to yeast are usually related to stimulation of cellulolytic and lactate-utilizing bacteria in the rumen, increased fiber digestion, and increased flow of microbial protein from the rumen which may be beneficial for feedlot cattle fed high-grain diets (Guedes et al., 2007; Mohammed et al., 2018; Cherdthong et al., 2021).

However, the use of yeast fermented cassava chip (YFCC) as a replacement for concentrate at 12 %CP not yet been investigated. Therefore, the objective of this study was to evaluate the effect of supplementation of yeast fermented cassava chip to replace concentrate with rice straw as a basal roughage on feed intake, rumen ecology and growth performance in crossbred Brahman cattle.

Materials and Method

The study was approved by the local ethics committee on animal experiments of animal ethics committee of Khon Kaen University (AEKKU 65/2567)

Preparation of yeast fermented cassava chip (YFCC):

This technique is based on the method developed by Oboh (2006) and Boonnop et al. (2008), which enriching nutritive value of cassava chip with yeast (*Saccharomyces cerevisiae*) fermentation. The method for synthesis of CCFY is as follows:

I. 20 g of yeast and 20 g sugar and were added with 100 ml distilled water then incubated at room temperature for 1 hour. (A)

II. Preparation of medium by add 20 g of molasses directly into a warring blender vessel flushed with O_2, then add distill 100 ml water and 48 g urea then pour solution and incubated at room temperature for 10 minutes. (B)

III. Adjusting pH media solution by 70% H_2SO_4 between 3.5 -.7.0 and continue mix with incubated for 1 hour.

IV. Remove yeast media solution in a flask from (A) into a medium (B) and continue flush O_2 for 60 hours.

V. After 60 hours, then transfer yeasts media solution 50 ml mixed with cassava chip 100 g and then covered by plastic bag for a minimum of 72 hours.

VI. Drying of cassava chip fermented yeast (CCFY) at 30 ºC for 24 hours before feeding to animals.

Animals, diets and experimental design:

Six, female one year old of crossbred Brahman cattle with an average weight of 250±10 kg were randomly divided into two groups according to receive two groups of Feed treatments by receiving concentrate at 12 %CP (T1) and yeast fermented cassava chip (T2) The composition of dietary treatments and rice straw were shown in Table 1.

Animals were housed in individual pens and individually fed concentrate dietary treatments at 1.5 %BW. All crossbred native cattle were fed rice straw *ad libitum* with feed access to clean water and mineral-salt block. Feed intake of concentrate and roughage were measured separately and refusals recorded. The experiment was run for 90 days, the first 15 days for treatment adaptation and for feed intake measurements while the last 7 days were for sample collections of faces and rumen fluid. Body weights were measured every 30 days during the experimental period, each time prior to feeding.

Data collection and sampling procedures:

Yeast fermented cassava chip concentrate and rice straw were sampled every 30 days and were composited by period prior to analyses. Composited samples were dried at 60 ºC and ground (1 mm screen using Cyclotech Mill, Tecator, Sweden) and then analyzed for DM, ash and CP content (AOAC, 1985), NDF, ADF and ADL (Goering and Van Soest, 1970).

Rumen fluid and blood samples were collected at 0, 2 and 4 h post-feeding on the last period. Approximately 200 ml of rumen fluid was taken from the middle part of the rumen by a stomach tube connected with a vacuum pump each time at the end of each period. Rumen fluid was immediately measured for pH and temperature using (HANNA instruments HI 8424 microcomputer) after withdrawal. Rumen fluid samples were then filtered through four layers of cheesecloth. Samples were divided into two portions. One portion was used for NH_3-N analyses where 5 ml of H_2SO_4 solution (1M) was added to 50 ml of rumen fluid. The mixture was centrifuged at 16,000 g for 15 minute and the supernatant stored at –20ºC prior to NH_3-N analysis using the micro Kjeldahl methods (AOAC, 1985). Another portion was fixed with10% formalin solution in normal saline (Galyean, 1989).

The total count of bacteria, protozoa and fungal zoospores were made using the methods of Galyean (1989) based on the use of a haematocytometer (Boeco). A blood sample (about 10 ml) was drawn from the jugular vein at the same time as rumen fluid sampling, separated by centrifugation at 5,000 g for 10 minutes and stored at -20 0C until analysis of blood urea nitrogen (BUN) according to the method of Crocker (1967).

Statistical analysis:

The means of each parameter measured were compared using Group T-test analyzed by

the analysis of variance procedure of SAS V.9 (2002).

Results and Discussion

Chemical composition of feeds:

The chemical compositions of concentrate diets (T1), yeast fermented cassava chip (T2) and rice straw are shown in Tables 1. Crude proteins of concentrate, CCFY and rice straw were at 12.1, 14.1 and 2.5%, respectively. Diets containing high levels of cassava chip based diets had a slightly higher non-structural carbohydrate (NSC) and lower NDF due to increased level of cassava chip in the diets.

Table 1 Chemical composition of concentrate, yeast fermented cassava chip and rice straw

Chemical composition (%)	Concentrate[1]	YFCC	Rice straw
DM	90.20	88.20	89.30
OM	89.30	87.30	86.40
CP	12.10	14.10	2.50
TDN	75.30	74.20	45.30
NDF	27.40	18.30	78.20
ADF	16.30	12.50	56.40
ME (Mcal/kg)	3.00	2.90	1.50
Price (Baht/kg)	9.20	7.50	1.20

DM = dry matter, CP = crude protein, OM = organic matter,
NDF = neutral detergent fiber, ADF = acid detergent fiber, TDN = total digestible of nutrients, ME = metabolizable energy, YFCC = yeast fermented cassava chip.
([1]Ingredients = concentrate composed of cassava chips 30%, corn meal30%, fine rice bran 6%, soybean meal 15%, palm meal 10%, urea 2%, molasses 5%, sulfur 0.5%, salt 0.5% and mineral mix 1%) as dry weight.

Effects of diets on feed intake, average daily gain, ruminal fermentation, and blood parameter:.

The effects of supplementation of yeast fermented cassava chip (YFCC) as replacement concentrate on feed intake in crossbred Brahman cattle are presented in Table 2. Feed intake were non-significantly different between treatments (P>0.05) and trend of total feed intake to higher in crossbred Brahman cattle receiving T1 than T2 (2.9 and 2.8 %BW). Average daily gain (ADG) was not differ among treatments (P>0.05). This result was in agreement with earlier works (Sommart et al., 2000; Khampa et al., 2011, 2014) which reported that inclusion of cassava chip in diets resulted in satisfactory animal performance and had no negative effects on animal health in finishing beef cattle and lactating dairy cow (Khampa et al., 2010; Kidane et al., 2022).

Table 2 Effects of supplementation of concentrate and yeast fermented cassava chip (YFCC) on feed intake, average daily gain (ADG), ruminal fermentation and blood metabolites in crossbred Brahman cattles

Item	T1	T2	P-value
Initial live body weight (kg)	250	254	0.914
Final live body weight (kg)	315	310	0.581
DM feed intake (kg/hd/day)			
Concentrate	4.7	-	-
YFCC	-	4.6	-
Rice straw	4.4	4.1	0.062
Total	9.1	8.7	0.087
DM feed intake (%BW)			
Concentrate	1.5	-	-
YFCC	-	1.5	-
Rice straw	1.4	1.3	0.117
Total	2.9	2.8	0.074
Aveage daily gain, (g/day)	550	542	0.126
Ruminal fermentation			
Temperature (^{0}C)	39.5	38.9	0.157
Ruminal pH	6.5	6.6	0.148
NH_3-N (mg%)	18.2	17.6	0.523
BUN (mg%)	8.3	9.5	0.426

T1 = Supplementation of concentrate at 12% CP.

T2 = Supplementation of yeast fermented cassava chip (YFCC).

NS = Non significant difference p>0.05

MD = dry matter

BUN = blood urea

Characteristics of rumen fermentation and blood metabolism:

Rumen ecology parameters were measured for temperature, pH and NH3-N (Table 2). In addition, blood urea nitrogen (BUN) was determined to investigate their relationships with rumen NH3-N and protein utilization. Rumen pH at 0, 2 and 4 h post-feeding was changed by dietary treatments, however, the pH values were quite stable at 6.5-6.6, but all treatment means were within the normal range which has been reported as optimal for microbial digestion of fiber and also digestion of protein (6.0-7.0) (Hoover, 1986; Polyorach & Wanapat, 2015; Wachirapakorn et al., 2016; Lunsin, 2018).

Ruminal NH3-N and BUN concentrations were altered by CCFY (T2) supplementation which containing high non starch carbohydrate based diets. As NH3-N is regarded as the most important nitrogen source for microbial protein synthesis in the rumen. In addition, the result obtained was closer to optimal ruminal NH3-N between at 15-30 mg% (Wanapat and Pimpa, 1999; Chanjula et al., 2004; Promkot et al., 2013; Supapong and Cherdthong, 2020) for increasing microbial protein synthesis, feed digestibility and voluntary feed intake in ruminant fed on low-quality roughages

Rumen microorganisms populations:

Table 3 presents rumen microorganism populations. The populations of bacteria, protozoa and fungal zoospores total bacteria direct counts were non significantly different (P>0.05) and trend of bacteria populations to higher numbers in crossbred Brahman cattle receiving diets CCFY (T2) than T1. In addition, the presented number of protozoa in the rumen trend to increased by CCFY supplementation contains cassava-based diets. In the experiment by Guedes et al. (2007) reported that yeast are usually related to stimulation of cellulolytic and lactate-utilizing bacteria in the rumen, increased fiber digestion, and increased flow of microbial protein from the rumen which may be beneficial for feedlot cattle fed high-grain diets (Gang et al., 2020; Suntara et al., 2020, 2021; Carpinelli et al., 2022).

Table 3 Effects of supplementation of concentrate and yeast fermented cassava chip (YFCC) on rumen microorganisms populations in crossbred Brahman cattle.

Item	T1	T2	*P-value*
Total direct counts (cell/ml)			
Bacteria ($x10^{12}$)	7.9	8.1	0.521
Protozoa			
Holotric ($x10^{4}$)	5.2	7.8	0.065
Entodiniomorph ($x10^{5}$)	4.2	4.7	0.147
Fungal zoospores ($x10^{6}$)	7.6	9.2	0.063

T1 = Supplementation of concentrate at 12% CP.

T2 = Supplementation of yeast fermented cassava chip (YFCC).

Conclusion

Based on this experiment, it could be concluded that using yeast fermented cassava chip (YFCC) as alternative diet to replace concentrate at 12 %CP could improved ruminal fermentation efficiency, microorganism populations and on negative effect on average daily gain in crossbred Brahman cattle.

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References

AOAC. 1985. Official methods of analysis. 14th Edition. Washington DC: Association of Official Analytical Chemists,

Boonnop, K., M. Wanapat, N. Ngamnit and S. Wanapat. 2008. Enriching nutritive value of cassava root by yeast fermentation. *In:* Proceedings of the graduate school congress x. Held at graduate school Khon Kaen University, Thailand.

Brossard, L., F. Chaucheyras-Durand, B. Michalet-Doreau and C. Martin. 2006. Dose effect of live yeasts on rumen microbial communities and fermentations during butyric latent acidosis in sheep: new type of interaction. Animal Science. 82(6), 829–836.

Carpinelli, N.A., J. Halfen, E. Trevisi, J.D. Chapman, E.D. Sharman, J.L. Anderson and J.S. Osorio. 2021. Effects of peripartal yeast culture supplementation on lactation performance, blood biomarkers, rumen fermentation, and rumen bacteria species in dairy cows. Journal of Dairy Science. 104(10), 10727–10743.

Chanjula, P., M. Wanapat, C. Wachirapakorn, S. Uriyapongson and P. Rowlinson. 2003. Ruminal degradability of tropical feeds and their potential use in ruminant diets. Asian-Australasian Journal of Animal Sciences. 16(2), 211–216.

Cherdthong, A., C. Suntara, W. Khota and M. Wanapat. 2021. Feed utilization and rumen fermentation characteristics of Thai-indigenous beef cattle fed ensiled rice straw with *Lactobacillus casei* TH14, molasses, and cellulase enzymes. Livestock Science. 245(1), 1-8.

Crocker, C. L. 1967. Rapid determination of urea nitrogen in serum or plasma without deproteinization. The American Journal of Medical Technology. 33(5), 361-365.

Fernandez, J.M., T. Sahlu, C.D. Lu, D. Ivey and M.J. Potchoiba. 1997. Production and metabolic aspects of nonprotein nitrogen incorporation in lactation rations of dairy goats. Small Ruminant Research. 26(2), 105–117.

Galyean, M. 1989. Laboratory procedure in animal nutrition research. Department of Animal and Life Science. New Mexico State University, USA.

Gang, G.U.O., S. Chen, L.I.U. Qiang, S. ZHANG, S. Tao, W. Cong, Y. WANG, Q. XU, and W. HUO. 2020. The effect of lactic acid bacteria inoculums on in vitro rumen fermentation, methane production, ruminal cellulolytic bacteria populations and cellulase activities of corn stover silage. Journal of Integrative Agriculture. 19(3), 838–847.

Goering, H. K. and P.J. Van Soest. 1970. Forage fiber analyses (apparatus, reagents, procedures, and some applications). Agriculture Handbook No. 379. Washington, D.C.: Agricultural Research Service, USDA.

Guedes, C.M., D. Gonçalves, M.A.M. Rodrigues and A. Dias-da-Silva. 2008. Effects of a *Saccharomyces cerevisiae* yeast on ruminal fermentation and fibre degradation of maize silages in cows. Animal Feed Science and Technology. 145(1–4), 27–40.

Hoover, W.H. 1986. Chemical factors involved in ruminal fiber digestion. Journal of Dairy Science. 69(10), 2755–2766.

Khampa, S., S. Chuelong, S. Kosonkittiumporn and P. Khejornsart. 2010. Manipulation of yeast fermented cassava chip supplementation in dairy heifer raised under tropical condition. Pakistan Journal of Nutrition. 9(10), 950–954.

Khampa, S., S. Ittharat and U. Koatdoke. 2011. Enrichment value of yeast-malate fermented cassava pulp and cassava hay as protein source replace soybean meal in concentrate on rumen ecology in crossbred native cattle. Pakistan Journal of Nutrition. 10(12), 1126-1131.

Khampa, S., U. Koatedoke, and S. Ittarat. 2014. Utilization of yeast fermented cassava pulp (YFCP) as diets on feed intake and rumen pH in dairy heifers. Prawarun Agricultural Journal. 11(1), 83–90.

Kidane, A., S.G. Vhile, S. Ferneborg, S. Skeie, M.A. Olsen, L.T. Mydland, M. Øverland and E. Prestl. 2022. *Cyberlindnera jadinii* yeast as a protein source in early-to mid-lactation dairy cow diets: Effects on feed intake, ruminal fermentation, and milk production. Journal of Dairy Science. 105(3), 2343–2353.

Lunsin, R. 2018. Effect of oil palm meal on nutrient utilization and milk production in lactating dairy cows fed with urea-treated rice straw. Agriculture and Natural Resources. 52(3), 285–289.

Mohammed, S.F., F.A. Mahmood and E.R. Abas. 2018. A review on effects of yeast (*Saccharomyces cerevisiae*) as feed additives in ruminants performance. Journal of Entomology Zoology Study. 6(5), 629–635.

Nagarajan, D., D.-J. Lee, C.Y. Chen, and J. S. Chang. 2020. Resource recovery from wastewaters using microalgae-based approaches: A circular bioeconomy perspective. Bioresource Technology. 30(12), 817-822.

Oboh, G. 2006. Nutrient enrichment of cassava peels using a mixed culture of *Saccharomyces cerevisae* and *Lactobacillus spp* solid media fermentation techniques. Electronic Journal of Biotechnology. 9(1), 46–49.

Polyorach, S., and M. Wanapat. 2015. Improving the quality of rice straw by urea and calcium hydroxide on rumen ecology, microbial protein synthesis in beef cattle. Animal Physiology Nutrition. 99(3), 449–456.

Promkot, C., M. Wanapat, and J. Mansathit. 2013. Effects of yeast fermented-cassava chip protein (YEFECAP) on dietary intake and milk production of Holstein crossbred heifers and cows during pre-and post-

partum period. Livestock Science. 154(2), 112–116.

SAS. 2002. SAS/STAT User's Guide. Version 9. Cary, North Carolina, USA: SAS Institute incorporated.

Sommart, K., M. Wanapat, P. Rowlinson, D.S. Parker, P. Climee, and S. Panishying. 2000. The use of cassava chips as an energy source for lactating dairy cows fed with rice straw. Asian-Australasian Journal of Animal Sciences. 13(8), 1094–1101.

Suntara, C., A. Cherdthong, S. Uriyapongson, M. Wanapat, and P. Chanjula. 2020. Comparison effects of ruminal Crabtree-negative yeasts and Crabtree-positive yeasts for improving ensiled rice straw quality and ruminal digestion using in vitro gas production. Journal of Fungi. 6(3), 1-20.

Suntara, C., A. Cherdthong, S. Uriyapongson, M. Wanapat, and P. Chanjula. 2021. Novel Crabtree negative yeast from rumen fluids can improve rumen fermentation and milk quality. Scientific Reports. 11(1), 6236-6242.

Supapong, C. and A. Cherdthong. 2020. Effect of sulfur and urea fortification of fresh cassava root in fermented total mixed ration on the improvement milk quality of tropical lactating cows. Veterinary Sciences. 7(3), 98-110.

Wachirapakorn, C., K. Pilachai, M. Wanapat, P. Pakdee, and A. Cherdthong. 2016. Effect of ground corn cobs as a fiber source in total mixed ration on feed intake, milk yield and milk composition in tropical lactating crossbred Holstein cows. Animal Nutrition. 2(4), 334–338.

Wanapat, M. 2003. Manipulation of cassava cultivation and utilization to improve protein to energy biomass for livestock feeding in the tropics. Asian-Australasian Journal of Animal Sciences. 16(3), 463–472.

Wanapat, M., and O. Pimpa. 1999. Effect of ruminal NH_3-N levels on ruminal fermentation, purine derivatives, digestibility and rice straw intake in swamp buffaloes. Asian-Australasian Journal of Animal Sciences. 12(6), 904–907.

Wanapat, M., S. Kang, N. Hankla, and K. Phesatcha. 2013. Effect of rice straw treatment on feed intake, rumen fermentation and milk production in lactating dairy cows. African Journal of Agricultural Research. 8(17), 1677–1687.

Wohlt, J.E., J.H. Clark, and F.S. Blaisdell. 1978. Nutritional value of urea versus preformed protein for ruminants. II. Nitrogen utilization by dairy cows fed corn based diets containing supplemental nitrogen from urea and/or soybean meal. Journal of Dairy Science. 61(7), 916–931.