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REGULAR ARTICLES

Improving the nutritive value of cassava bioethanol waste using fermented yeast as a partial replacement of protein source in dairy calf ration

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Abstract

The aim of this work was to evaluate the influence of yeast (*Saccharomyces cerevisiae*)–fermented cassava bioethanol waste (YECAW) on feed utilization, ruminal fermentation, and microbial population in dairy calves fed a concentrate diet at 1% body weight (BW). Four male Holstein Friesian crossbred calves with an initial BW of 109 ± 6.23 kg were used in this research. The experimental design was a 4 × 4 Latin squared design and the dietary treatments were four levels of YECAW supplementation at 0%, 5%, 10%, and 20% concentrate mixture. The YECAW product contained CP at 25.1% dry matter (DM) and NDF and ADF at 65.2 and 40.6% DM, respectively. Inclusion of YECAW did not alter feed intake of rice straw, total intake, nutrient intake, and digestion coefficients ($P > 0.05$). Ruminal pH and temperature, ruminal ammonia-nitrogen, and blood urea-nitrogen (BUN) were not significant by YECAW levels supplementation ($P > 0.05$). Increasing YECAW levels did not adversely affect the population of bacteria, protozoa, and fungi and values ranged from 6.5 to 7.0×10^{12}, 3.2 to 4.0×10^{5}, and 6.9 to 7.4×10^{3} cells/ml, respectively ($P > 0.05$). Feeding of YECAW to dairy calves did not affect the total VFA, acetic acid (C2), propionic acid (C3), or butyric acid (C4) proportion ($P > 0.05$) which ranged from 102.6 to104.6 mmol/l, 70.7 to 72.0, 17.8 to 20.2, and 9.1 to 10.3 mol/ 100 mol, respectively. Therefore, feeding of YECAW is recommended because no adversely affect the utilization of feed and rumen characteristics and might be alternative protein source for ruminants.

Keywords Cassava waste · Ruminal microorganism · Ruminant · Feed intake · Rumen fermentation

Introduction

Feeding management of dairy calves is essential to their survival and productivity and is aimed at providing the balanced nutrients needed to maintain a stable and efficient microbial population. Bioethanol manufacturing is an industry that depends almost completely on cassava (Sriroth et al. 2012). During cassava processing, a large waste stream is produced. This residue consists of some dissolved solids, but the major composition of the drained solids is starch and minerals. Cassava bioethanol waste (CBW) is a by-product of bioethanol production from raw cassava root. CBW contains some fibers, and crude protein (CP) content is about 11.0–14.0% dry matter (DM), which suggests its use as a nutrient for ruminant animals. Phoemchalard et al. (2014) indicated that CBW could be incorporated into the diet of cattle without adversely affecting growth and nutrient digestibility. In addition, Cherdthong et al. (2016) revealed that supplementation of 10% CBW in total mixed rations (TMR) did not affect feed use, ruminal fermentation, nutrient digestibility, or blood urea-nitrogen (BUN) in goat. Accordingly, CBW is used to feed and fatten goat and might be practically used as a new roughage choice for goat production (Cherdthong et al. 2016).

Cultured yeast has been successfully introduced to ruminant diets to improve the nutritive value of feedstuffs. Boonnop et al. (2009) report that adding yeast to fermented fresh cassava root could improve CP content from 3.2 to 21.1% DM, while Polyorach et al. (2014) shows that cassava chips fermented by yeast can increase CP content from 2.2 to 47% DM and could improve feed utilization and animal performance. However, there are still limited studies about using CBW fermented with culture of *S. cerevisiae* in dairy calf ration.

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Therefore, the aim of this work was to evaluate the influence of yeast (*Saccharomyces cerevisiae*)–fermented cassava bioethanol waste (YECAW) on feed utilization, ruminal fermentation, and microbial population in dairy calves fed a concentrate diet at 1% BW.

Materials and methods

Experiment location and animal care

This experiment was conducted at the Division of Beef Cattle and Buffaloes, Khon Kaen University, Khon Kaen province, Thailand (16.46° N 102.82° E; altitude 169 m above sea level). The management of dairy calves used in the study and all related procedures were performed according to the Guidelines of the Ethics of Animal Experimentation of the National Research Council of Thailand with the permission of the Animal Ethics Committee of Khon Kaen University.

Dietary preparation

Cassava bioethanol waste (CBW) was collected from a bioethanol production factory, sun-dried for 2 days and used as an ingredient in YECAW production. YECAW used in the present work was detailed by Polyorach et al. (2014) who, in brief, stimulated *S. cerevisiae* using 100-ml distilled water mixed with 20-g bakers' yeast and 20-g cane sugar and stored for 1 h at room temperature (solution A). Solution B was created by adding of 8-g molasses in 100-ml distilled water, which was then supplemented with 64 g urea. H_2SO_4 was used to control the pH at 3.5 to 5. Solutions A and B were mixed in a 1 to 1 ratio, and an air pump (600 W) provided oxygen for 66 h thereafter. Finally, CBW was mixed with the yeast culture solution at a ratio of 1.3 g DM to 1 ml. After fermenting for 72 h in a solid state under shade, the product was sun-dried for 48 h and used in the concentrate diet. The ingredients and chemical composition of the experimental diets are presented in Table 1.

Animals, design, and managements

Four male Holstein Friesian crossbred calves with initial body weights (BW) of 109 ± 6.23 kg were used in this research. The experimental design was a 4 × 4 Latin squared design and the dietary treatments were four levels of YECAW supplementation at 0%, 5%, 10%, and 20% concentrate mixture. A concentrate composition was fed to the animals at 1% BW, and rice straw was fed ad libitum. The diets were offered twice daily at 07:00 and 16:00 and clean fresh water was freely available. All dairy calves was fed in individual houses. This work was evaluated separately for four periods each period

Table 1 Ingredient and chemical composition of experimental diets (% of dry matter)

Item	Levels of YECAW (%DM)				YECAW	Rice straw
	0	5	10	20		
Ingredients, %DM						
Cassava chip	45.0	43.0	41.0	39.0		
Soybean meal	14.0	12.0	10.0	8.0		
YECAW*	0.0	5.0	10.0	20.0		
Rice bran	12.6	11.7	12.7	9.0		
Palm kernel meal	11.0	11.0	10.0	8.9		
Coconut meal	11.0	11.0	10.0	9.0		
Urea	1.4	1.3	1.3	1.1		
Molasses	2.0	2.0	2.0	2.0		
Salt	1.0	1.0	1.0	1.0		
Sulfur	1.0	1.0	1.0	1.0		
Mineral premix	1.0	1.0	1.0	1.0		
Chemical composition						
Dry matter (%)	90.6	90.1	90.8	91.3	93.4	92.4
Organic matter (%DM)	94.8	93.0	91.5	90.4	87.5	86.5
Ash (%DM)	5.2	7.0	8.5	9.6	12.5	13.5
Crude protein (%DM)	14.9	14.6	14.6	14.7	25.1	2.1
Neutral detergent fiber (%DM)	14.6	20.5	24.1	27.6	65.2	79.5
Acid detergent fiber (%DM)	8.9	12.1	14.4	17.0	40.6	54.5

**YECAW* yeast-fermented cassava bioethanol waste

consisting of 21 days. Feed intake was recorded for 21 days and samples collection of diets, rumen fluid, feces, and blood was conducted during the last 7 days.

Sample collection and assay procedure

Experimental diets were sampled daily during each period for chemical analysis. Feed offered and refusal samples were collected at morning and afternoon feedings during the last 7 days of each period. Fecal samples were collected at 07:00 or 16:00 by rectal sampling. The samples were dried at 60 °C, ground by machine, and analyzed using the AOAC (1995) method for dry matter (DM), CP, ash, and acid-insoluble ash (AIA). Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were determined using the method of Van Soest et al. (1991). Calculation of nutrient digestibility was performed using the equation of Van Keulen and Young (1977).

On the last day of each period, blood samples (8 ml) were taken at the jugular vein at 0 h post feeding and 4 h post feeding for analysis of BUN according to Crocker (1967). EDTA was added to blood sample as an anticoagulant at 12 mg, and plasma was divided by centrifugation at 500×*g* for 10 min. The rumen fluid (50 ml) was collected 0 and 4 h post feeding via a stomach tube linked to a vacuum pump. Rumen pH and temperature were measured immediately using a movable pH and temperature meter (HANNA Instruments HI 8424 microcomputer, Singapore). Rumen fluid samples were divided into three parts. The first portion (5 ml) was analyzed for direct counts of bacteria and protozoal population using Galyean (1989)'s methods. In a second portion, 45 ml of rumen fluid was mixed with 5 ml of 1 M H_2SO_4 and centrifuged at 16,000×g for 15 min, and the supernatant was measured ammonia-nitrogen (NH_3-N) concentration (AOAC 1995) and volatile fatty acids (VFA) analysis using HPLC (Samuel et al. 1997).

Statistical methods

All data were subjected to ANOVA according to a 4 × 4 Latin square design using GLM procedure of SAS (1996). Data were analyzed using the model:

$$Y_{ijk} = \mu + M_i + A_j + P_k + \varepsilon_{ijk}$$

where Y is the single observation, μ is the overall mean, M is the YECAW levels ($i = 1, 2, 3, 4$), A is the effect of calf ($j = 1, 2, 3, 4$), P is the period ($k = 1, 2, 3, 4$), and ε is the residual effect. The results are presented as mean values and standard error of the means. Means were compared using Duncan's new multiple range test. A significance was declared at $P < 0.05$ as representing statistically significant differences.

Results and discussions

Chemical composition of diets

Table 1 presents the data related to feed ingredients and chemical composition. The YECAW product contained CP at 25.1% DM and NDF and ADF at 65.2 and 40.6% DM, respectively. Previous works indicated that the unfermented CBW consisted of CP content at 11 to 14% DM (Sriroth et al. 2012; Cherdthong et al. 2016). However, in this study, CBW fermented with yeast solution could increase CP to 11.1–14.1% DM. The CP increase possibly be related to the inclusion of urea (60 g) and molasses (8 g) in solution B, which may increase nitrogen content. In addition, Oboh and Akindahinsi (2003) revealed that high protein concentration could be related to the ability of the yeast to excrete enzymes into the substrate during its metabolic activities, which would lead to yeast growth. Thus, yeast cells could also be another microbial protein source and lead to increased CP content in YECAW. IN agreement with Boonnop et al. (2009) who noted that adding yeast to fermented cassava chips improved CP from 2.0 to 30.4% CP, whereas Polyorach et al. (2014) showed that cassava chips fermented by yeast can improve CP level by as much as 47% and could improve feed utilization and ruminant performance. This high protein YECAW product could very well serve as an alternative protein source in ruminant and may provide low cost feed (Boonnop et al. 2009). Concentrate diets were prepared to be isonitrogenous at 14.6–14.9% CP, and urea was included to balance the CP concentration. Fiber contents (NDF and ADF) were linearly increased according to increasing YECAW inclusion.

Feed utilization efficiency

The effects of YECAW on feed utilization efficiency in dairy calves are shown in Table 2. Inclusion of YECAW did not alter feed intake of rice straw and total intake ($P > 0.05$). Total intake ranged from 87.1 to 89.8 g/kg $BW^{0.75}$. Similarly, Phoemchalard et al. (2014) reported that no difference in the consumption of dry matter intake was found when supplementing with either 15% or 30% unfermented CBW in yearling heifers. In contrast, Cherdthong et al. (2016) indicated that fattening goats with unfermented CBW in TMR higher than 10% DM could reduce nutrient intake and digestibility. This might be due to the quality of CBW which contains higher fiber and lower CP when compared with YECAW. It may also be related to differences in animal species. Increasing YECAW did not affect nutrient intake ($P > 0.05$), and intake values of OM and CP were 3.0 to 3.2 kg/day and 2.6 to 2.9 kg/day, respectively. Digestion coefficients of DM, OM, CP, NDF, and ADF were not changed among YECAW levels and ranged from 69.4 to 66.0, 71.2 to

Table 2 Effect of levels of yeast-fermented cassava bioethanol waste (YECAW) in concentrate diets on intake and nutrient digestibility in dairy calf

Items	Levels of YECAW (%DM)				SEM	*P* value
	0	5	10	20		
kg/d	1.4	1.5	1.4	1.55	0.11	0.34
g/kg $BW^{0.75}$	54.5	53.9	52.3	52.1	2.45	0.66
kg/d	0.9	1.1	1.0	1.0	–	–
g/kg $BW^{0.75}$	35.2	35.2	35.0	35.0	–	–
kg/d	2.4	2.7	2.4	2.6	0.08	0.11
g/kg $BW^{0.75}$	89.8	89.1	87.3	87.1	2.98	0.44
Nutrients intake (kg/day)						
Organic matter	3.0	3.2	3.1	3.0	2.05	0.26
Crude protein	2.7	2.9	2.7	2.6	1.98	0.39
Neutral detergent fiber	0.2	0.2	0.2	0.2	0.02	0.48
Acid detergent fiber	1.6	1.8	1.7	1.6	1.05	0.12
Nutrients digestibility						
Dry matter (%)	69.4	67.4	66.7	66.0	3.01	0.15
Crude protein (%DM)	66.8	67.2	65.6	66.1	2.49	0.37
Organic matter (%DM)	71.2	69.5	68.6	68.1	3.68	0.26
Neutral detergent fiber (%DM)	54.6	52.3	53.0	52.7	2.06	0.09
Acid detergent fiber (%DM)	43.1	42.6	42.7	43.0	1.98	0.11

68.1, 66.8 to 65.6, 54.6 to 52.3, and 43.1 to 42.6% DM, respectively. However, feeding of YECAW could improve DM digestibility when compared with that demonstrated by Phoemchalard et al. (2014) who indicated that DM digestibility ranged from 49 to 53% DM when yearling heifers were fed unfermented CBW. Improving of CBW with yeast could increase the quality of the by-product and enhance feed utilization. These results indicated that YECAW could be incorporated into concentrate diets with no adverse effect on feed intake and digestibility in animals.

Rumen ecology, rumen microbes, and blood urea-nitrogen

Table 3 presents the effect of YECAW levels on ruminal fermentation, NH_3-N concentration, and microbial population in dairy calves. Ruminal pH and temperature were not significant by YECAW levels supplementation ($P > 0.05$). The pH and temperature are stable from 6.8 to 7.1 at 38.8 °C to 39.0 °C, respectively. The rumen pH and temperature range for all YECAW doses were suitable for ruminal condition and microorganism activity to break down the diet (Cherdthong et al. 2015). Rumen NH_3-N concentration was not significantly different among treatments ($P > 0.05$), with values ranging from 15.0 to 15.7 mg/dl. This is similar to the report from Cherdthong et al. (2016), who found no differences in ruminal NH_3 in goats when 10–20% CBW was added to the basal diet. In addition, BUN was also measured to conduct the relationship with rumen NH_3-N and protein utilization. It was demonstrated that BUN did not change among treatments ($P > 0.05$). Increasing YECAW levels did not adversely affect the population of bacteria, protozoa, and fungi and values

Table 3 Effect of yeast-fermented cassava bioethanol waste (YECAW) levels on ruminal fermentation, NH_3-N concentration, rumen microbes, and blood urea-nitrogen concentration of dairy calf

Item	Levels of YECAW (%DM)				SEM	*P* value
	0	5	10	20		
Ruminal pH	6.8	6.9	6.9	7.1	0.87	0.84
Ruminal temperature (°C)	39.0	38.9	38.8	39.1	2.65	0.21
NH_3-N concentration (mg/dl)	15.0	15.4	15.0	15.7	1.12	0.58
Blood urea-nitrogen concentration (mg/dl)	11.2	11.3	11.0	12.1	0.99	0.11
Ruminal microbes (cells/ml)						
Bacteria ($\times 10^{12}$)	6.6	6.8	6.5	7.0	2.58	0.09
Protozoa ($\times 10^5$)	3.7	3.2	4.0	3.7	1.98	0.26
Fungal zoospore ($\times 10^3$)	7.4	7.4	6.9	7.4	3.99	0.17

ranging from 6.5 to 7.0×10^{12}, 3.2 to 4.0×10^{5}, and 6.9 to 7.4×10^{3} cells/ml, respectively ($P > 0.05$). In addition, the populations of fungal zoospores and protozoa were not altered when yeast-fermented cassava chip proteins were tasted in laboratory (Polyorach et al. 2014). Conversely, Wanapat et al. (2011) indicated that feeding cassava chips fermented with yeast enhanced the bacterial population when compared with the YECAW group. This could be because yeast-fermented cassava chip protein products supply sufficient factors for bacterial growth such as carbon skeletons, amino acids, and minerals. Thus, changes to the bacterial population were not found in the present study.

Characteristics of ruminal volatile fatty acid

The effects of YECAW levels on VFA profiles in the rumen of dairy calves are presented in Table 4. Fermentation of YECAW by ruminal microbes results in the production of microbial CP, gases, and VFAs (Chaucheyras-Durand et al. 2008; Doto and Liu 2011). Feeding of YECAW to dairy calves did not affect the total VFA, acetic acid (C2), propionic acid (C3), or butyric acid (C4) proportion ($P > 0.05$) which ranged from 102.6 to104.6 mmol/l, 70.7 to 72.0, 17.8 to 20.2, and 9.1 to 10.3 mol/100 mol, respectively. Similarly, Cherdthong et al. (2018) indicated that the suitable VFA proportion in the rumen has a concentration of C2 at 65 to 70%, C3 at 20 to 25%, and C4 at 10 to 15%, respectively. Moreover, the ratios of C2:C3 and C2 plus C4:C3 were also similar among YECAW levels ($P > 0.05$), which ranged from 3.5 to 4.1 and 4.0 to 4.6, respectively. These results indicated that YECAW can be used as alternative ingredient in ruminant diets and does not negatively affect the production of VFA in the rumen. However, yeast fermented with other feedstuffs that contain higher quality when compared with CBW such as cassava chips or fresh cassava root could enhance VFA production in the rumen. Polyorach et al. (2014) noted that yeast-fermented cassava chip protein in concentrate diets significantly increased total VFA and C3 while decreasing C2 to C3. Furthermore, Boonnop et al. (2009) suggested that yeast-fermented cassava chip protein enhanced ruminal fermentation by enhancing the C3 intermediate and increasing ruminal microorganism production in laboratory experiments. This could be due to some essential factors contained in yeast-fermented cassava chip protein products that activated ruminal microbes, particularly *Megasphaera elsdenii* or *Selenomonas ruminantium* which are representative of lactate-utilizing bacteria (Lynch and Martin 2002).

Table 4 Effect of yeast-fermented cassava bioethanol waste (YECAW) levels on volatile fatty acid profiles in rumen of dairy calf

Items	Levels of YECAW (%DM)				SEM	*P* value
	0	5	10	20		
Total VFA (mmol/l)	104.6	102.6	103.6	104.2	4.65	0.36
VFA profiles (mol/100 mol)						
Acetic acid (C2)	70.7	71.2	71.4	72.0	3.20	0.58
Propionic acid (C3)	20.2	19.1	18.6	17.8	2.01	0.95
Butyric acid (C4)	9.1	9.8	10.1	10.3	1.25	0.91
C2:C3	3.5	3.7	3.8	4.0	0.56	0.52
C2 + C4:C3	4.0	4.2	4.4	4.6	0.69	0.82

Conclusion

Inclusion of YECAW at 20% in concentrate diets did not affect feed utilization, rumen fermentation, and ruminal microorganisms in in dairy calves fed 1% of concentrate diet. Thus, feeding of YECAW is recommended because it has controlled environmental contamination and might be alternative protein source for ruminants.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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