ผลของการเสริมผลิตภัณฑ์ผสมไบโอติน (BMP) ต่อจลนศาสตร์การผลิตแก๊ส และการย่อยได้ของอาหารผสมสำเร็จ (TMR)

In vitro gas production and degradability of total mixed ration influenced by biotin mixed product supplementation

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บทคัดย่อ: การวิจัยครั้งนี้มีวัตถุประสงค์เพื่อศึกษาผลของการเสริมผลิตภัณฑ์ผสมไบโอติน (BMP) ต่อจลนศาสตร์การผลิตแก๊ส และการย่อยได้ของอาหารผสมสำเร็จ (TMR) วางแผนทดลองแบบ 3x3 แฟคทอเรียลใน CRD โดยแบ่งการศึกษาเป็นสองปัจจัยคือ ปัจจัยที่ 1 สูตรอาหารผสมสำเร็จ (TMR) 3 สูตร ที่มีสัดส่วนอาหารข้นต่ออาหารหยาบ (C:R) ได้แก่ 50:50, 60:40, และ 70:30 ปัจจัยที่ 2 การเสริมผลิตภัณฑ์ผสมไบโอตินสามระดับ (0, 0.5, และ 1 เปอร์เซ็นต์ของวัตถุแห้งหรือไบโอตินบริสุทธิ์เสริมที่ระดับ 0, 0.2, และ 0.4 มิลลิกรัมต่อกิโลกรัมของ TMR) นำอาหารทดลองบรรจุประมาณ 0.2 กรัม ลงในขวดแก้วขนาด 20 มิลลิลิตร บรรจุสารละลายของเหลวจากกระเพาะรูเมนจากโคนม แล้วนำเข้าบ่มที่อุณหภูมิ 39 องศาเซลเซียส เป็นเวลา 96 ชั่วโมง จากการศึกษาพบว่า ปริมาณผลผลิตแก๊ส ประสิทธิภาพการผลิตแก๊ส ค่าการย่อยสลายได้ของวัตถุแห้ง (DMD) และการผลิตกรดไขมันสายสั้นเพิ่มขึ้นเป็นเส้นตรงเมื่อสัดส่วนในอาหารเพิ่มขึ้น ($P<0.05$) อย่างไรก็ตามปริมาณแอมโมเนีย-ไนโตรเจน ไม่แตกต่างกันอย่างมีนัยสำคัญระหว่างสัดส่วนอาหารข้นต่ออาหารหยาบ ($P>0.05$) การเสริม BMP ส่งผลต่อปริมาณผลผลิตแก๊ส ประสิทธิภาพการผลิตแก๊ส และค่าการย่อยสลายได้ของวัตถุแห้ง (DMD) เพิ่มขึ้นเป็นเส้นตรงเมื่อเพิ่มระดับ BMP ในสูตรอาหาร TMR กับ 50 เปอร์เซ็นต์ของอาหารข้น ในขณะที่สูตรอาหาร TMR ที่ 60 และ 70 เปอร์เซ็นต์ของอาหารข้น พบว่าสูงสุดในใน 0.5 เปอร์เซ็นต์ ของ BMP ($P<0.05$) อย่างไรก็ตามปริมาณแอมโมเนีย-ไนโตรเจนและกรดไขมันสายสั้นไม่มีผลอย่างมีนัยสำคัญจากการเสริม BMP ($P>0.05$) ผลของการศึกษาแสดงให้เห็นว่า การเสริม BMP เป็นทางเลือกเพิ่มขีดความสามารถค่าการย่อยสลายได้ของวัตถุแห้ง และ 0.5 เปอร์เซ็นต์ของผลิตภัณฑ์ผสมไบโอติน หรือ 0.02 มิลลิกรัมต่อกิโลกรัม เป็นระดับที่แนะนำในสูตรอาหารผสมสำเร็จ

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ABSTRACT: The objective of this study was to determine the effect of biotin mixed product (BMP) supplementation on gas production kinetics and ruminal degradability of total mixed ration (TMR) diets. This experiment was arranged by 3x3 factorial arrangement in CRD. Two factors were 1) three concentrate and roughage (C:R) ratios in TMR 50:50, 60:40 and 70:30) and 2) three levels of BMP supplementation (0, 0.5 and 1% of dry matter or pure biotin supplementation at 0, 0.2 and 0.4 mg/kg of TMR). Approximately 0.2 g of feed samples were weighed into 50 mL bottles and incubated with artificial rumen fluid at 39°C for 96 h. This experiment was performed according to intro gas production technique. The results showed that the gas production, effective gas production (EP), DM degradability (DMD) and short chain fatty acid production were linearly increased when the proportion of concentrate in diet was increased ($P<0.05$). However, NH3-N concentration was not significantly different among various C:R ratio ($P>0.05$). Supplementation of BMP affected gas production, effective gas production and DMD. There were linearly increase when increased level of BMP in TMR with 50% of concentrate while in TMR with 60 and 70 % of concentrate showed highest in 0.5% of BMP ($P<0.05$). However, NH3-N and short chain fatty acid were not significantly affected by BMP supplementation ($P>0.05$). The results indicated that BMP supplementation is alternative way to increased capability of DM degradability and 0.5% of biotin mixed product or 0.02 mg/kg is a recommended level in TMR.
Keywords: gas production, degradability, total mixed ration, biotin, supplementation

Introduction

In the present, there are shortages of good-quality feeds for feeding of ruminants, especially in the tropics. Rumen microbes play a crucial role in the digestion of nutrients in the rumen and convert to high quality microbial proteins. Recent studies in this regard indicated that major cellulolytic organisms in the rumen including bacteria like Ruminococcus species and Bacteroides species and anaerobic fungi like Neocallimastix have specific requirements for thiamine, riboflavin, niacin, pyridoxine, biotin, folic acid, and B12 (Ashwin and Srinivas, 2019). Biotin, a water-soluble B vitamin, is an essential nutrient for both rumen bacteria and the animal. Apart from being indispensable for gluconeogenesis, fatty acid synthesis, and protein synthesis (Dakshinamurti and Chauhan, 1988). In addition, most cellulolytic rumen bacteria require biotin for growth (Baldwin and Allison, 1983). An in vitro study reported that biotin synthesis was reduced by about 50% as the concentration of dietary forage decreased from 80% forage to 50% forage (Da Costa Gomez et al., 1998). Having a high concentration of grains can create an acid rumen and in turn decrease feed utilization instead of synthesis of ruminal biotin due to a decrease in the growth of cellulolytic microbes (Rosendo et al., 2003). In previous studies, many researchers found that supplementation of biotin can increased ruminal degradability and products. Abel et al. (2001) found that biotin can improve fermentation of fiber in the rumen. According to Milligan et al. (1967) reported that supplemental of biotin in diet increased fiber digestion in vitro that may continue effect to increase intake and milk yield. Majee et al. (2003) found that digestibility, intake and milk production were higher for cows supplemented with 20 mg biotin per day compared to control cows. Therefore, ruminant diets with high amounts of concentrate that may increase feed utilization due to biotin supplementation (Zimmerly and Weiss, 2001). Thus, the objective of this study was to determine the effect of biotin on gas production kinetics and ruminal degradability of TMR.

Material and methods

The experiment was arranged by 3x3 factorial arrangement in CRD. Two factors were 1) three ratio of concentrate:roughage in TMR (50:50, 60:40 and 70:30) and 2) three levels of biotin mixed product (BMP) supplementation (0, 0.5 and 1.0 % of dried TMR as convers to pure biotin = 0, 0.2 and 0.4 mg/kg). The TMR were mixed by using concentrate containing 18% crude protein (CP) and 2.70 Mcal metabolizable energy (ME)/kgDM) and rice straw in a ratio as followed dietary treatments and then added three levels of BMP supplementation (0, 0.5 and 1.0 % of dried TMR as convers to pure biotin = 0, 0.2 and 0.4 mg/kg) before drying in oven at 60oC for 48 h and grinding through a 2-mm screen for in vitro gas technique and degradability test.

The method used for in vitro gas production technique described by Menke and Steingass (1988). Three fistulated Holstein Friesian (420 ± 30 kg) were used as donors for rumen fluid inoculum in this experiment Preparation of artificial saliva was done according to the method of Menke and Steingass (1988). The 0.2 g of the air dried sample was incubated in 50 mL bottle which containing 20 mL of buffer mineral solution and 10 mL of rumen fluid for 96 h at 39°C. During the incubation, the cumulative gas production was measured at 1, 2, 4, 6, 8, 12, 24, 36, 48, 72 and 96 h then fitted to the model of Ørskov and McDonald (1979) as follows: y = a + b (1-exp(-ct)); a = the gas production from the immediately soluble fraction, b = the gas production from the insoluble fraction, c = the gas production rate constant for the insoluble fraction (b), t = incubation time, (/a/ + b) = the potential extent of gas production, y = gas produced at time "t". Digestibility of DM (DMD) was determined at a time points (24 and 48 h after incubation). After incubated time the mixed liquids in each bottle were removed and filtrated then divided to two portions, first portion was dried at 105°C for 12 h and burning at 550°C for 4 h to determine DM and ash. DMD was calculated as difference weighing between before and after incubation, corrected by a blank which consisted of four flask containing only buffered rumen fluid. Short chain fatty acid production was calculated by the equation; Short chain fatty acid (SCFA, mol) = 0.0239Gv-0.0601, while Gv = net gas production (ml/200mg, DM) at 24 h of incubation. A second portion were filtered through four layers of cheesecloth then the filtrated fluid was mixed with 10 ml of 50% H2SO4 solution as used for Ammonia-Nitrogen (NH3-N) analysis. The mixed sample was centrifuged at 16,000×g for 15 min and the supernatant was stored at -20°C prior to NH3-N measurement according to the method of Bremner and Keeney (1965).

All obtained data were subjected to the analysis of variance (ANOVA) procedures of SAS (1989) according to a 3×3 factorial arrangement in CRD. Treatment means were compared using Duncan's New Multiple Range Test (Steel and Torrie 1980). The statistical model was: $Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_k + e_{ijk}$ where; Y_{ijk} = Observation in treatment combination ij and replicated j, μ = Over all sample mean, αi = effect of factor A , β_j = effect of factor B, $\alpha\beta_i$ = effect of interaction AB , e_{ijk} = error.

Table 1 Percentage of feed ingredients of experimental concentrate diets and chemical composition.

Ingredients	Kg/100kg
Cassava chip	57.0
Rice bran	8.50
Soybean meal	15.0
Palm kernel meal	14.0
Salt	0.40
Dicalcium phospate	1.50
Premixed	0.50
Sulfur	0.30
Molasses	1.50
Urea	1.30

Chemical composition	% of DM	
	Concentrate	Rice straw
Organic matter	93.4	88.2
Crude protein	18.3	3.33
Ether extract	5.70	0.62
NDF	40.1	81.1
ADF	14.5	57.7
Ash	6.57	11.8
ME[1], Mcal/kgDM	2.65	1.46

[1] Calculated according to Harris et al. (1972), NDF=neutral detergent fiber, ADF= acid detergent fiber

Results and discussion

The patterns of cumulative gas production of various C:R ratio and BMP supplementation were presented in Table 2. The results showed that the gas production kinetics that calculated as b and d values and effective gas production (EP) were influenced by C:R ratio and BMP supplementation. There were linearly increased when the level of concentrate and biotin were increased (figure 1; a and b) ($P<0.05$). Moreover, we found that b, d values and EP were linearly increased when increased level of BMP in TMR with 50% of concentrate while in TMR with 60 and 70 % of concentrate showed highest in 0.5% of BMP ($P<0.05$) (figure 1; d, e and f). NH3-N production was not influenced by C:R ratio and BMP supplementation ($P>0.05$) (Table 3). DMD and SCFA production were linearly increased when the proportion of concentrate in diet was increased ($P<0.05$) (Table 3). Supplementation of BMP affected DMD. There were linearly increase when increased level of BMP in TMR with 50% of concentrate while in TMR with 60 and 70% of concentrate showed highest in 0.5% of BMP ($P<0.05$). These are related with an accumulated gas production (figure 1; d, e and f). However, SCFA was not significantly different when compared between the level of BMP supplementation ($P>0.05$) (Table 3).

Table 2 Effects of C:R ratio and biotin on gas production kinetic.

C:R ratio	50:50			60:40			70:30			SEM	P-value		
BMP, %	0.0	0.5	1.0	0.0	0.5	1.0	0.0	0.5	1.0		A	B	A*B
Gas production characteristics													
a	-0.19	-2.19	-2.27	-3.82	-4.12	-2.93	-3.72	-4.94	-4.26	1.70	<0.01	0.11	0.14
b	41.4^{a}	45.0^{a}	49.9^{ab}	53.4^{b}	57.3^{bc}	50.6^{b}	54.8^{b}	61.9^{c}	53.9^{b}	2.71	<0.01	0.05	0.01
c	0.06	0.08	0.06	0.07	0.07	0.08	0.08	0.07	0.07	0.01	0.35	0.40	0.10
d	41.6^{a}	47.2^{ab}	52.2^{b}	57.2^{b}	61.4^{c}	53.5^{b}	58.5^{b}	66.8^{c}	58.1^{b}	2.43	<0.01	0.05	0.01
Effective gas production potential (EP)	25.0^{a}	28.1^{a}	28.3^{a}	29.8^{ab}	32.2^{b}	30.4^{b}	32.8^{b}	33.5^{b}	30.0^{b}	1.39	<0.01	0.05	0.03

a = the gas production from the immediately soluble fraction; b = the gas production from the insoluble fraction; c, the gas production rate constant for the insoluble fraction (b); d= /a/ + b, the gas potential extent of gas production.
[abc] Mean in the same row with different superscript differ (P<0.05)

Table 3 Effects of R:C ratio and biotin on ruminal production and degradability.

C:R ratio	50:50			60:40			70:30			SEM	P-value		
BMP, %	0.0	0.5	1.0	0.0	0.5	1.0	0.0	0.5	1.0		A	B	A*B
NH_3-N (mg%)													
24 h	11.9	10.5	12.6	12.9	12.8	13.1	11.9	10.8	13.2	0.76	0.48	0.16	0.33
48 h	13.5	14.5	12.2	14.9	13.0	13.3	14.5	15.1	13.6	0.92	0.56	0.44	0.42
DM Degradation, %													
24 h	53.5a	59.3a	58.5^{ab}	60.8^{b}	67.9^{c}	63.8^{b}	67.2^{c}	70.9^{c}	68.3^{c}	1.45	<0.01	0.04	<0.01
48 h	72.3^{a}	73.2^{a}	73.0^{a}	70.9^{a}	73.3^{a}	71.8^{a}	75.4^{ab}	80.2^{b}	76.4^{ab}	1.52	<0.01	0.21	0.05
Short chain fatty acid, mol/L	0.70^{a}	0.81^{b}	0.79^{b}	0.87^{b}	0.94^{c}	0.87^{b}	0.97^{c}	0.99^{c}	0.83	0.03	<0.01	0.28	0.01

[abc] Mean in the same row with different superscript was differ (P<0.05)

Based on these results, it could be indicated that increasing proportion of concentrate in TMR can increase gas production, effective gas production, DMD and SCFA production. High gas production related with high ability of DM degraded in rumen which may cause to increase SCFA production. Yang et al. (2001) reported that the DM and OM digestibilities were linearly increased as the C:R ratio increased. Increasing the C:R ratio increases the DM and OM digestibility in cow and sheep (Moss et al., 1995; Ramos et al., 2009), owing to the forage has a generally higher NDF content than the concentrate. Structural carbohydrates are usually less digestible than non- structural carbohydrates, the total digestibility decreases with increasing proportions of forage in the diet (Moe and Tyrrell, 1979). Supplementation of BMP increased gas production, effective gas production and DM degradability and showed high effect when supplemented at 0.5% of BMP in TMR with 60 and 70 % of concentrate. These indicated that biotin supplementation is alternative way to increased ability of DMD in the rumen. Abel et al. (2001) found that biotin can improve fermentation of fiber in the rumen. Milligan et al. (1967) reported that supplementation of biotin in diet increased fiber digestion in vitro that may continue effect to increase intake and milk yield. Because of biotin is a specific essential vitamin for major cellulolytic organisms (Ashwin and Srinivas, 2019) and most cellulolytic rumen bacteria require biotin for growth (Baldwin and Allison, 1983). Therefore, supplemented biotin in ruminant diet observes a response to increase cellulolytic bacteria and DMD (Zimmerly and Weiss, 2001).

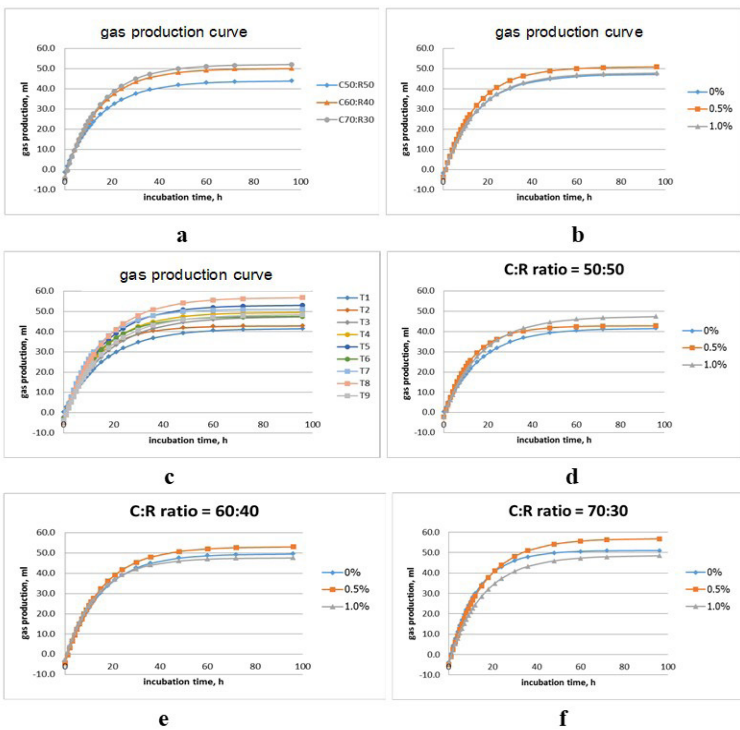

Figure 1. In vitro gas production for the effect by (a) C:R ratio (b) biotin mixed product (c) combination treatment between C:R ratio and biotin mixed product (d, e and f).

Conclusion

Base on the results, increasing proportion of concentrate in TMR can increase gas production, effective gas production, DM degradability and short chain fatty acid production but do not affect NH3-N production. Supplementation of biotin as BMP increase gas production, effective gas production and DM degradability and showed high effect when supplemented at 0.5% of BMP in TMR with 60 and 70% of concentrate but do not affect NH3-N and short chain fatty acid production. In conclusion, biotin supplementation is alternative way to increase DM degradability in the rumen and 0.5% of BMP 0.02 mg of biotin/kg of diet is a recommended level in TMR.

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