



Effects of replacing rice bran with tamarind seed meal in concentrate mixture diets on the changes in ruminal ecology and feed utilization of dairy steers

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Abstract

Feed ingredients costs have been impacting the production cost. Attempts have been made to use local feed resources in order to reduce feed costs. The objective of this study was to identify effect of using crushed tamarind seed meal (TSM) in concentrate mixture on rumen fermentation, dry matter intake, and digestibility of dairy steers. Four rumen-fistulated dairy steers were used in a 4 × 4 Latin Square Design. Four levels of TSM were used to replace rice bran (RB) in the concentrate mixtures in four treatments (T1 = 0% replacement of RB, T2 = 30% replacement of RB, T3 = 60% replacement of RB, T4 = 100% replacement of RB). The findings revealed that replacement of TSM for RB resulted in similar digestibility of nutrients and intakes ($P > 0.05$). However, rumen fermentation parameters were remarkably improved, namely total VFA and the concentration of C3 especially at the highest level of replacements (100%, T4), ($P < 0.05$). Rumen protozoal population was found lowered in all replacements, especially those in higher levels of TSM replacement. Consequently, the rumen methane productions were significantly reduced. TSM can be a promising energy source to replace rice bran, hence lowering the cost of concentrate mixture.

Keywords Tamarind seed meal · Rumen fermentation · Feed resource

Introduction

Feed is an important factor in animal agriculture. If farmers can reduce feed costs, it would increase the income and gain more profit. In the dry season of tropical countries including Thailand, feeds are usually scarce in terms of quantity and quality, especially roughages for ruminants. Most farmers can use different kinds of agricultural crops, crop residuals as well as by-products as ruminants feeds. Some of these feeds have high nutritional values and readily used as animal feed while the others may need to be processed for better utilization. Methane (CH₄) is the ruminal gas production during the anaerobic fermentation (Steinfeld et al. 2006). It is one of the main Green

House Gases (GHG), which has been increased by approximately 40% from 1970 to 2004 (IPCC 2007). It accounts for up to 15% loss of the dietary energy intake. Hristov et al. (2013) and Cieslak et al. (2016), interestingly reported on some potential and promising rumen methane mitigation methods such as the use of feeds with condensed tannins and saponins or leguminous forages as well as the using suitable level of grain. However, the economy impact needs to be carefully considered (Kara et al. 2015). Tamarind (*Tamarindus indica*) was originated from Africa but found in many tropical countries (Souza et al. 2018). Tamarind seed is a by-product of the tamarind processing. It contains high level of soluble carbohydrate which could be incorporated as a source of energy for ruminants. Marangoni et al. (1988) reported that tamarind seeds contain 15% protein and 60% carbohydrate. Bhatta et al. (2001) found that tamarind seed husk contain 15% of tannins as well. It was also found that the use of tamarind seed coat in concentrate could reduce methane production in an in vitro study (Bhatta et al. 2001). However, information on the use of tamarind seeds in ruminant feeds, especially to replace rice bran, is lacking. The objective of this study was to evaluate the effect of tamarind seed meal in replacing rice bran on the changes in ruminal ecology and feed utilization of dairy steers.

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Materials and methods

Animals, diets, and experimental design

All experimental animals were treated with vitamin A, D3, E injection and dewormed before imposing respective treatments. They were raised in individual pen of 3×4 m. size where water and mineral block were available all the times.

Four rumen-fistulated dairy steers with body weight of 250 ± 12 kg were assigned to a 4×4 Latin square design. Four dietary treatments were formulated to contain tamarind seed meal (TSM) at 0, 6, 12, and 20% of DM in the concentrate mixture. Each treatment group was used to replace rice bran at 0, 30, 60, and 100% of DM, respectively. The experiment was carried out for four periods. Each of which was lasting for 21 days. The first 14 days were used for feed adaptation and intake measurement. The last 7 days were used for sample collection using total collection method while the animals were on metabolism crates. Each steer was weighed at the beginning and of each period. Tamarind seeds were collected from tamarind processing group of Koksa-ngud village, Thailand, and sun-dried for two consecutive days and then ground (1 mm screen using the Cyclotech Mill, Tecator, Sweden) into powder form as TSM.

Sampling procedures and chemical analysis

Feed, feces, and urine samples were taken during the experimental period and then stored at 4°C for later chemical analysis. Samples were divided into two parts; the first part was analyzed for DM while the second part was for ash and crude protein (CP) according to AOAC (2012). Acid detergent fiber (ADF) was determined by AOAC method (2012) and was expressed inclusive of residual ash. Neutral detergent fiber (NDF) was estimated according to Van Soest et al. (1991) with addition of α -amylase but without sodium sulfite and the results were expressed with residual ash. Details of samplings of rumen fluid for immediate pH measurement, volatile fatty acids (VFA) analysis (Samuel et al. 1997), blood urea nitrogen (Crocker 1967), protozoal population count (Galyean 1989), and methane production estimation (Moss et al. 2000) were according to report by Wanapat et al. (2014).

Statistical analysis

Data collected were subjected to ANOVA for 4×4 Latin square design (SAS 2013). Data were analyzed using the model: $Y_{ijk} = \mu + M_i + A_j + P_k + \varepsilon_{ijk}$; where Y_{ijk} , observation from animal j , receiving diet i , in period k ; μ , the overall of mean; M_i , the mean effect of different levels of TSM replacing

Table 1 Ingredient and chemical composition of concentrates, rice straw and tamarind seed meal used in the experiment

Items	TSM replacing rice bran (%)				Rice straw	TSM ¹
	0	30	60	100		
Ingredients, kg^{-1}						
Cassava chip	500	500	500	500		
Rice bran	200	140	80	0		
Tamarind seed meal	0	60	120	200		
Coconut meal	100	100	100	100		
Palm meal	100	100	100	100		
Urea	30	30	30	30		
Molasses	40	40	40	40		
Sulfur	10	10	10	10		
Mineral mixed	10	10	10	10		
Salt	10	10	10	10		
Chemical composition, %						
Dry matter	84.3	84.4	84.4	84.5	92.5	91.0
% of DM						
Organic matter	88.9	89.4	89.9	90.6	86.2	87.3
Ash	11.0	10.5	10.0	9.3	13.8	12.7
Crude protein	16.0	15.9	15.9	15.8	2.1	12.3
Neutral detergent fiber	21.8	22.9	24.0	25.4	77.0	31.0
Acid detergent fiber	12.6	12.9	13.2	13.6	56.0	18.6
Condensed tannins	–	–	–	–	–	9.7
Price (USD/kg) ¹	0.33	0.31	0.29	0.28		

TSM, tamarind seed meal, ¹ 1 USD = 31.36 Thai Baht

rice bran ($i = 0, 30, 60, 100\%$ of DM replacing rice bran); A_j , the effect of animal ($j = 1, 2, 3, 4$); P_k , the effect of period ($k = 1, 2, 3, 4$); and ε_{ijk} , the residual effect. Treatment means were compared using Duncan's New Multiple Range Test (Steel and Torrie 1980).

Results and discussion

Chemical composition of concentrates, rice straw, and tamarind seed meal

Chemical composition of concentrate mixture, rice straw, and tamarind seed meal are shown in Table 1. The crude protein content of concentrate mixture ranged between 15.8 and 16.0% of DM. The CP content of TSM was lower than earlier findings by Marangoni et al. (1988) who reported that tamarind seeds contain 15% CP and 60% carbohydrate, while CT content was also lower than the study of Bhatta et al. (2001) who reported that CT was found at 15% of DM.

Feed intake and digestibility of nutrients

The use of tamarind seed meal in the concentrate diet did not affect ($P > 0.05$), feed intake (Table 2). This result was similar to earlier report by Bhatta et al. (2000), who found that the use of tamarind seeds at 7.5% of DM did not influence feed intake in lactating cows. The digestibilities of nutrients (DM, OM,

Table 2 Effect of replacing rice bran with tamarind seed meal in concentrate mixture on feed intake and nutrients digestibility in dairy steers

Items	TSM replacing rice bran (%)				SEM	P value
	0	30	60	100		
Rice straw intake						
kg/day	3.8	3.6	3.9	3.9	0.21	ns
g/kg BW ^{0.75}	51.1	49	52.2	53	2.22	ns
Concentrate intake						
kg/day	2.5	2.3	2.5	2.4	0.09	ns
g/kg BW ^{0.75}	33.5	33	34	33	0.92	ns
Total intake						
kg/day	6.3	5.9	6.5	6.4	0.28	ns
g/kg BW ^{0.75}	84.6	82	86.2	85.6	2.31	ns
Nutrients digestibility, %						
Dry matter	64.6	63.9	60.9	57.2	4.2	ns
Organic matter	61.7	60.3	57.7	54.1	4.4	ns
Crude protein	83.4	82.8	81.4	78.4	2.01	ns
A neutral detergent fiber	58.7	57.6	54.4	52.9	2.56	ns
Acid detergent fiber	51.8	50.7	46.5	44.7	3.6	ns

SEM, standard error of the means; ns, non-significant difference

CP, NDF, and ADF) were not changed among treatments ($P > 0.05$). Bhatta et al. (2000) reported that the use of tamarind seed peels in concentrate diet did not affect the digestibility of nutrients in milking cows. However, Souza et al. (2018) demonstrated that the inclusion of tamarind residue in cassava silage could increase DM and NDF intake in lambs. Nevertheless, high level of CT may reduce nutrient digestibility of the feed (protein, carbohydrates, and fats) while low level of CT not more than 5%DM would be beneficial with protein to by-pass to the lower gut (Kamra et al. 2006; Naumann et al. 2017).

Characteristics of ruminal fermentation parameters and blood metabolite

There was no significant difference on ruminal pH ($P > 0.05$) when rice bran was replaced by TSM at all levels (Table 3).

Table 3 Effect of replacing rice bran with tamarind seed meal in concentrate mixture on ruminal pH, NH₃-N concentration, blood urea nitrogen, and ruminal microbiome in dairy steers

Items	TSM replacing rice bran (%)				SEM	P value
	0	30	60	100		
Ruminal pH						
0 h-post feeding	6.45	6.52	6.72	6.56	0.09	ns
4	6.69	6.46	6.60	6.48	0.11	ns
Mean	6.58	6.54	6.65	6.54	0.09	ns
Temperature, °C						
0 h-post feeding	38.4	38.6	38.3	38.3	0.07	ns
4	38.5	38.5	38.5	38.3	0.26	ns
Mean	38.4	38.6	38.5	38.3	0.15	ns
NH₃-N, mg/dL						
0 h-post feeding	20.7	20.0	21.0	19.5	1.21	ns
4	20.5	20.7	21.9	21.5	1.18	ns
Mean	20.5	20.3	21.5	20.5	0.95	ns
BUN, mg/dL						
0 h-post feeding	17.3	17.5	18.0	17.0	0.80	ns
4	16.0	15.5	15.7	16.0	0.32	ns
Mean	17.3	17.2	16.7	16.8	0.52	ns
Protozoal population, × 10⁵ cells/mL						
0 h-post feeding	9.0	8.8	8.4	8.3	0.77	ns
4	11.1 ^a	7.3 ^b	6.0 ^{bc}	5.1 ^c	0.56	0.01
Mean	10.1 ^a	7.9 ^b	7.4 ^b	6.7 ^b	0.38	0.01
Fungal zoospores, × 10⁴ cells/mL						
0 h-post feeding	4.9	5.0	4.9	5.3	0.52	ns
4	5.3	5.1	5.1	5.5	0.42	ns
Mean	5.1	5.1	5.0	5.4	0.31	ns

BUN, blood urea nitrogen; SEM, standard error of the means; ns, non-significant difference

^{abc} Values within the row with different superscripts are significantly different ($P < 0.05$)

The ruminal pH ranged from 6.54 to 6.65, which was reported to be the optimum level for microbial activity in the rumen, according to Wanapat (1990), who stated that the optimal ruminal pH level ranged between 6.5 and 7.0 could make the rumen ecology suitable for microbial activity. The temperature was not significantly different among treatments and ranged between 38.3 and 38.6 °C. The concentrations of NH₃-N and BUN were not altered among treatments ($P > 0.05$) when the steers received TSM and ranged between 20.5 to 21.5 and 16.7 to 17.3 mg/dL, respectively. The NH₃-N concentrations were similar to the data reported by Wanapat and Pimpa (1999) in which the values obtained were in good range for improving rumen fermentation by rumen microorganisms. Therefore, it can be stated that TSM supplementation did not have any negative effect on the concentration of BUN. Rumen fungal zoospores were not affected among treatments ($P > 0.05$). While the groups with TSM replacing rice bran could lower protozoal population as compared with the

control group ($P < 0.05$), criminal protozoal counts were suppressed by the addition of plants containing secondary compounds in agreement with earlier findings (Pilajun and Wanapat 2013; Moate et al. 2014; Ampapon et al. 2015; Foiklang et al. 2016a). This could be explained by the fact that CT in TSM could play the key role in reducing protozoal population and methanogens (Foiklang et al. 2016b). Likewise, the protozoa can generate hydrogen in the rumen as a substrate for methane synthesized by the existing methanogens. Therefore, the suppression in protozoal population could lead to a significant reduction in methanogens and, consequently, methane production, as well (Jayanegara et al. 2014). Total VFA and propionic acid production was significantly ($P < 0.05$) improved when the animals received the concentrate diet containing TSM (Table 4). Total VFA and propionic acid concentrations were higher in the group of TSM replacing rice bran at 100% ($P < 0.05$) while acetic acid (C₂), butyric acid (C₄), and C₂:C₃ ratio were similar among

Table 4 Effect of replacing rice bran with tamarind seed meal in concentrate mixture on volatile fatty acids and methane production in dairy steers

Items	TSM replacing rice bran (%)				SEM	P value
	0	30	60	100		
Total VFA, mmol/L						
0 h-post feeding	102.1	102	102.1	102.6	2.79	ns
4	114.6 ^a	117.6 ^{ab}	121.4 ^{ab}	130.4 ^c	3.01	0.04
Mean	108.3 ^a	110.1 ^{ab}	111.8 ^{ab}	116.3 ^c	2.49	0.03
Acetic acid (C ₂), mol/100 mol						
0 h-post feeding	62.8	62	61.2	61	0.29	ns
4	57.6	55.2	54.9	54.3	0.35	ns
Mean	60.2	58.6	58	57.8	0.19	ns
Propionic acid (C ₃), mol/100 mol						
0 h-post feeding	26.7	26.9	27.8	27.9	0.69	ns
4	30.1 ^b	33.2 ^{ab}	34.6 ^{ab}	34.8 ^a	0.98	0.01
Mean	28.4 ^b	30.1 ^{ab}	31.2 ^{ab}	31.4 ^a	0.78	0.01
Butyric acid (C ₄), mol/100 mol						
0 h-post feeding	10.5	11.1	11.0	11.1	0.85	ns
4	12.3 ^a	11.6 ^a	11.1 ^{ab}	10.3 ^b	1.18	0.04
Mean	11.4	11.3	11.0	10.7	0.97	ns
CH ₄ , mol/100 mol TVFA						
0 h-post feeding	25.12	24.91	24.31	24.24	0.52	ns
4	22.56 ^a	20.34 ^{ab}	19.36 ^b	19.28 ^b	0.71	0.01
Mean	23.83 ^a	22.63 ^{ab}	21.83 ^b	21.75 ^b	0.58	0.01
C ₂ :C ₃ ratio						
0 h-post feeding	2.38	2.30	2.23	2.23	0.09	ns
4	1.93	1.68	1.58	1.60	0.10	ns
Mean	2.15	1.96	1.88	1.90	0.09	ns

VFA, volatile fatty acids; SEM, standard error of the means; ns, non-significant difference

¹ Calculated CH₄ production by Moss et al. (2000) (mol/100 mol TVFA) = 0.45(% C₂) – 0.275(% C₃) + 0.40(% C₄)

^{abc} Values within the row with different superscripts are significantly different ($P < 0.05$)

treatments ($P > 0.05$). Previous studies reported that the CT had influenced rumen fermentation parameters (Anantasook et al. 2014; Foiklang et al. 2016a). Effect of TSM on promoting VFA production exhibited a pronounced level when animals were fed on high level of fibrous diet. Similar change in ruminal total VFA and propionic acid production by plant containing CT (grape pomace powder) has been reported by Foiklang et al. (2016b). The calculated CH₄ production was significantly reduced when TSM was included in the concentrate mixture ($P < 0.05$). Numerous recent studies revealed that biomass containing secondary compounds and their combinations appeared to be effective in suppressing methane production (Anantasook et al. 2014; Moate et al. 2014; Foiklang et al. 2016b). It has been reported that the use of plant containing condensed tannins had remarkably reduced ruminal CH₄ production, regardless of feed used and the pattern of VFA production (Gemedá and Hassen 2015).

Conclusions and recommendation

Concentrate mixtures containing which TSM resulted in increasing rumen fermentation efficiency, total VFA and propionic acid concentration. Additionally, it has reduced ruminal protozoal population and methane production. Based on these results, it could be suggested that TSM has high potential to be used as an alternative energy source to replace rice bran in the concentrate mixtures up to 100%. It is promising in the areas where tamarind fruit-tree planting is relevant.

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Compliance with ethical standards

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

Research involving human participants and/or animals All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Informed consent Informed consent was obtained from all individual participants included in this study.

References

- Ampapon, T., Wanapat, M. and Kang, S.C., 2015. Rumen metabolism of swamp buffaloes fed rice straw supplemented with cassava hay and urea. *Trop. Anim. Health and Prod.* 48, 779–784.
- Anantasook, N., Wanapat, M. and Cherdthong, A., 2014. Manipulation of ruminal fermentation and methane production by supplementation of rain tree pod meal containing tannins and saponins in growing dairy steers. *J. Animal Physiol. and Anim. Nutr.* 98(1), 50–55.
- AOAC(Official Methods of Analysis). 2012., Association of Official Analytical Chemists, 19th ed. Gaithersburg, MD.
- Bhatta, R., Krishnamoorthy, U. and Mohammed, F., 2000. Effect of feeding tamarind (*Tamarindus indica*) seed husk as a source of tannin on dry matter intake, digestibility of nutrients and production performance of crossbred dairy cows in mid-lactation. *J. Anim. Feed Sci. and Tech.*, 82, 67–74.
- Bhatta, R., Krishnamoorthy, U. and Mohammed F., 2001. Effect of tamarind (*Tamarindus indica*) seed husk tannins on *in vitro* rumen fermentation. *J. Anim. Feed Sci. and Tech.*, 90, 143–152.
- Cieslak, A., Zmora, P., Matkowski, A., Nawrot-Hadzik, I., Pers-Kamczyc, E., El-Sherbiny, M., Bryszak M. and Szumacher-Strabel, M., 2016. Tannins from *Sanguisorba officinalis* affect *in vitro* rumen methane production and fermentation. *J. Anim. and Plant Sci.*, 26(1), 54–62
- Crocker, C.L., 1967. Rapid determination of urea nitrogen in serum or plasma without deproteinization. *The American J. Med. Tech.*, 33(5), 361–365.
- Foiklang, S., Wanapat, M. and Norrapoke, T., 2016a. Effect of grape pomace powder, mangosteen peel powder and monensin on nutrient digestibility, rumen fermentation, nitrogen balance and microbial protein synthesis in dairy steers. *Asian–Australas. J. Anim. Sci.*, 29(10), 1416–1423.
- Foiklang, S., Wanapat, M. and Norrapoke, T., 2016b. *In vitro* rumen fermentation and digestibility of buffaloes as influenced by grape pomace powder and urea treated rice straw supplementation. *Anim. Sci. J.* 87(3), 370–377.
- Galyean, M., 1989. Laboratory Procedure in Animal Nutrition Research. Dept. of Animal and Range Science, New Mexico State University, USA.
- Gemedá, B.S. and Hassen, A., 2015. Effect of Tannin and Species Variation on *in vitro* digestibility, gas, and methane production of tropical browse plants. *Asian– Australas. J. Anim. Sci.*, 28, 188–199.
- Hristov, A.N., Oh, J. and Firkins, J.L., 2013. Mitigation of methane and nitrous oxide emissions from animal operations: I. A review of enteric methane mitigation options. *J. Sci.*, 91, 5045–5069.
- IPCC, 2007. Climate Change 2007: Synthesis Report. Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, Pachauri, R.K and Reisinger, A. (eds.)]. IPCC, Geneva, Switzerland, 104 pp.
- Jayanegara, A., Wina, E. and Takahashi, J., 2014. Meta-analysis on methane mitigating properties of saponin-rich sources in the rumen: Influence of addition levels and plant sources. *Asian–Australas. J. Anim. Sci.*, 27, 1426–1435.
- Kamra, D.N., Agarwal, N. and Chaudhary, L.C. 2006. Inhibition of ruminal methanogenesis by tropical plants containing secondary compounds. *Int. Congr.*, 1293, 156–163.
- Kara, K., Güçlü, B.K. and Baytok, E., 2015. Comparison of nutrient composition and antib methanogenic properties of different *Rosaceae* species. *J. Anim. Feed Sci. and Tech.*, 24, 308–314.

- Marangoni, A., Alli, I. and Kermasha, S., 1988. Composition and properties of seeds of the tree legume *Tamarindus indica*. *J. Food Sci.*, 35, 1452–1455.
- Moate, P.J., Williams, S.R.O., Torok, V.A., Hannah, M.C., Ribaux, B.E., Tavendale, M.H., Eckard, R.J., Jacobs, J.L., Auldist, M.J. and Wales, W.J., 2014. Grape marc reduce methane emissions when fed to dairy cows. *J. Dairy Sci.* 97, 5073–5087.
- Moss, R., Jouany, I.P. and Newbold, J., 2000. Methane production by ruminants: its contribution to global warming. In *Ann. de Zootech.*, 49(9), 231–253.
- Naumann, H.D., Tedeschi, L.O., Zeller, W.E. and Huntley N.F., 2017. The role of condensed tannins in ruminant animal production: advances, limitations and future directions. *R. Bras. Zootec.*, 46(12), 929–949.
- Pilajun, R. and Wanapat, M., 2013. Microbial population in the rumen of swamp buffalo (*Bubalus bubalis*) as influenced by coconut oil and mangosteen peel supplementation. *J. Anim. Physiol. and Anim. Nutr.*, 97, 439–445.
- Samuel, M., Sagathevan, S., Thomas, J. and Mathen, G., 1997. An HPLC method for estimation of volatile fatty acids in ruminal fluid. *Ind. J. Dairy Sci.*, 67, 805–807.
- SAS, 2013. *User's Guide: Statistic, Version 9.4th Edition*. SAS Inst. Inc., Cary, NC, USA.
- Souza, C.M., Oliveira, R.L., Voltolini, T.V., Menezes, D.R., dos Santos, N.J.A., Barbosa, A.M., Silva, T.M., Pereira, E.S. and Bezerra, L.R., 2018. Lambs fed cassava silage with added tamarind residue: Silage quality, intake, digestibility, nitrogen balance, growth performance and carcass quality. *J. Anim. Feed Sci. and Tech.*, 235, 50–59.
- Steel, R.G.D. and Torrie, J.H., 1980. *Principles and Procedures of Statistics: A Biometrical Approach*, 2nd ed. McGraw-Hill Book Co. NY, USA.
- Steinfeld, H., Gerber, P., Wassenaar, T., Castel, V., Rosales, M. and de Haan, C., 2006. Livestock's role in climate change and air pollution. 79–123. In: *Livestock's long shadow: environmental issues and options* (eds. Steinfeld, H., P. Gerber, T. Wassenaar, V. Castel, M. Rosales, and C. de Haan), FAO, Rome, Italy.
- Van Soest, P.J., Robertson, J.B. and Lewis, B.A., 1991. Methods for dietary fiber neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. *J. Dairy Sci.*, 74, 3583–3597.
- Wanapat, M., 1990. *Nutritional Aspects of Ruminant Production in Southeast Asia with Special Reference to Thailand*. Dept. of Animal Science, Faculty of Agriculture, Khon Kaen Univ., Khon Kaen. pp. 217.
- Wanapat, M. and Pimpa, O., 1999. Effect of ruminal NH₃-N levels ruminal fermentation, purine derivatives, digestibility and rice straw intake in swamp buffaloes. *Asian–Australas. J. Anim. Sci.*, 12, 904–907.
- Wanapat, M., Gunun, P., Anantasook, N. and Kang, S., 2014. Changes of rumen pH, fermentation and microbial population as influenced by different ratios of roughage (rice straw) to concentrate in dairy steers. *J. Agric. Sci.*, 152(4), 675–685.