PJN
ISSN 1680-5194

# PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
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Pakistan Journal of Nutrition 14 (9): 620-624, 2015
ISSN 1680-5194


# Effects of Using Indian Mulberry Leaves as Feed Additives on Feed Digestion, Ruminal Fermentation and Milk Production in Dairy Cattle

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**Abstract:** The study considers the effects of the use of Indian mulberry leaves as feed additives on dairy cattle performances. Eight Holstein-Friesian crossbreeds with an average of 475.7 kg/BW were used. The experiment utilized 4 x 4 Replicated Latin Square Design (LSD) that consisted of 4 levels of Indian mulberry leaves additives: 0, 7.5, 10 and 12.5 g/kg DM in total mixed ration (TMR) diet based on rice straw was used as the main roughage source. The trial consisted of four periods of 21 days each. The results from this study showed that there was no effect on DMI. However, the digestibility of DM showed significant difference ($p<0.05$) with increasing levels of Indian mulberry leaves, except in the digestibility of CP, EE, NDF and ADF where there was no significant difference ($p<0.05$). There were no effects on ruminal pH, total VFA, acetate (C2), propionate (C3), butyrate (C4) and the C2/C3 ratio ($p>0.05$) (6.8, 100.8, 57.9, 29.1, 13.2 mol/L and 2.0, respectively). Milk production, milk composition, blood glucose and blood urea nitrogen did not show significant difference ($p>0.05$). However, Indian mulberry leaves at 10 g/kg DM could be used in dairy cattle feeding to improve the digestibility of feed DM and maintaining ruminal pH without affecting ruminal fermentation and milk production.

**Key words:** Feed intake, performance, plant herb

## INTRODUCTION

The secondary plant metabolites are now looked upon as potential sources of compounds that when fed to dairy cattle will favorably alter the ruminal fermentation without causing overall inhibition of fermentation. A blend of essential oils reduced the rate of deamination of amino acid and inhibited the growth of hyper-ammonia producing bacteria, resulting in reduced ammonia nitrogen concentration. Eugenol at 500 mg/L reduced ammonia nitrogen and branched-chain volatile fatty acid (VFA) concentration (McIntosh *et al*., 2003; Castillejos *et al*., 2006). Anti-methanogenic effects of garlic and its active components were the result of the direct inhibition of Archaea microorganisms in the rumen (Busquet *et al*., 2005). Condensed tannin at 20-40 mg/kg DM slowed down protein degradation in rumen and improved protein utilization by animals (Acamovic and Brooker, 2005). Honey *et al*. (2012) reported that Indian mulberry leaf, or *Morinda citrifolia* leaf, contained polysaccharide, quinine-glycoside and flavonoid substants and anthraquinone. The substances could inhibit growth of *staphylococcusaureus*, *pseudomonas aeruginosa*, *proteus morgaii*, *bacillus subtilis*, *escherichia coli*, *helicobactor pylori* and *salmonella* (EFSA, 2008; Takashima *et al*., 2007). Also, it has been reported to have a broad range of therapeutic effects including antibacterial, antifungal, antiviral, antitumor, antihelmin, analgesic, hypotensive, anti-inflammatory and immune enhancing effects (Wang *et al*., 2002; Hemwimon *et al*., 2007). The green fruit and leaves were traditionally used in Polynesian and Asian cultures to treat menstrual cramps, bowel irregularities, diabetes, liver diseases and urinary tract infection. In the local cuisine of Asian food, the leaves are used as a green vegetable and the fruit is added as a salad ingredient. They are believed to promote food digestion and act as food laxative. EFSA (2008) reported that Indian mulberry leaves could be eaten at 1.29 g/d for adult males, or 18.4 mg/kg bodyweight for a 70 kg adult. Yulistiani *et al*. (2015) found that mulberry foliage was a potential supplement of fermentable energy and protein for sheep fed with treated rice straw at 1.2% of BW. However, a rare document in animals was reported. Therefore, secondary plant metabolites were highly sought for use in manipulation of ruminal fermentation to enhance nutrient utilization. Consequently, the objective of this study was to observe the effects of the addition of Indian mulberry powder to the dairy cattle feed to determine the effects on feed intake, digestibility and dairy cattle performances.

## MATERIALS AND METHODS

**Animal and treatments:** Eight 98.4% purebred Holstein-Friesian lactating dairy cows with average body weight of 475.7±30.2 kg in mid-lactation (144±35 day in milk) of the second to third lactation were used. The experiment

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was used as 4 x 4 Replicated Latin Square Design (LSD), corresponding to the addition of Indian mulberry leaf powder in the levels of 0, 7.5, 10 and 12.5 g/kg of DM on TMR with rice straw as the main roughage source. All diets were formulated to contain 15% CP and 67.8% TDN (Table 1) using the KCF 2006 software of Pattarajinda and Duangjinda (2006). Each cow was weighed before the start of experimental period and at the end to find body weight (BW) changes. The duration of this experiment was 4 periods, each lasting 21 days.

**Measurements, sample collection and chemical analysis:** Weekly composites of TMR and orts were collected from daily samples of about 0.5 kg and stored at -20°C. TMR diet samples were dried for 48 h at 60°C. Then, the diet samples were ground to pass through a 1 mm screen using a Wiley Mill (Thomas-Wiley, Philadelphia, PA) and analyzed for DM, CP, ether extract and ash (AOAC, 1985) and for NDF and ADF (Van Soest *et al*., 1991). Cows were housed individually in pens with free access to water. Cows were offered TMR diet 2 times a day (7.00 am and 4.00 pm). Feed offered and orts were measured and recorded daily during the period of the experiment to calculate feed intake. Cows were weighed on the starting and ending date of the experimental period. Cows were milked twice daily at 4.00 am and 3.00 pm and milk production recorded. Milk samples were collected on days 18 and 19 of each period and analyzed for fat, protein, lactose, total solid (TS) and solid not fat (SNF) using Milkosonic S-L90 procedures.

On day 15 of each period, 20 g of $Cr_2O_3$ was included in all diets as an external marker. Fecal samples were collected at 5 h intervals and composited for nutrient and chromium analysis to estimate dry matter and feed digestibility according to Maynard and Loosli (1975). Blood samples were collected on day 21 of each period from the coccygeal vein at hourly intervals (0, 1, 2 and 3 h) in 10 ml tubes. After, each tube was immediately placed on ice until centrifugation at 3,000 rpm for 15 min and after centrifuging, blood serum was stored at -20°C before being analyzed for blood glucose and blood urea nitrogen using an automated chemistry analyzer (Hitachi 912). Also, on day 21 of each period rumen fluid was collected at hourly intervals (0, 1, 2 and 3 h) by a suction tube and ruminal fluid samples were obtained by straining ruminal contents through 2 layers of cheesecloth. The pH was immediately determined by using a pH meter (Electrochemical Analyzer, Consort model C933P) and then preserved by the addition of 5 ml of 1 $MH_2SO_4$ solution to 50 ml of rumen fluid and stored at -20°C. Prior to analysis, the ruminal fluid samples were thawed and centrifuged at 3,500 rpm for 15 min at 4°C. The supernatant part was separated for analysis in order to find volatile fatty acids (VFA) using a HPLC (Instruments by Waters model 600E controller; Waters model 484 UV detector) according to the method of Samuel *et al*. (1997).

**Statistical analysis:** The data from the experiment was analyzed for variance following the Latin Square Design (LSD) experiment using Proc GLM (SAS, 1996). Means were compared by using Duncan's New Multiple Range Test, with level of significance set at $p<0.05$.

## RESULTS

**Nutrient composition of diets:** For the nutrient composition of TMR with different levels of added Indian mulberry leaf powder, it was found that CP was very similar at around 15% and DM, ether extract, NDF, ADF and ash (64.7, 3.4, 35.7, 22.2 and 10.7%, respectively) were all similar to the numbers that had been calculated, meaning that the animals had the same intake close to the calculation and met the requirements in all groups of animal (Table 1).

**Dry matter intake and body weight changes:** The DMI of dairy cows was not significant ($p>0.05$). However, when considering the numbers, it was found that the addition of 12.5 g/kg DM Indian mulberry leaf powder reduced the DMI. When considering the DMI as a percentage of BW (DMI, %BW), it was found that the addition of Indian mulberry leaf powder to ration did not lead to the difference of DMI as a percentage of BW (3.4, 3.4, 3.4 and 3.3%, respectively, $p>0.05$). Likewise, with the DMI as a percentage of $BW^{0.75}$ (DMI, $\%BW^{0.75}$), it was found that all treatments were not statistically significant ($p>0.05$) as well (2.5, 2.5, 2.5 and 2.4, respectively) (Table 3). The average initial body weight was 475.7 kg and the average final weight was 486.6 kg. The BW had increased in all treatment groups (13.1, 11.2, 10.3 and 9.9 kg, respectively) (Table 3).

**Nutrient digestibility:** It was found that for the addition of leaf powder at the levels of 0, 7.5, 10 and 12.5 g/kg DM. TMR, the best digestibility responses to leaf powder were found at the levels of 10 and 12.5 g/kg DM. (78.2 and 79.4). Also the digestion of feed DM increased as the percentage of supplemental dose increased. The digestion of CP, ADF and Ash tended to increase, meaning that Indian mulberry played a certain role in promoting ruminal feed digestion even if animals were fed with low-quality roughage such as rice straw. On the other hand, the digestibility of EE and NDF showed no statistically significant difference ($p>0.05$) (Table 2).

**Ruminal pH:** Ruminal pH was not affected by Indian mulberry leaves in any of the treatments, both before and after feeding. The average rumen pH levels at 0, 1, 2 and 3 h after feeding were 6.8, 6.8, 6.9 and 6.9, respectively (Table 3).

Table 1: Formulation and calculated nutrient composition (%, DM) in TMR diets

| Ingredient | % DM |
|---|---|
| Corn | 14.3 |
| Soybean meal | 12.0 |
| Rice bran | 8.0 |
| Cassava chip | 5.0 |
| Cane sugar | 7.0 |
| Salt | 1.0 |
| Minerals | 0.3 |
| Urea | 1.3 |
| Sulfur | 0.1 |
| Brewer's grain | 13.0 |
| Rice straw | 38.0 |
| **Nutrient composition (%)** | |
| DM | 63.1 |
| CP | 15.0 |
| TDN | 67.9 |
| NDF | 37.7 |
| ADF | 21.3 |
| Ether extract | 3.1 |

DM: Dry matter, CP: Crude protein, NDF: Neutral detergent fiber, ADF: Acid detergent fiber

Table 2: Nutrient analysis of TMR diets with differing levels of Indian mulberry leave powder (%DM basis)

| | ---------- Level of Indian mulberry leaves -------- | | | |
|---|---|---|---|---|
| Item | 0 | 7.5 | 10 | 12.5 |
| DM | 64.3 | 63.8 | 65.0 | 65.6 |
| CP | 14.5 | 14.9 | 15.6 | 15.5 |
| Ether extract | 3.4 | 3.0 | 3.5 | 3.8 |
| NDF | 35.7 | 36.2 | 35.5 | 35.4 |
| ADF | 21.5 | 22.1 | 22.6 | 22.5 |
| Ash | 10.8 | 11.0 | 10.6 | 10.3 |

DM: Dry matter, CP: Crude protein, NDF: Neutral detergent fiber, ADF: Acid detergent fiber

**Volatile fatty acid production in the rumen:** There were no effects on total VFA (101.7, 99.2, 101.5 and 100.9, respectively), acetate concentration (57.8, 56.7, 57.0 and 58.5%, respectively), propionate concentration (28.5, 30.0, 29.7 and 29.7%, respectively), butyrate concentration (13.7, 13.3, 13.2 and 11.7, respectively) and C2:C3 ratio (2.0, 1.9, 2.0 and 2.0, respectively) (Table3).

**Blood glucose and blood urea nitrogen (BUN):** There were no differences in blood glucose concentrations (BG) before and after feeding at 1, 2 and 3 hours post feeding. BG concentrations for the treatments were 59.5, 58.9, 59.2 and 59.3 mg/dL, respectively (Table 4). Blood urea nitrogen concentrations (BUN) were not affected. The average BUN levels at 1, 2 and 3 hours post feeding were 17.3, 18.3, 19.4 and 18.9 mg/dL, respectively.

**Milk production and milk composition:** The levels of Indian mulberry leaf powder in the diets did not affect milk production and milk composition, average milk yield, fat, protein, lactose, TS and SNF (11.5, 3.5, 2.9, 4.1, 11.6 and 8.1%, respectively; p>0.05) (Table 5).

Table 3: Dry matter intake, body weight (BW) change, ruminal pH and VFA of lactating dairy cows fed differing levels of Indian mulberry leave powder in TMR diets

| | - Level of Indian mulberry leaves - | | | | | |
|---|---|---|---|---|---|---|
| Item | 0 | 7.5 | 10 | 12.5 | SEM | p-value |
| DMI kg/d | 16.3 | 16.4 | 16.6 | 15.8 | 0.20 | 0.50 |
| BW (%) | 3.4 | 3.4 | 3.4 | 3.3 | 0.05 | 0.94 |
| $BW^{0.75}$ (%) | 2.5 | 2.5 | 2.5 | 2.4 | 0.03 | 0.94 |
| Initial BW, (kg) | 473.3 | 475.8 | 478.3 | 474.5 | 1.50 | 0.54 |
| Final BW, (kg) | 486.4 | 487.0 | 488.6 | 484.4 | 3.30 | 0.62 |
| BW Change, (kg) | 13.1 | 11.2 | 10.3 | 9.9 | 1.58 | 0.49 |
| **Digestion (%)** | | | | | | |
| DM | $70.2^{c}$ | $75.7^{bc}$ | $78.2^{ab}$ | $79.4^{a}$ | 0.12 | 0.02 |
| CP | 82.5 | 80.3 | 81.5 | 81.2 | 0.35 | 0.14 |
| Ether extract | 87.4 | 88.6 | 87.1 | 88.4 | 0.26 | 0.20 |
| NDF | 77.6 | 80.8 | 78.3 | 76.9 | 0.32 | 0.18 |
| ADF | 78.0 | 76.3 | 80.6 | 78.5 | 0.38 | 0.12 |
| **Ruminal pH** | | | | | | |
| 0-h pre feeding | 6.9 | 7.0 | 6.9 | 7.1 | 0.03 | 0.08 |
| 1-h post feeding | 6.9 | 6.9 | 6.8 | 6.8 | 0.03 | 0.71 |
| 2-h post feeding | 6.9 | 6.7 | 7.0 | 6.9 | 0.04 | 0.11 |
| 3-h post feeding | 6.8 | 6.9 | 6.7 | 6.8 | 0.04 | 0.80 |
| 1-3 h post feeding | 6.8 | 6.8 | 6.9 | 6.9 | 0.02 | 0.88 |
| **Total VFA, mmol/L** | | | | | | |
| 0-h pre feeding | 98.3 | 103.9 | 101.4 | 101.8 | 0.78 | 0.20 |
| 1-h post feeding | 97.4 | 103.2 | 96.9 | 96.6 | 1.18 | 0.20 |
| 2-h post feeding | 103.4 | 98.5 | 102.3 | 103.8 | 0.67 | 0.14 |
| 3-h post feeding | 100.2 | 100.0 | 102.8 | 102.7 | 0.99 | 0.67 |
| 1-3 h post feeding | 101.7 | 99.2 | 101.5 | 100.9 | 0.87 | 0.54 |
| **Molar proportion of VFA (mol/100 mol)** | | | | | | |
| Acetate (C2) | 57.8 | 56.7 | 57.0 | 58.5 | 0.43 | 0.60 |
| Propionate (C3) | 28.5 | 30.0 | 29.7 | 29.7 | 0.29 | 0.46 |
| Butyrate (C4) | 13.7 | 13.3 | 13.2 | 11.7 | 0.33 | 0.30 |
| C2/C3 | 2.0 | 1.9 | 2.0 | 2.0 | 0.03 | 0.21 |

Values with different letters in the same row differ significantly ($p<0.05$) DMI: Dry matter intake, BW: Body weight, $BW^{0.75}$ Metabolic of the body weight, DM: Dry matter, CP: Crude protein, NDF: Neutral detergent fiber, ADF: Acid detergent fiber, VFA: Volatile fatty acids

Table 4: Blood glucose and blood urea nitrogen of lactating dairy cows fed differing levels of Indian mulberry leave powder in TMR diets

| | - Level of Indian mulberry leaves - | | | | | |
|---|---|---|---|---|---|---|
| Items | 0 | 7.5 | 10 | 12.5 | SEM | p-value |
| **Glucose, (mg/dL)** | | | | | | |
| 0-h pre feeding | 56.0 | 55.0 | 55.4 | 56.0 | 0.69 | 0.94 |
| 1-h post feeding | 59.4 | 56.0 | 57.0 | 56.5 | 1.00 | 0.65 |
| 2-h post feeding | 57.4 | 60.4 | 59.5 | 57.9 | 1.00 | 0.50 |
| 3-h post feeding | 61.8 | 60.4 | 61.1 | 63.4 | 0.46 | 0.17 |
| 1-3-h post feeding | 59.5 | 58.9 | 59.2 | 59.3 | 0.63 | 0.99 |
| **BUN, (mg/dL)** | | | | | | |
| 0-h pre feeding | 16.0 | 16.9 | 17.6 | 17.7 | 0.49 | 0.60 |
| 1-h post feeding | 17.2 | 17.3 | 19.3 | 18.6 | 0.38 | 0.18 |
| 2-h post feeding | 17.9 | 18.3 | 19.4 | 18.6 | 0.39 | 0.54 |
| 3-h post feeding | 18.9 | 19.4 | 19.6 | 19.6 | 0.68 | 0.44 |
| 1-3-h post feeding | 17.3 | 18.3 | 19.4 | 18.9 | 0.45 | 0.40 |

BUN: Blood urea nitrogen

Table 5: Milk production and milk composition of lactating dairy cows differing levels of Indian mulberry leave powder in TMR diets

| | Level of Indian mulberry leaves | | | | | |
|---|---|---|---|---|---|---|
| Item | 0 | 7.5 | 10 | 12.5 | SEM | p-value |
| Milk yield, (kg/d) | 11.4 | 11.7 | 11.6 | 11.5 | 0.15 | 0.91 |
| Milk 4% FCM, (kg/d) | 10.5 | 10.9 | 10.6 | 10.6 | 0.32 | 0.63 |
| Milk Fat, (%) | 3.4 | 3.5 | 3.4 | 3.6 | 0.04 | 0.58 |
| Milk Protein | 2.9 | 2.9 | 3.0 | 3.0 | 0.18 | 0.59 |
| Lactose | 4.2 | 4.1 | 4.2 | 4.2 | 0.20 | 0.45 |
| Milk TS, (%) | 11.2 | 11.5 | 11.8 | 12.1 | 0.05 | 0.93 |
| Milk SNF, (%) | 7.8 | 8.0 | 8.1 | 8.5 | 0.02 | 0.34 |

4% FCM: Milk yield (0.4+0.15% Fat), TS: Total solids, SNF: Solids not fat

## DISCUSSION

Indian mulberry leaf powder naturally has a bitter flavor. When adding in higher proportions, it may cause a bitter effect that animals may decrease intake; however, among the treatments, high dosage levels might not be used as the effect was not found. Also, the rice straw feeding as main roughage source had gut-fill effect from bulky density effect. Besides moisture, bulk density and gut distention may affect DMI (Eastridge, 2006). Cows in mid-lactation normally have started to increase their body weight by increasing their intake. Consequently, the responses to Indian mulberry leaves may have lower effects by intake of animals.

The DMI in animals supplies nutrients for production, improving body weight and maintenance. For the DM digestibility in this study, increasing nutrients digestion had the same effects as increasing dosage of all treatment groups. The findings of digestibility percentage by using $Cr_2O_3$ method had given higher number compared to other methods because it was measured in particulate form only (Yulistiani *et al*., 2015; Kung *et al*., 2003).

Controlling effects of flavonoid and anthraquinone substants could have been a major role via anti-oxidation, anti-inflammation and inhibition of growth rate of ruminal microbial and methanogenic bacteria (Takashima *et al*., 2007; Kung *et al*., 2003) that might partially support feed digestion and maintain rumen function (Yupakarn and Pattarajinda, 2012).

The ruminal pH levels were in normal range (6.8-6.9), consistent with the report by Van Soest (1994) which mentioned that the ruminal pH should have the pH of between 6.0-7.0. However, hourly analysis found that supplemental Indian mulberry leaf powder could help to maintain ruminal pH to have alkali balance even when the animal was fed more concentrated feed ratio (62%). After feeding at 1 and 2 hours, the pH did not drop down lower than 6.5. Bach *et al*. (2005) reported that ruminal pH above 6.5 would promote the growth rate of ruminal microorganism and promote protease activity.

VFA value was in normal range as France and Siddons (1993) mentioned. Feeding at levels of 10 and 12.5 g/kg DM had numerically higher total VFA production than that of lower supplemental levels that was related to DM intake and digestion of intake nutrients. In the first hour, more acetate was produced than others, meaning that Indian mulberry may play a certain role in increasing in roughage digestion. Krause and Combs (2003) reported that concentration of VFA production was dependent on amount of intake and concentrate ratio and particle size of fiber the higher the proportion of concentrate ratio, the higher the propionate production. In this study, propionate and acetate still produced more during the first 2 h post feeding when compared to butyrate. Sutton *et al*. (2003) reported that feeding cattle from 60% of concentrate to 90% of concentrate produced double concentration of propionate and decreased concentrations of acetate and butyrate (at ratios of 67.0:19.0:13.0 to 50.1:37.0:9.1, respectively, $p<0.05$). Consequently, for the intake based on long form of rice straw with inclusion of 38% of diets, the ruminal digestion and VFA production of the experiment seemed to have normal production. Average acetate to propionate ratio was 1.95. The ratio was quite in normal range because acetate and propionate were also highly produced in both acids during the first to third hours after feeding.

Blood glucose concentrations (BG) in this study were consistent with those of Mudron *et al*. (2005) which stated that the normal range of blood glucose level was between 43.2-68.4 mg/dL. Glucose levels of treatment groups elevated after meal through 3 h. when compared to those of control group in which the glucose levels had dropped down in the second hour before going up and had more fluctuation levels than those of supplemental groups. It might be an effect from Indian mulberry because the higher the feed DM digestion, the more consistent the glucose concentration was found.

Blood urea nitrogen concentrations from the experiment were within the normal range of 4-25 mg/dL (Kohn *et al*., 2005). All treatment groups had a linear fashion and had numerically higher levels of BUN than that of control group, according to digestion of DM and digestion of high protein content.

Milk production correlated with that of cow in mid-lactation status and the amount of DMI (Beede and Collier, 1986). Also, for milk compositions, there was no statistically significant ($p>0.05$) difference, but the treatment groups had higher percentages. The DMI among treatments had low intake and cow in mid-lactation status may affect milk production and composition because the animal did not need that much requirement for milk production and instead increased her body weight during this period. Increases in feed digestion in levels of 10 and 12.5 g/kg DM could not support milk production because this experiment used low quality roughage, so the amount of nutrient production could be too low in concentration to support high milk production.

**Conclusion:** The use of Indian mulberry leaf powder as dairy cow feed additive could possibly increase the effectiveness in supporting feed digestion, especially in roughage feed. The addition of Indian mulberry leaf powder in the level of 10 g/kg DM in the TMR can increase the digestion of DM without affecting ruminal fermentation and milk production. One way that this seems to be an advantage is it could help to maintain ruminal pH ecology. Green roughage sources would be included in further studies.

## ACKNOWLEDGEMENT

This study was supported by the Higher Education Research Promotion and National Research University

Project of Thailand, Office of the Higher Education Commission, through the Food and Functional Food Research Cluster of Khon Kaen University. The facilities were supported by the Thermo-tolerant Dairy Cattle Research Group, Roi Et Agricultural Research and Training Center, KKU.

## REFERENCES

Acamovic, T. and J.D. Brooker, 2005. Biochemistry of plant secondary metabolites and their effects in animals In Symposium on Plants as animal foods: a case of catch 22 Proceed. of the Nutr. Soc., 64: 403-412.

AOAC, 1985. Official Methods of Analysis, 14th Edn.,Washington, DC: Association of Official Analytical Chemists.

Bach, A., S. Calsamiglia and M.D. Stem, 2005. Nitrogen metabolism in the rumen. J. Dairy Sci., (E. Suppl.) 88: E9-E21.

Beede, D.K. and M. Collier, 1986. Potential nutritional strategies for intensively managed cattle during thermal stress. J. Anim.Sci., 62: 543-553.

Busquet, M., S. Calsamiglia, A. Ferret, M.D. Carro and C. Kamel, 2005. Effect of garlic oil and four of its compounds on rumen microbial fermentation. J. Dairy Sci., 88: 4393-4404.

Castillejos, L., S. Calsamiglia and A. Ferret, 2006. Effect of essential oil active compounds on rumen microbial fermentation and nutrient flow in *in vitro* systems. J. Dairy Sci., 89: 2649-2658.

Eastridge, M.L., 2006. Major advances in applied dairy cattle nutrition. J. Dairy Sci., 89: 1311-1323.

European Food Safety Authority (EFSA), 2008. Safety of leaves from Morindacitrifolia L. EFSA J., 769: 1-17.

France, F. and R.C. Siddons, 1993. Volatile fatty acid production. In: Forbes, J.M. (Eds.), Quantitative Aspects of Ruminant Digestion and Metabolism. Wallingford, UK, pp: 107-121.

Hemwimon, S., P. Pavasant and A. Shotipruk, 2007. Microwave-assisted extraction of antioxidative anthraquinones from roots of *Morinda citrifolia*. Sep. Sci. Technol., 54: 44-50.

Honey, J., B. Neha, B. Ranjan, S. Birendra and A. Thakur, 2012. Scientific basis of Noni Plant (Morinda citrfolia). Asian J. Pharm. Sci., 2: 45-47.

Kohn, R.A., M.M. Dinneen and E. Russek-Cohen, 2005. Using blood urea nitrogen to predict nitrogen excretion and efficiency of nitrogen utilization in cattle, sheep, goats, horses, pigs and rats. J. Anim. Sci., 83: 879-889.

Krause, K.M. and D.K. Combs, 2003. Effects of forage particle size, forage source and grain fermentability on performance and ruminal pH in mid lactation cows. J. Dairy Sci., 86: 1382-1397.

Kung, J.L., K.A. Smith, A.M. Smagala, K.M. Endres, C.A. Bessett, N.K. Ranjit and J. Yaissle, 2003. Effects of 9,10 anthraquinone on ruminal fermentation, total-tract digestion and blood metabolite concentrations in sheep. J. Anim. Sci., 81: 323-328.

Maynard, L.A. and J.K. Loosli, 1975. Animal Nutrition, 6thEdn., McGraw-Hill book company, New York.

McIntosh, F.M., P. Williams, R. Losa, R.J. Wallace, D.A. Beever and C.J. Newbold, 2003. Effect of essential oil on ruminal metabolism and their protein metabolism. Appl. Environ. Microbiol., 69: 5011-5014.

Mudron, P., J. Rehage, H.P. Sallmann, M. Holtershinken and H. Scholz, 2005. Stress response in dairy cows related to blood glucose. Acta Vet., 74: 37-42.

Pattarajinda, V. and M. Duangjinda, 2006. Dairy Feed and Least Cost Feed Formulation Program. Department of Animal Science. Faculty of Agriculture, KhonKaen Universitry, KhonKaen.

Samuel, M., S. Sagathewan, J. Thomas and G. Maten, 1997. An HPLC method for estimation of volatile fatty acids of ruminal fluid. Ind. J. Anim. Sci., 69: 805-807.

SAS, 1996. SAS User's Guide: Statistics, Version 6 ed. SAS Inst., Inc., Cary, NC.

Sutton, J.D., M.S. Dhanoa, S.V. Morant, J. France, D.J. Napper and E. Schuller, 2003. Rates of production of acetate, propionate and butyrate in the rumen of lactating dairy cows given normal and low-roughage diets. J. Dairy Sci., 86: 3620-3633.

Takashima, J., Y. Ikeda, K. Komiyama, M. Hayashi, A. Kishida and A. Ohsaki, 2007. New constituents from the leaves of Morinda citrifolia. Chem. Pharm. Bull. (Tokyo), 55: 343-345.

Van Soest, P.J., 1994. Nutritional ecology of the ruminant, 2nd ed. Cornell University Press, Ithaca.

Van Soest, P.J., J.B. Robertson and B.A. Lewis, 1991. Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci., 74: 3583-3597.

Wang, M.Y., B.J. West, C.J. Jensen, D. Nowicki, C. Su, A.K. Palu and G. Anderson, 2002. *Morinda citrifolia*: A literature reviewand recent advances in Noni research. Acta Pharmacol Sin., 23: 1127-1141.

Yulistiani, D., Z.A. Jelan, J.B. Liang, H. Yaakub and N. Abdullah, 2015. Effects of Supplementation of Mulberry (Morus alba) Foliage and Urea-rice Bran as Fermentable Energy and Protein Sources in Sheep Fed Urea-treated Rice Straw Based Diet. Asian Aust. J. Anim. Sci., 28: 494-501.

Yupakarn, W. and V. Pattarajinda, 2012. The studies of using dry herbs and extract oil to improve *in vitro* dairy cattle feed digestibility. KHON KAEN AGR. J., 40: 137-140.