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## ผลของการใช้ใบไชยาในอาหารต่อการย่อยได้และโลหิตวิทยาในโครุน

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**บทคัดย่อ:** การศึกษาครั้งนี้เป็นการทดสอบผลของการใช้ใบไชยาในอาหารต่อการย่อยได้ของโภชนาและค่าโลหิตวิทยาในโคคนมระยะรุน โดยใช้โคนมระยะรุนเพศเมียจำนวน 4 ตัว อายุเฉลี่ย  $300.75 \pm 83.47$  วัน น้ำหนักตัวเฉลี่ย  $181.75 \pm 42.74$  กิโลกรัม ถูกเลี้ยงในคอกซังเดี่ยว ถูกแบ่งออกเป็น 2 กลุ่ม ตามแผนการทดลองแบบสลับ กลุ่มละ 2 ชั้้ (ในแต่ละระยะ) ได้แก่ กลุ่มควบคุมที่ได้รับอาหารขัน (16% โปรตีน) และกลุ่มที่ใช้ใบไชยาแห้งที่ระดับ 5% ของอาหารขัน การให้อาหารขันของทั้งสองกลุ่มให้ในปริมาณ 0.75% ของน้ำหนักตัว แบ่งเป็นกลุ่มละ 2 ชั้้ต่อการทดลอง จำนวน 2 ระยะๆ ละ 21 วัน โดยชั้นน้ำหนักเริ่มต้นและเก็บตัวอย่างเลือดในวันที่ 1 ก่อนเริ่มให้อาหารทดลองและน้ำหนักสุดท้ายรวมถึงเก็บตัวอย่างเลือดในวันที่ 21 ของแต่ละระยะของการทดลอง ส่วนการย่อยได้มีระยะเวลาในการปรับ 17 วัน และทำการเก็บข้อมูล 4 วัน โดยใช้การหาการย่อยได้แบบใช้สารบ่งชี้ พบว่า การตากแห้งทำให้ปริมาณไชยาในเดือขยะลดลงเหลือ 97.2 ppm จากปริมาณสต 567 ppm และเมื่อนำไปใช้ในอาหารที่ระดับ 5% ของอาหารขันนั้น ไม่พบผลกระทบต่อการกินได้และน้ำหนักตัวที่เพิ่มขึ้น ( $P>0.05$ ) และค่าการย่อยได้ของรตถแห้งและโปรตีนก็ไม่พบความแตกต่างกันทางสถิติ ( $P>0.05$ ) ค่าโลหิตวิทยาที่สำคัญได้แก่ ค่าเม็ดเลือดแดงดีดแน่น ค่าจำนวนเซลล์เม็ดเลือดแดง ค่าฮีโมโกลบิน และค่าจำนวนของเซลล์เม็ดเลือดขาวไม่มีความแตกต่างกันยกเว้นระดับของลิโนฟิโซทีโนโคที่ได้รับใบไชยา มีค่าต่ำกว่ากลุ่มควบคุมอย่างมีนัยสำคัญทางสถิติ ( $P<0.05$ ) นอกจากนี้เมื่อวัดระดับของเอนไซม์ ALT AST ในชีรัม และระดับของอัลบูมิน ซึ่งเป็นดัชนีที่บ่งชี้ถึงความผิดปกติของเนื้อเยื่อตับนั้น ไม่มีความแตกต่างกันทางสถิติระหว่างกลุ่มควบคุมและกลุ่มที่ได้รับใบไชยา ซึ่งผลจากการศึกษาครั้งนี้สรุปได้ว่าการใช้ใบไชยาแห้งทัดแทนอาหารขันที่รับ 5% ไม่ส่งผลกระทบต่อการกิน การเจริญเติบโต การย่อยได้และสุขภาพของโคนมระยะรุน ดังนั้นการใช้ใบไชยาแห้งเป็นอีกทางเลือกที่จะนำมาเป็นแหล่งวัตถุดิบโปรตีนได้

**คำสำคัญ:** ค่าโลหิตวิทยา ใบไชยา โคนมระยะรุน การย่อยได้ของโภชนา

#ผู้รับผิดชอบบทความ

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## Effect of Feeding Chaya (*Cnidoscolus chayamansa*) Leaf on Nutrient Digestibility and Blood Profiles in Growing Dairy Cattle

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**Abstract:** The aim of this study was to investigate the effect of feeding dried chaya (*Cnidoscolus chayamansa*) leaf on nutrient digestibility and blood profiles in growing dairy cattle. Four Holstein-Friesian growing heifers averaging  $181.75 \pm 42.74$  kg of body weight and  $300.75 \pm 83.47$  d of age were randomly assigned to a change over design in two periods with two treatments as follows: control group and replacing concentrate with 5% chaya leaf. The concentrate was given 0.75% of BW with fresh pangola grass as roughage. There were 17 d adaptation in each period, followed by 4 d measurement period for fecal sample collection. Blood collection was collected in each cow: pre and post-trial via jugular venipuncture. The result of this study revealed that cyanide content in dried chaya leaf was 97.2 ppm. Compared to control group, body weight gain, average daily gain, dry matter intake and G : F were not significantly different ( $P>0.05$ ). There were no significant differences in dry matter and crude protein digestibility between groups. The 5% chaya leaf had no impact on PCV, RBC, hemoglobin, MCV, MCH and MCHC values. While lymphocytes of the cow fed 5% dried chaya leaf was significantly lower ( $P<0.05$ ) than lymphocytes of the control group. Blood chemistry serum such as total protein, albumin, AST and ALT of 5% chaya leaf were not significantly different from the control group. This study indicates that the replacement of concentrate with 5% dried chaya leaf can be used as a feed ingredient since it has no effect on growth and health in growing dairy cattle.

**Keywords:** Blood profiles, Chaya leaf, Growing dairy cattle, Nutrient digestibility

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### Introduction

Currently, the climate change affects crop, such as maize and soybean, which results in the low production. That result is the cause of rising feed prices. To find out an alternative feed source from fodder tree is interesting because it

is cheaper. Chaya (*Cnidoscolus chayamansa*) is an Euphorbiaceae shrub like cassava. The origin of chaya is in the Yucatán Peninsula of Mexico. Then it is distributed in Guatemala and Central America. Chaya is a drought tolerant and fast-growing plant. In addition, chaya leaf has high

protein content around 23.6–29% DM (Donkoh et al., 1999; Totakul et al., 2021b), iron 4.7 mg/100 g and ascorbic acid 142.11 mg/100 g (Chikezie et al., 2016). Chaya plant is also beneficial to health because it contains phenolic compound (Kuri-GarcÃ-a, 2017). It was reported that using chaya in pellet form increased dry matter and protein digestibility in *in vitro* gas production technique (Totakul et al., 2021a) and beef cattle (Totakul et al., 2021b). Feeding high level of chaya in place of concentrate did not show negative effect on feed intake and digestibility in growing goat (Kumar et al., 2010). Although, chaya leaf consists of cyanogenic glycoside 2.37–4.25 mg/100 g of dry weight, heat treatment such as sun drying can decreased cyanogenic glycoside (Gonzalez-Laredo, 2003).

Hematology or blood biochemical profiles is one of the key indicators of animal health. It is noted that feeding chaya leaf caused the change in mean corpuscular volume (MCV), neutrophil, lymphocyte, eosinophil and platelets counts in rabbit (Lawal et al., 2010). Alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein and albumin are the parameters related to liver function. Feeding chaya leaf extract showed anti-inflammatory and hepato protective effect (Pérez-González et al., 2018). The serum of total protein and albumin increased in broiler fed chaya leaf (Oni et al., 2017). Therefore, the aim of this study was to investigate the effect of feeding dried chaya leaf on nutrient digestibility and blood profiles in growing dairy cattle.

## Materials and Methods

### Ethics statements

This study was approved by the ethics committee of Maejo University Animal Care and Use (Approval no. MACUC002P/2565), which is relevance to animal ethics for scientific proposes of National Research Council of Thailand.

### Chaya leaf preparations

Chaya leaves (*Cnidoscolus chayamansa*) were harvested and dried in the sun for 2-3 d. Afterwards, the dried chaya leaves were ground and placed into plastic bags. The dried chaya leaf was analyzed for chemical composition such as dry matter, crude protein, and crude fiber according to Association of Official Analytical Chemists (1984) methods.

### Determination of Hydrocyanic acid

Hydrocyanic acid was determined by using alkaline titration. First, ten grams of ground chaya leaves were immersed in 200 mL of distilled water for 12 h. Second, the mixture was distilled using 20 mL of 2.5% sodium hydroxide as a trap and then continued the distillation until approximately 150 mL of solution was collected. After that, the solution was added to 8 mL of 6 mol of ammonia hydroxide ( $\text{NH}_4\text{OH}$ ) solution and 2 mL of 5% potassium iodide (KI) solution. 0.02 mol of silver nitrate ( $\text{AgNO}_3$ ) was used for titration until the solution became cloudy white, which was the end point. The cyanide content was calculated from

$$1 \text{ mL of } 0.02 \text{ mol } \text{AgNO}_3 = 1.08 \text{ mg HCN}$$

### Animals and Experimental design

Four Holstein-Friesian growing heifers averaging (Mean  $\pm$  SD)  $181.75 \pm 42.74$  kg of body weight and  $300.75 \pm 83.47$  d of age were randomly assigned to a change over design in two periods with two treatments as follow: control group and replacing concentrate with 5% chaya leaf. The concentrate was given 0.75% of BW with fresh pangola grass as roughage. The initial and final BW were measured on days 1 and 21 respectively in each period. The digestibility

was also measured. There were 17 d adaptation in each period, followed by 4 d feed and fecal collection period. All cows were reared in the dairy cattle farm at Maejo University Phrae campus (Phrae province) and fed at 0700 and 1700 h daily. The feed and faecal samples were collected during the periods to determine dry matter, crude protein and acid insoluble ash. The apparent digestibility was calculated using the following formula:

$$\text{Digestibility} = 1 - \frac{(\text{Nutrient concentration in feces (%DM)} \times \text{AIA in diet (%DM)})}{(\text{Nutrient concentration in diet (%DM)} \times \text{AIA in feces (%DM)})}$$

### Blood collection and analyzation

Blood collection was collected in each cow: pre and post-trial (day 1 and 21 from each period). 12 mL of blood was collected via jugular veinpuncture in each cow, 2 mL of blood sample was placed in vacuum tube containing potassium ethylenediaminetetraacetic acid (BD Vacutainer®, Becton Drive, Franklin Lakes, USA) for a complete blood count. Another 10 mL of blood was placed in vacuum tube and separated by centrifuged at  $4,200 \times g$  for 5 min to collect serum. All blood samples and serum were stored at temperature below  $4^{\circ}\text{C}$  and analysed within 24 h. after collecting.

Complete blood count such as packed cell volume (PCV), red blood cell (RBC), hemoglobin, MCV, mean cell hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets, white blood cell (WBC),

neutrophils, eosinophil, lymphocytes and monocytes was determined by automated haematology analyser (NIHON KOHDEN MEK-6550K Series) and serum chemistries such as total protein, albumin, ALT and AST were determined by automate blood chemistry analyser (Automatic Clinical Chemistry BA400 Analyzer).

### Statistical analysis

Growth performances such as body weight gain, average daily gain, dry matter intake and gain to feed ratio (G:F) were analysed using Student's t test. Similarly, nutrient digestibility and blood profiles were also analysed.

## Results

### Chemical composition of experimental feed

Dry matter, crude protein and crude fiber of experimental feed are shown in Table 1. The

dried chaya leaf in this experiment contains about 26.27% of crude protein. The cyanide content in dried chaya leaf is 97.2 ppm. Compare with fresh leaf which was collected and analysed, the fresh leaf is 567 ppm.

#### Body weight gain and digestibility

Compared to control group, body weight gain (BWG), average daily gain (ADG), dry matter intake (DMI) and G : F were not significantly different. Likewise, DM and CP digestibility had no significant difference between groups (Table 2).

#### Blood profiles

There were no differences in PCV, RBC, hemoglobin, MCV, MCH, MCHC and platelets between the control and 5% dried chaya leaf. In addition, WBC, neutrophils, eosinophil and monocytes did not differ between groups. However, 5% dried chaya leaf decreased lymphocytes ( $P<0.05$ ) (Table 3).

Blood biochemical profiles such as total protein, albumin, AST and ALT did not differ between groups (Table 4).

**Table 1** Chemical composition of experimental feed

Item	Concentrate	Pangola grass	Dried chaya leaf
DM, %	92.00	35.30	91.70
CP, %DM	17.95	7.77	26.27
CF, %DM	–	34.42	14.04
HCN, ppm	–	–	97.2

DM = dry matter; CP = crude protein; CF = crude fiber; HCN = hydrocyanic acid

**Table 2** Effect of 5% dried chaya leaf substitute concentrate feed on growth performance and dry matter (DM) and crude protein (CP) digestibility in growing dairy cattle (Mean±SD)

Item	Group		P-value
	Control	5% Chaya	
BWG (Kg)	9.87±7.19	12.00±8.44	0.71
ADG (Kg/d)	0.39±0.20	0.47±0.29	0.67
DMI (Kg DM/d)	3.72±0.39	3.72±0.40	1.00
DMI (%BW)	2.09±0.23	2.14±0.28	0.83
G:F	0.11±0.07	0.13±0.08	0.70
Nutrient digestibility (%)			
DM	51.31±8.07	52.59±11.87	0.86
CP	57.70±12.08	57.50±9.79	0.98

BWG = body weight gain; ADG = average daily gain; DMI = dry matter intake; G:F = gain to feed ratio;

DM = dry matter; CP = crude protein

**Table 3** Blood haematology of growing dairy cattle fed 5% dried chaya leaf substitute concentrate feed (Mean±SD)

Parameter	Group		SEM	P-value	Reference*
	Control	5% Chaya			
PCV, %	28.25±4.35	28.75±2.87	1.21	0.854	21-38
RBC ( $10^6/\mu\text{L}$ )	7.10±1.26	7.27±1.13	0.39	0.843	4.9-10
Haemoglobin (g/dL)	8.95±1.21	9.12±0.71	0.33	0.812	8.4-14
MCV, fL	40.50±2.52	40.50±3.79	1.05	1.00	36-50
MCH, pg	12.67±0.99	12.58±1.43	0.40	0.912	11-19
MCHC, %	31.37±0.53	31.07±0.98	0.26	0.610	31-43
Platelets, $10^3/\mu\text{L}$	340.75±142.67	265.50±147.77	47.95	0.759	160-800
WBC, $10^3/\mu\text{L}$	20.95±4.25	18.57±5.43	1.66	0.517	4.9-13.3
Neutrophils, $10^3/\mu\text{L}$	9.73±2.14	10.81±3.98	1.10	0.648	1.0-6.3
Eosinophil, $10^3/\mu\text{L}$	0.83±0.62	1.68±0.61	0.29	0.191	0.1-1.5
Lymphocytes, $10^3/\mu\text{L}$	8.59±1.73 <sup>a</sup>	6.17±0.55 <sup>b</sup>	0.62	0.037	1.6-8.1
Monocytes, $10^3/\mu\text{L}$	0.97±0.60	1.13±0.81	0.23	0.764	0.1-0.8

PCV = packed cell volume; RBC = red blood cell; MCV = mean corpuscular volume; MCH = mean cell hemoglobin; MCHC = mean corpuscular hemoglobin concentration; WBC = white blood cell

<sup>a,b</sup>Means in the same row with different superscripts differ significantly ( $p < 0.05$ ).

\*Kraft and Dürr (2005); Wood and Quiroz-Rocha (2010); George et al. (2010) cited by Roland et al. (2014)

**Table 4** Total protein, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST) in serum in growing dairy cattle fed 5% dried chaya leaf substitute concentrate (Mean±SD)

Parameter	Group		SEM	P-value
	Control	5% Chaya		
Total protein, g/dL	5.73±0.40	6.10±0.37	0.14	0.217
Albumin, g/dL	2.97±0.46	3.02±0.39	0.14	0.874
AST, U/L	53.67±20.82	44.25±15.75	12.21	0.523
ALT, U/L	24.50±4.80	26.00±2.94	1.33	0.613

### Discussion

In the current study, CP of chaya leaf was 26.27% DM. This finding agreed with Donkoh et

al. (1999) and Totakul et al. (2021) who stated that chaya leaf had CP between 23.6–29% DM. In comparison to fresh chaya leaf, dried chaya

leaf has low concentration of hydrocyanic acid (HCN). In this study, the HCN in dried chaya leaf was 97.2 ppm which was lower than toxic level of HCN in animal. Under this study, the HCN in dried chaya leaf was low. Hence, the HCN content which is 97.2 ppm is safe in forage. This is due to the fact that the HCN content of less than 100 ppm in wet basis is safe for the animal. In addition, the dried chaya leaf in this study was given 1.29% of DMI. As a result, the cow was received 0.035 mg/kg of BW which was lower than toxic level in previous data. Kumar (1992) reported that the lethal dose of HCN for cattle and sheep was 2-4 mg/kg of BW. In the present experiment, the DMI, BWG and G:F in cattle fed with dried chaya leaf were not different from the control group. Kumar (2010) indicated that the replacement of concentrate with chaya leaf at 50-75% did not affect the DMI and CP digestibility in goat. Supplementing chaya leaf meal pellet at 4-8% did not affect total intake, while supplementing at 8% increased DM and CP digestibility in growing crossbred bulls (Totakul et al., 2021b). Under this work, the digestibility of DM and CP did not show the difference between groups. These results were possibly related to a very small amount of dried chaya leaf substitute. Nevertheless, the hematology profiles in this study was in the same normal range as previous reports (Kraft and Dürr, 2005; Wood and Quiroz-Rocha; 2010; George et al., 2010 cited by Roland et al., 2014), except that WBC and neutrophil were higher in both two groups. This result may be related with the age

of cow. The highest WBC was observed in young horses (Chineke et al., 2006) and chicken (Addass et al., 2012). Moreover, the lymphocytes were decreased in rabbit administrated with Chaya leaf extract (Lawal et al., 2010). In this study, blood lymphocytes of cattle fed 5% dried chaya leaf which served as a substitute for concentrate were lower than the control. However, the blood lymphocytes of both two groups were normal range of healthy cow. The total protein, albumin, AST and ALT are indicators of animal health. High value of ALT and AST especially related with the abnormality of liver function. According to this study, the total protein, albumin, AST and ALT in serum of cattle fed 5% dried chaya leaf did not differ from the control group. It was reported that cattle received low level of cyanide did not show any sign of toxicity or change in level of liver enzyme. Cope (2021) suggested low amount of cyanide could be detoxified by the liver and excreted through urine. This study recommends that level of supplementation under the experiment has no negative effect on cattle health.

In conclusion, based on this experiment, the replacement of concentrate with 5% dried chaya leaf can be used as an alternative feed ingredient to reduce feed costs without affecting performance and health in growing dairy cattle.

### Conflict of Interest

The authors declare that there are no conflicts of interest.

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