การสร้างแบบสำหรับการคำนวณผลผลิตจุลินทรีย์โปรตีนจากกระเพาะรูเมนของแพะ โดยใช้อนุพันธ์พิวรีนในน้ำปัสสาวะ

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บทคัดย่อ

การศึกษานี้ประกอบด้วย 3 การทดลองย่อยและทำการทดลองที่ฟาร์มสัตว์ทดลองของมหาวิทยาลัย Putra Malaysia เมือง Serdang รัฐ Selangor ประเทศมาเลเซีย เพื่อสร้างแบบสำหรับการคำนวณผลผลิตจุลินทรีย์โปรตีนจากกระเพาะรูเมนของแพะ

การทดลองที่ 1 แพะเพศผู้พันธุ์ Ferral จำนวน 6 ตัว (น้ำหนัก 40.2 $\pm$ 4.6 กก.) นำมาศึกษาหาปริมาณอนุพันธุ์พิวรีนพื้นฐาน (endogenous purine derivatives) ที่หลั่งในน้ำปัสสาวะสภาวะอดอาหาร

การทดลองที่ 2 แพะเพศผู้พันธุ์ Ferral จำนวน 4 ตัว (น้ำหนัก 39.6$\pm$1.8 กก.) นำมาศึกษาหาสัดส่วนระหว่างปริมาณอนุพันธุ์พิวรีนในเลือดกับปริมาณอนุพันธุ์พิวรีนที่หลั่งในน้ำปัสสาวะ โดยใช้ [^{14}C]-Uric acid เป็น marker แพะทดลองจะได้รับปริมาณอาหาร 2 ระดับ คือ 40% และ 80% ของปริมาณที่กินได้อย่างเต็มที่ อาหารที่ใช้ในการทดลองนี้เป็นอาหารสำเร็จรูป ประกอบด้วย 40 % ใบปาล์มแห้งกับ 60 % อาหารข้น (OPFC) โดยมีแผนการทดลองเป็นแบบ incomplete 2 $\times$ 4 Latin square

การทดลองที่ 3 ของเหลวในกระเพาะรูเมนแพะเพศผู้พันธุ์ Ferral จำนวน 4 ตัวที่ถูกฆ่า นำมาปั่นแยกเอาส่วนที่เป็นจุลินทรีย์มาศึกษาหาสัดส่วนระหว่างปริมาณพิวรีนไนโตรเจนของจุลิน-ทรีย์ต่อปริมาณไนโตรเจนทั้งหมดของจุลินทรีย์ทุกชนิดจากกระเพาะรูเมน

ผลการศึกษาพบว่าค่าเฉลี่ยอนุพันธุ์พิวรีนพื้นฐาน ซึ่งได้แก่ อัลแลนตอย กรดยูริก แซนทีน ไฮโปแซนทีนที่หลั่งในน้ำปัสสาวะขณะอดอาหารมีค่าเท่ากับ 202 $\pm$ 17 ไมโครโมลต่อหนึ่งกิโลกรัมเมตตาโบลิกของร่างกาย (μmol/kg$^{0.75}$), เปอร์เซนต์เฉลี่ยของ [^{14}C]-tracer ที่พบในน้ำปัสสาวะหลังจากฉีด [^{14}C]-Uric acid เข้าไปในเลือดแล้ว มีค่าเท่ากับ 83 $\pm$ 2.0 % (cv=6.88 ช่วง 76.3-91.4% จำนวนแพะ 8 ตัว) โดยที่ปริมาณการกินได้ของอาหารทั้งสองระดับไม่มีอิทธิพลต่อการพบ [^{14}C]-tracer ในน้ำปัสสาวะ และสัดส่วนของจุลินทรีย์พิวรีนไนโตรเจนต่อไนโตรเจนทั้งหมดของจุลินทรีย์จากกระเพาะรูเมนมีค่าเฉลี่ย 0.085.

ผลการศึกษานี้ สามารถสร้างแบบความสัมพันธ์ระหว่างอนุพันธ์พิวรีนที่หลั่งในน้ำปัสสาวะกับจุลินทรีย์พิวรีนที่ดูดซึมในลำไส้เล็ก โดยใช้เปอร์เซนต์ที่พบของ [^{14}C]-tracer กับ อนุพันธุ์พิวรีนพื้นฐานที่หลั่งในน้ำปัสสาวะ ได้ดังนี้ $Y= 0.83X+0.202\times BW^{0.75}$ ซึ่ง

Y = อนุพันธ์พิวรีนที่หลั่งในน้ำปัสสาวะ (มิลลิโมล/วัน)

X = จุลินทรีย์พิวรีนที่ดูดซึมที่ลำไส้เล็ก (มิลลิโมล/วัน)

$BW^{0.75}$ = Metabolic body weight (kg)

ดังนั้น จุลินทรีย์ไนโตรเจนจากกระเพาะรูเมน(MNpd g/d) สามารถคำนวณได้จากจุลินทรีย์พิวรีนที่ดูดซึมที่ลำไส้เล็ก(X) ได้ดังนี้

$$MNpd = \frac{70 \times X}{0.085 \times 0.83 \times 1000} = 0.992 \times X \text{ (กรัมไนโตรเจน/วัน)}$$

ซึ่ง 0.085 คือ สัดส่วนของอนุพันธุ์พิวรีนไนโตรเจนของจุลินทรีย์ต่อไนโตรเจนทั้งหมดของจุลินทรีย์จากกระเพาะรูเมน, 0.83 คือ ค่าเฉลี่ยของการย่อยได้จุลินทรีย์พิวรีน และ 70 คือ ปริมาณไนโตรเจนในพิวรีน (มิลลิกรัมไนโตรเจน/มิลลิโมล) การศึกษานี้สรุปว่า แบบสำหรับการคำนวณจุลินทรีย์โปรตีนจากกระเพาะรูเมนในแพะนี้เป็นประโยชน์ที่สามารถนำไปเปรียบเทียบคุณค่าของอาหารที่ใช้ในฟาร์มได้

Development of Equation Based on Urinary Purine Derivatives to Estimate Rumen Microbial Protein Production in Goats

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Abstract

Three experiments were conducted at the farm of the Universiti Putra Malaysia, Serdang, Selangor, Malaysia, to establish a model as an index for estimating rumen microbial protein production. In Experiment 1, six Ferral male goats (wt. 40.2±4.6 kg) were used to determine the endogenous purine derivatives (PD) excreted in the urine by fasting. In Experiment 2, four Ferral male goats (wt. 39.6±1.8 kg) were used to measure the proportion of plasma PD excreted in the urine by using [^{14}C]-uric acid as a marker at two levels of feed intake (40% and 80 % voluntary intake), using an incomplete 2 × 4 Latin square experimental design. The feed consisted of 40 % oil palm frond and 60 % concentrate (OPFC). In Experiment 3, four Ferral male goats fed (OPFC) were slaughtered and rumen contents were taken for measurements of purine and total nitrogen contents of mixed rumen microbes.

The results showed that endogenous PD (allantoin, uric acid, xanthine and hypoxanthine) excreted in the urine obtained by the fasting trial was 202±17 μmol/kg $BW^{0.75}$ d^{-1}. The average percentage recovery of plasma PD excretion in the urine by using [^{14}C]-uric acid as a marker was 83±2.0 % (*cv*=6.88, ranged 76.3-91.4 %, *n*=8). Percentage recovery was not affected by levels of feed intake. The ratio of purine N: total N in the mixed rumen liquid associated bacteria (LAB) was 0.085.

In this study, a preliminary model for goats was established by using the information from the recovery of labeled PD [^{14}C]-uric acid and the fasting PD excretion. The model obtained was $Y = 0.83X + 0.202 \times BW^{0.75}$, where

Y = PD excretion in the urine (mmol/d)

X = PD absorption at small intestine (mmol/d)

$BW^{0.75}$ = Metabolic body weight (kg)

Thus the microbial nitrogen based on total PD (MNpd) can be calculated as follows:

$$MNpd = \frac{70 \times X}{0.085 \times 0.83 \times 1000} = 0.992 \times X \text{ (g/d)}$$

where 0.085 is the ratio of purine-N: total N in mixed rumen microbes, 0.83 is the average of digestibility of microbial purine from published results, and 70 is the N content of purines (mg N/mmol). The equation can be useful in comparing the nutritive value of diets at farm level.

Key words: Purine derivatives, Rumen, Microbial protein, [^{14}C]-uric acid, Goats

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Introduction

It is generally known that urinary purine derivatives (PD) can be used as an alternative method for predicating rumen microbial protein in the ruminant livestock. Predication equations have been developed for sheep [1, 2] and cattle [3]. There are some studies on the use of urinary purine derivatives (PD) to predict rumen microbial protein synthesis in goats in the temperate region [4, 5 and 6], but none in the tropics.

Three experiments were therefore conducted to develop a predication model for goats in the tropics. The objective of the first experiment was to estimate the rate of basal PD (endogenous PD) excretion in the urine. The objective of the second experiment was to measure the proportion of plasma PD excretion in the urine (using nuclear technique), and the objective of the third experiment was to determine the proportion of purine-N and total-N in mixed rumen microbes of goats.

Materials and Methods

The experiments in this study were conducted according to the procedures described by IAEA-TECDOC 945 [7]. In the first experiment, 6 male Ferral goats (40.2±4.6 kg) were fed pellets consisting of 40% oil palm frond and 60% concentrate (OPFC) on dry matter basis. The animals were fed 1.6 % DM of body weight daily for 3 weeks. The amount of feed offered was reduced stepwise within 2 days (60 % and 30 %), before fasting for 6 days. Total urine out put of each animal was collected daily in plastic bags containing 200 ml of 15% (v/v) H_2SO_4 (to maintain final pH of urine below 3). Daily urine was collected over a total period of 15 days, 9 days before fasting and 6 days during fasting. The volume of urine samples were recorded, then kept at –20 ^{o}C prior to PD analysis [8]. In the second experiment, 4 male Ferral goats (39.6±1.8 kg) were fed OPFC at 40 % and 80 % of voluntary feed intake. The experiment was design as a 2×4 incomplete Latin square (Table I).

Table I The experimental design by 2×4 incomplete Latin square at 40 and 80 % voluntary feed intake

Period	Goat no. 1	Goat no. 2	Goat no. 3	Goat no. 4
1	-	**80**	-	**40**
2	**40**	-	**80**	-
3	-	**40**	-	**80**
4	**80**	-	**40**	-

The experiment consisted of 4 periods, where each period (19 days) was divided into12 days of dietary adaptation and 7 days of samples collection. On day 11, each animal was placed in a metabolic crate. Each crate was fitted with containers for separate urine and faecal collection.

On the first day of each samples collection period (day 13), the double-lumen polyurethane catheter (Duo Certofix®, B. Braun) was inserted into the jugular vein of each animal. On day 14, the animals were injected with sterile 30 μCi [2,8- ^{14}C] uric acid in 30 ml of 0.9 % NaCl solution via the double-lumen polyurethane catheter.

Total urine out put of each animal was collected daily into plastic bag containing 200 ml of 15% (v/v) H_2SO_4 (final pH of urine collected below 3). The urine was collected at 24 h interval on days 13, 18 and 19, at 3 h interval on days 14 and 15, and at 12 h interval on days 16 and 17. The

volume of urine samples were recorded, then kept at –20 $^{\circ}$C prior to PD analysis and determination of specific radioactivity of [^{14}C] by using the Liquid Scintillation Counter [Packard Tri-CARB 4530].

In the third experiment, approximately 2 – 4 kgs of rumen contents were taken immediately from the rumen of four goats after slaughtering (3 h after the morning feed). These animals had been fed with OPFC *ad libitum* approximately 1 month before slaughtering. Rumen content samples were squeezed through 8 layers of surgical gauze to separate rumen fluids and particles. The liquid portion was immediately used for microbial isolation [9, 10], prior to purine bases [11] and total N [12] analyses.

Results

Basal PD Excretion

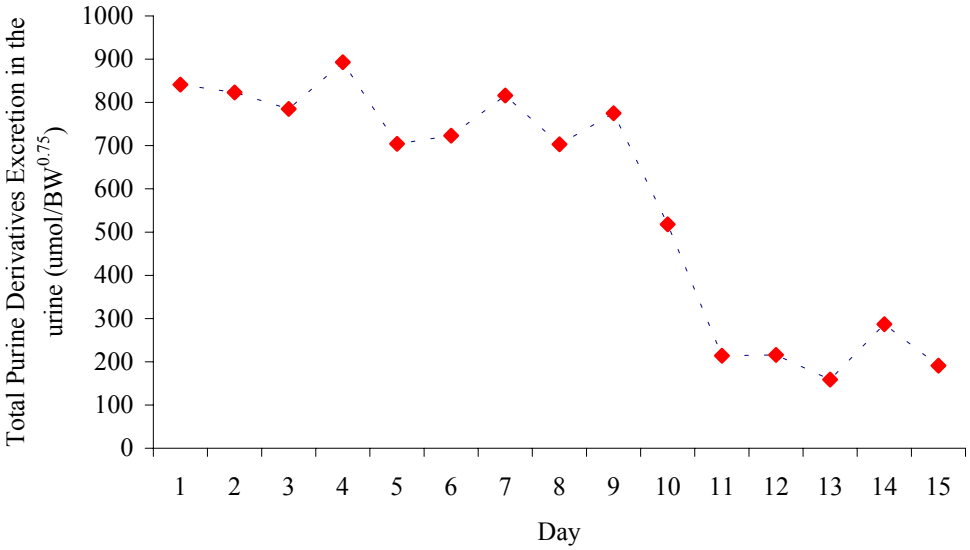

Figure 1 **Excretion of Urinary PD in Goats during Pre-fasting and Fasting**

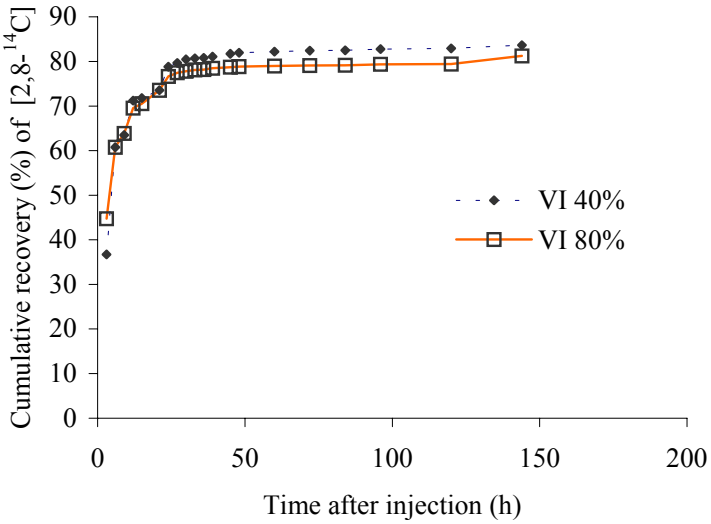

Figure 2 Cumulative Recovery of [2,8 –^{14}C Uric acid] in the Urine of Goats fed with Two Level of Voluntary Intake.

The daily PD excretion in the urine of goats during pre-fasting and fasting periods is shown in *Figure 1*. The average daily endogenous PD excretion in Goats during Fasting Periods urine was 202±17 µmol/d. The proportion of purine derivatives to total PD in the urine were allantoin 73.9%, uric acid 9.5%, xanthine and hypoxanthine 16.6%.

Proportion of Plasma PD Excretion in Urine (Nuclear Technique)

The results showed that there were no differences (p>0.05) in cumulative recovery of [14-C]-tracer excretion in the urine between different levels of feed intake. Total recovery of PD in the urine was 83.65 and 81.26 % for feed intake at 40 and 80 %, respectively (*Figure 2*). The proportion of plasma PD excreted in the urine is defined as 'b coefficient' in the relationship between PD excretion in the urine and microbial purine absorption. Thus, the PD excretion in urine: Y=b(E+X) where Y is PD excretion in the urine, E= endogenous PD excretion in the urine and X is the microbial purine absorption at the small intestine.

The Proportion of Purine-N to Total-N in Mixed Rumen Microbes of Goats

The chemical composition (% OM basis) of mixed rumen liquid associated bacteria (LAB) isolated from goats is presented in Table II. The average amount of RNA, total purines and total-N mixed rumen microbes were 9.77, 10.82 %, 10.43%, respectively and the ratio obtained for purine –N to total-N mixed rumen microbes was 8.51 %.

Table II **Percentage Chemical Composition of Mixed Rumen Microbes Based on OM and the Ratios between Total Purine-N to Total N of Mixed Rumen Microbes of Goats**

Items	Goats	*Standard Error* [2/]
DM	96.11	±0.73
Ash	12.03	±0.37
OM [3/]	89.27	±0.30
Nitrogen [4/]	10.43	±0.33
RNA [5/]	9.77	±0.52
Purine $bases_{Yeast}$ [6/]	10.82	±0.58
A+G (mmol/g) [7/]	1.21	±0.02
PurineN: N [8/]	8.51	±0.46

[1/] Number of animals =4 (each animal = 2 replications)
[2/] Standard error of difference
[3/] DM-Ash
[4/] Nitrogen was analysed by the method described by Kjeldahl method (12)
[5/] RNA was analysed by the method described by (11)
[6/] Calculated based on 1.107 mmol purine/g yeast RNA (9)
[7/] Purine bases was analysed by the method described by using HPLC (10)
[8/] Purine N/÷total Nitrogen

The Equation Based on Recovery of Labeled PD [^{14}C-Uric acid] and the Endogenous PD Excretion.

The model developed for goats based on the recovery of labeled PD [^{14}C-uric acid] and the endogenous PD excretion is $Y= 0.83X+0.202\times BW^{0.75}$ $[0.83(X+0.243\times BW^{0.75})]$.

where Y is PD excretion (mmol/d), proportion PD excretion in the urine is 0.83±2.0. [Averaged (mean±SE)from *Figure 2*], X is microbial purine absorption in mmol/d. and $BW^{0.75}$ is the metabolic body weight (kg).

Calculation of Intestinal Flow of Microbial N

It is assumed that the ratio of purine and protein in mixed microbial population is constant, then PD excretion can provide an index for calculation of intestinal flow of microbial nitrogen.

The following factors are used for calculation of intestinal flow of microbial N (g/d) from the microbial purines absorbed (x mmol/d): absorbed =
[(PD excreted in the urine(mmol/d) – Endogenous PD in the urine ($0.243\times BW^{0.75}$) mmol/d)]÷0.83

i) Digestibility of microbial purine is assumed to be 0.83 (13).

ii) The N content of purines is 70 mg N/mmol.

iii) The ratio of purine-N: total N in mixed rumen microbes is taken as 8.51:100 (Table II).

$$\text{Microbial N (gN/d)} = \frac{X\times (\text{mmol/d})\times 70}{0.085\times 0.83\times 1000} = 0.992\times X$$

Discussion and Conclusion

Urinary purine derivatives (PD) is often used a marker to estimate microbial purines absorption, hence microbial protein supply to the intestine. This technique is less exhaustive as animals do not require digestive tract surgery. The equation can be useful in comparing the nutritive of diets at farm level. The equation derived from this study is

$$\text{Microbial N (gN/d)} = \frac{X \times (\text{mmol/d}) \times 70}{0.085 \times 0.83 \times 1000} = 0.992 \times X .$$

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