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OPART PIMPA

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DOCTOR OF PHILOSOPHY
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By

OPART PIMPA

Thesis Submitted to the Graduate School, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

January 2002

UPM

I wish to dedicate this thesis to my beloved and respected parents, teachers and supervisors who had enriched my knowledge and experience. I also wish to dedicate this thesis to all the buffaloes and cattle used for my experiments.

Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

URINARY PURINE DERIVATIVES EXCRETION AS A METHOD FOR ESTIMATION OF RUMEN MICROBIAL PROTEIN PRODUCTION IN SWAMP BUFFALOES AND ZEBU CATTLE

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January 2002

Chairman: Associate Professor Liang Juan Boo, Ph.D.

Faculty: Agriculture

Prediction equations based on urinary purine derivatives (PD) excretion rate as an index to predict rumen microbial protein production have been developed for European cattle and sheep. However, there is evidence to suggest that those equations may not be applicable directly to tropical swamp buffaloes (*Bubalus bubalis*) and zebu cattle (*Bos indicus*). To establish similar equations for the above two species of ruminant, five studies were conducted. In the first study, endogenous PD excretion rate determined by fasting procedure for swamp buffaloes and the Malaysian indegenous KK cattle (zebu cattle) were 199 and 300 μmol/kg$^{0.75}$ /day, respectively. Urinary PD excretion rate per kg digestible organic matter intake (DOMI) for buffaloes (8.19 mmol/kg DOMI) was significantly lower than that for KK cattle (15.45 mmol/kg DOMI). The second study examined the relationship between daily urinary PD excretion (Y, mmol) and exogenous purine bases (PB) supply via duodenal infusion (X, mmol/day). The relationship obtained were $Y = 0.12X + 12.78$ ($r^2 = 0.45$) for buffaloes and $Y = 0.85X + 7.15$ ($r^2 = 0.62$) for KK cattle, suggesting that 12% and 85% of the supplied exogenous purine were excreted in the urine of buffaloes and

zebu cattle, respectively. In the third study, labelled [8-^{14}C] uric acid marker was used to test the hypothesis that the lower recovery rate of urinary PD in swamp buffaloes was due to their higher recycling of plasma PD as compared to KK cattle. The averaged non-renal PD loss of plasma PD for swamp buffaloes and KK cattle did not differ significantly. The result thus rejected the above hypothesis. A follow-up study was conducted to examine whether the lower urinary PD excretion rate for buffaloes could be due to lower absorption in the small intestine as compared to cattle. The results indicated that purine absorption rate for buffaloes as percent of disappearance of the first segment of small intestine was not significantly different from that of zebu cattle. However a significantly larger quantity of digesta and PB was noted in the last 3 segments of the small intestine of buffaloes than of cattle. The above results suggested that the lower urinary PD excretion rate in the buffaloes could be partially due to their lower absorption rate in the small intestine as compared to cattle. Based on the endogenous and PD excretion rate obtained in this thesis, the relationships between daily urinary PD excretion (Y, mmol) and daily microbial purine supply (X, mmol) were $Y = 0.12X + 0.20\ kg^{0.75}$ for buffaloes and $Y = 0.85X + 0.30\ kg^{0.75}$ for zebu cattle. The equation for zebu cattle is similar to those published for European cattle. Due to the extremely low recovery rate (12%), the proposed equation for buffaloes should be used with great caution. Based on the above equations, microbial N yield for buffaloes and zebu cattle can be calculated from the following equation;

$$\text{Microbial N (gN/day)} = \frac{X\ (\text{mmol/day}) \times 70}{0.073 \times 0.83 \times 1000} = 1.16X$$

In the above equation, digestibility of microbial purine was assumed to be 0.83 and N content of purine was 70 mg N/mmol. The ratio of purine N: total N in mixed rumen microbes was taken as 7.3: 100 for buffaloes and zebu cattle.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

TERBITAN PURINA URINA SEBAGAI SATU KAEDAH UNTUK MENGANGGARKAN PENGHASILAN PROTEIN MIKROB RUMEN KERBAU DAN LEMBU ZEBU

Oleh

OPART PIMPA

Januari 2002

Pengerusi: Prof. Madya Dr. Liang Juan Boo

Fakulti : Pertanian

Kadar pengeluaran terbitan purina dalam urina (PD) telah digunakan sebagai indeks bagi menganggar penghasilan protein mikrob rumen ternakan ruminan. Persamaan dihasilkan untuk lembu dan bebiri Europah tetapi ia tidak boleh digunakan secara langsung pada kerbau (*Bubalus bubalis*) dan lembu (*Bos indicus*) tropikal. Oleh itu, lima eksperimen telah dijalankan bagi mendapatkan persamaan untuk kerbau dan lembu. Dalam eksperimen pertama, kadar pengeluaran PD endogenus untuk kerbau dan lembu KK yang ditentukan dengan prosedur berpuasa adalah 199 μmol/kg $^{0.75}$/hari, dan 300 μmol/kg $^{0.75}$/hari, masing-masing. Kadar pengeluaran PD urin per kg bahan organik hadaman (DOMI) kerbau (8.19 mmol/kg DOMI) didapati lebih rendah (P<0.05) dari lembu KK (15.45 mmol/kg DOMI). Eksperimen yang kedua mengkaji pertalian di antara pengeluaran PD urin harian (Y, mmol) dan bes purina eksogenus (PB) yang di suntik melalui infusi duodenal (X, mmol/hari). Pertalian yang didapati bagi kerbau adalah Y = 0.12X + 12.78 (r^2 = 0.45) manakala bagi lembu KK adalah Y = 0.85X + 7.15 (r^2 = 0.62), mencadangkan bahawa 12% purina eksogenus yang dibekalkan dikeluarkan oleh kerbau,

sementara lembu mengeluarkan 85%. Dalam kajian ketiga, asid urik berlabel (8–^{14}C) digunakan bagi menguji hipotesis yang menyatakan bahawa kadar pemulihan bagi PD urina kerbau disebabkan kitaransemula plasma PD yang lebih tinggi berbanding lembu KK. Purata nilai kehilangan bukan-renal untuk kerbau dan lembu tidak berbeza. Oleh itu, keputusan ini menolak hipotesis tersebut. Kajian seterusnya dijalankan untuk meneliti samada kadar pengeluaran PD yang rendah pada kerbau disebabkan oleh penyerapan purina yang rendah di usus kecil kerbau. Hasil kajian menunjukkan bahawa kadar penyerapan purina kerbau tidak berbeza dengan lembu. Walaupun begitu, kuantiti PD dalam digesta kerbau didapati lebih tinggi berbanding lembu pada tiga bahagian akhir usus kecil. Keputusan ini mencadangkan bahawa perbezaan di antara kedua spesis ruminan ini mungkin disebabkan oleh kadar penyerapan purina yang rendah di usus kecil kerbau. Berdasarkan keputusan tersebut, pertalian di antara pengeluaran harian PD dalam urin (Y, mmol) dan bekalan harian purina mikrobial (X, mmol) bagi kerbau adalah $Y = 0.12X + 0.20$ kg $^{0.75}$ dan bagi lembu adalah, $Y = 0.85X + 0.30$ kg$^{0.75}$. Berdasarkan kedua persamaan di atas, penghasilan N mikrob boleh dianggarkan dengan persamaan di bawah:

$$\text{Mikrobial N (gN/hari)} = \frac{X \text{ (mmol/hari)} \times 70}{0.073 \times 0.83 \times 1000} = 1.16X \text{ bagi kerbau dan lembu}$$

Persamaan ini menganggap penghadaman purina mikrobial adalah 0.83 dan kandungan N purina adalah 70 mg N/mmol. Nisbah kadar N purina : N keseluruhan dalam campuran mikrob rumen diambil sebagai 7.3 : 100 bagi kerbau dan lembu. Persamaan bagi pembekalan purin mikrobial dengan menggunakan pengeluaran PD dalam urin untuk lembu KK adalah sama dengan persamaan untuk lembu Europah, Oleh kerana kadar kedapatan semula untuk kerbau (12%) sangat rendah maka, persamaan ini perlu digunakan secara berhati-hati.

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My special thanks to my beloved wife Benchamaporn Pimpa for her inspiration, moral encouragement and sacrifices during the course of this study. Finally, I am grateful to the cattle and buffaloes used in this study.

I certify that an Examination Committee met on 15th January 2002 to conduct the final examination of Opart Pimpa on his Doctor of Philosophy thesis entitled "Urinary Purine Derivatives Excretion as a Method for Estimation of Rumen Microbial Protein Production in Swamp Buffaloes and Zebu Cattle" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of Examination Committee are as follows:

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The thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy.

AINI IDERIS, Ph.D.
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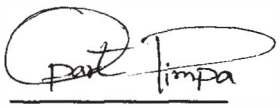
Date:

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.



OPART PIMPA

Date : 14 February 2002

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LIST OF ABBREVIATIONS

µCi	Micro Curi
µmol	Micromol
ADF	Acid Detergent Fiber
ADG	Average Daily Gain
AEP	2-aminoethylphosphonic acid
ATP	Adenine Triphosphate
BCP	Bacterial plus Protozoal Crude Protein
BW	Body Weight
C	Creatinine
°C	Degree Celsius
CF	Crude Fiber
CMC	Carboxy methyl Cellulose
Con	Concentrate
CP	Crude Protein
Cr-EDTA	Chromium-Ethylene diamine Tetra acetic acid
CV	Coefficient of Variation
DAPA	Diaminopimelic acid
DDMI	Digestible Dry Matter Intake
DM	Dry Matter
DMI	Dry Matter Intake
DNA	Deoxyribonucleic acid
DOM	Digestible Organic Matter
DOMI	Digestible Organic Matter Intake
DOMR	Digestible Organic Matter apparently fermented in the Rumen
DOMS	Digestible Organic Matter in the Stomach
dpm	Disintegrations per minute
EE	Ether Extract
FB	Fluid Associated Bacteria
FM	Fluid Associated Microbes
GE	Gross Energy
GFR	Glomerular Filtration Rate
h	Hour
HPLC	High Pressure Liquid Chomatography
IMP	5-inosine-monophosphate
Kg	Kilogram
$Kg^{0.75}$	Metabolic Body Weight
KJ	Kilo-Joule
KK Cattle	Kedah Kelantan Cattle
L	Liter