UNIVERSITI PUTRA MALAYSIA

URINARY PURINE DERIVATIVES AS INDEX FOR ESTIMATION OF RUMINAL MICROBIAL NITROGEN PRODUCTION IN SHEEP AND GOATS

THONGSUK JETANA.

IB 2005 3

URINARY PURINE DERIVATIVES AS INDEX FOR ESTIMATION OF RUMINAL MICROBIAL NITROGEN PRODUCTION IN SHEEP AND GOATS

THONGSUK JETANA

DOCTOR OF PHILOSOPHY
UNIVERSITI PUTRA MALAYSIA

2005

URINARY PURINE DERIVATIVES AS INDEX FOR ESTIMATION OF RUMINAL MICROBIAL NITROGEN PRODUCTION IN SHEEP AND GOATS

By

THONGSUK JETANA

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

November 2005

DEDICATION

To

Mae and Mear

Abstract of thesis presented to the Senate of the University Putra Malaysia
in fulfilment of the requirements for the degree of Doctor of Philosophy

URINARY PURINE DERIVATIVES AS INDEX FOR ESTIMATION OF RUMINAL MICROBIAL NITROGEN PRODUCTION IN SHEEP AND GOATS

By

THONGSUK JETANA

November 2005

Chairperson : **Professor Norhani Bt. Abdullah, PhD**

Institute : **Bioscience**

Microbial-N production in the rumen can be estimated by using urinary purine derivatives (PD) as an index. A series of experiments were conducted to establish the relationship between urinary PD (allantoin, uric acid, xanthine and hypoxanthine) and feed intake, endogenous PD excretion and recovery rate of plasma PD in sheep and goats. Studies on factors affecting PD excretion; xanthine oxidase and uricase activities of the plasma, liver and intestinal mucosa cells; uric acid kinetic; and purine-N:total-N ratio of rumen microbes were also conducted. Male Poll Dorset Cross sheep and male Ferral goats were used. The animals were fed a diet consisting of 40% oil palm frond and 60% concentrate (OPFC). Four sheep (40.2±2.8kg) and four goats (39.6±1.8 kg) were used to measure urinary PD excretion at 40%, 60%, 80% and 95% of voluntary intake (VI). The proportion of plasma PD excreted in the urine was determined by using [^{14}C]-uric acid as a marker at 40% and 80 % of VI. Endogenous PD excretion was determined by fasting in six sheep (55.4±5.1 kg) and six goats (40.2±4.6kg). The results showed that sheep excreted significantly (p<0.05) higher PD and creatinine than goats

when compared at the same level of feed intake. However, the coefficient of the relationship between PD and DOMI in goats (12.57 mmol/kg DOMI) was similar to that in sheep (12.49 mmol/kg DOMI). The proportion of allantoin to total PD in goats (86%) was higher than that in sheep (60%). The distribution pattern of enzymes (xanthine oxidase and uricase) activities in the plasma, liver and intestinal mucosal cells were similar in both animal species, but uricase activity of the intestinal mucosa cells in sheep was significantly higher ($p<0.05$) than in goats. The average daily urinary endogenous PD excretion obtained by the fasting trial for sheep (201±35 μmol/kg$W^{0.75}d^{-1}$) was similar to that for goats (202±17 μmol/kg $W^{0.75}d^{-1}$). The average percentage of total recovery of plasma PD excreted in the urine determined by using [^{14}C]-uric acid in sheep (77±2.8 %) was not significantly different from that in goats (83±2.0 %). In the uric acid kinetics study, total tracer recovered reached a peak value of about 74.2% at 12 h for goats, and 74.4% at 15 h for sheep. The conversion efficiency of [^{14}C]-uric acid to allantoin in the plasma pool was higher ($p<0.05$) in goats than in sheep, with a peak value of 40% recovery at 12 h post injection for goats and 33.5% at 15 h post injection for sheep. By 15 h, no [^{14}C]-uric acid was detected in the urine of both animal species. The rates of [^{14}C]-allantoin and [^{14}C]-uric acid excretions in the urine of sheep (31.0 and 88.0% h^{-1}, respectively) were significantly ($p<0.05$) faster than those of goats (19.0 and 64.7% h^{-1}, respectively), but the rates of total [^{14}C]-tracer were not significantly different between the two animal species (42.5% h^{-1} and 30.3% h^{-1} for sheep and goats, respectively). The primary compartment size in the plasma (V_1) was significantly ($p<0.05$) larger in sheep (24.4 ±3.01 mg C) than in goats (17.5±1.28 mg C) and the secondary compartment size in the tissue (V_2) of sheep was also larger (129±21.6 mg C) than that of goats (65.7±23.7 mg C). The

volume of distribution (L) was 45% higher in sheep (0.898 L) than in goats (0.490 L). However, the net flux tended to be higher in goats (20.3±3.82 mg C) than in sheep (16.1±2.0 mg C). Hence, the results indicated that differences exist between sheep and goats in uric acid/allantoin kinetics. The equations established for sheep and goats based on the recovery of labeled PD [^{14}C]-uric acid and endogenous PD excretion to determine the absorption of purines (X mmol/d) estimated from PD excretion in the urine (Y mmol/d) for sheep was $Y=0.77X+0.201\times BW^{0.75}e^{-0.20X}$ and for goats $Y= 0.83X+0.202\times BW^{0.75}e^{-0.20X}$. The purine-N:total-N ratios of mixed rumen liquid-associated bacteria and solid-associated bacteria for sheep were 11.2 and 10.4, and those for goats were 8.5 and 10.0, respectively. The proposed equations to estimate rumen microbial-N production based on PD excretion was 0.753X for sheep and 0.992X for goats.

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

DERIVATIF PURINA URIN SEBAGAI INDEKS UNTUK MENGANGGAR PENGHASILAN NITROGEN MIKROB RUMEN BEBIRI DAN KAMBING

Oleh

THONGSUK JETANA

November 2005

Pengerusi : Profesor Norhani Abdullah, PhD

Institut : Biosains

Pengasilan N-mikrob dalam rumen boleh di anggarkan menggunakan derivatif purina (DP) urin sebagai indeks. Satu siri eksperimen telah dijalankan untuk menghasilkan persamaan antara DP (alantoin, asid urik, xantin dan hipoxantin) dan pengambilan makanan, pengkumuhan DP endogen, dan kadar perolehan semula DP plasma untuk bebiri dan kambing. Kajian keatas faktor yang mempengaruhi pengkumuhan DP; aktiviti enzim xantin oksidase dan urikase plasma, hati dan sel mukosa usus; kinetik asid urik; nisbah purina-N:total-N mikrob rumen juga dilakukan. Bebiri jantan kacukan silang Poll Dorset dan kambing Ferral digunakan. Haiwan diberi diet terdiri dari 40% pelepah daun kelapa sawit dan 60% konsentrat (OPFC). Empat bebiri (40.2±2.8kg) dan empat kambing (39.6±1.8 kg) digunakan unutk mengukur pengkumuhan DP pada 40%, 60%, 80% and 95% pengambilan secara sukarela (IV). Bahagian DP plasma yang dikumuhkan dalam urin ditentukan menggunakan [^{14}C]-asid urik sebagai traser pada dua tahap VI (40% dan 80%). Derivatif purina endogen yang dikumuhkan dalam urin ditentukan dalam enam bebiri jantan (55.4±5.1kg) dan enam kambing jantan (40.2±4.6kg) semasa berpuasa. Hasil kajian menunjukan bebiri

mengumuhkan DP dan kreatinin dalam urin lebih banyak (p<0.05) daripada kambing apabila dibandingkan pada tahap pengambilan makanan yang sama. Koefisien hubungan diantara DOMI dan DP untuk kambing (12.49 mmol/kg DOMI) adalah serupa dengan bebiri (12.57 mmol/kg DOMI). Bahagian alantoin dalam DP total untuk kambing (86%) adalah lebih tinggi daripada bebiri (60%). Purata pengumuhan DP endogen urin semasa berpuasa untuk bebiri (201±35 μmol/kg $W^{0.75}$ h^{-1}) adalah sama dengan kambing (202±17 μmol/kg $W^{0.75}$ h^{-1}). Taburan aktiviti enzim (xantin oksidase dan urikase) untuk plasma, hati dan usus kecil adalah sama pada kedua-dua spesis haiwan, tetapi aktiviti urikase di sel mukosa usus adalah lebih (p<0.05) tinggi untuk bebiri daripada kambing. Purata peratus perolehan semula DP plasma dalam urin dengan menggunakan ^{14}C-asid urik untuk bebiri (77±2.8%) adalah sama seperti kambing (83±2.0%). Dalam kajian kinetik asid urik, jumlah traser yang diperolehi semula mencapai nilai puncak 74.2% pada 12 j untuk kambing dan 74.4% pada 15 j untuk bebiri. Efisiensi penukaran [^{14}C]-asid urik ke alantoin dalam gembeling plasma adalah lebih tinggi (p<0.05) untuk kambing dari bebiri dengan nilai 40% perolehan semula pada 12 j selepas suntikan untuk kambing dan 33.5% pada 15 j selepas suntikan untuk bebiri. Pada masa 15 j, tiada [^{14}C]-asid urik dikesani dalam urin kedua spesis haiwan. Kadar pengkumuhan [^{14}C]-alantoin dan [^{14}C]-asid urik dalam urin bebiri (31.0 dan 88.0% j^{-1}, masing masing) adalah lebih cepat ($p<0.05$) daripada kambing (19.0 and 64.7% h^{-1}, masing masing), tetapi kadar untuk total [^{14}C]-traser tidak berbeza antara kedua spesis haiwan (42.5% j^{-1} dan 30.3% j^{-1} untuk bebiri dan kambing, masing-masing). Saiz ruang primer dalam plasma (V1) adalah lebih besar ($p<0.05$) untuk bebiri (24.4±3.01mg C) daripada kambing (17.5±1.28mg C) dan saiz ruang sekunder dalam tisu (V2) pada bebiri juga adalah lebih besar (129±21.6mg C)

daripada kambing (65.7±23.7mg C). Isipadu taburan (L) adalah 45% lebih tinggi untuk bebiri (0.898 L) daripada kambing (0.490 L). Walaupun demikian, fluks bersih adalah lebih tinggi untuk kambing (20.3±3.82mg C) daripada bebiri (16.1±2.0μg C). Oleh itu, keputusan menunjukkan perbezaan wujud di antara bebiri dan kambing dalam kinetik asid urik/allantoin. Persamaan berdasarkan perolehan semula DP [^{14}C]-asid urik dan pengkumuhan DP endogen untuk menentukan penyerapan purina (X mmol/h) dengan menggunakan nilai pengkumuhan DP dalam urin (Y mmol/h) untuk bebiri ialah $Y=0.77X+0.201\times BW^{0.75}e^{-0.20X}$ dan kambing $Y= 0.83X+0.202\times BW^{0.75}e^{-0.20X}$. Nisbah purina-N:total-N bakteria campuran dalam cecair dan pepejal rumen untuk bebiri ialah 11.2 dan 10.4, dan untuk kambing ialah 8.5 dan 10.0, masing masing. Persamaan yang disyorkan untuk menganggar N-mikrob rumen berdasarkan pengkumuhan DP adalah 0.753X untuk bebiri dan 0.992X untuk kambing.

ACKNOWLEDGEMENTS

I would like to express my deep appreciation and sincere gratitude to the chairman of the Supervisory Committee, Professor Dr. Norhani Abdullah and the other members of supervisory committee, Professor Dr. Ho Yin Wan and Associate Professor Dr. Liang Juan Boo, for their invaluable guidance, advices and encouragement throughout the course of this study.

I wish to thank Universiti Putra Malaysia for providing me with a scholarship, and Chulalongkorn University for granting me the study leave to pursue my PhD degree.

I am much indebted to Associate Professor Dr. John Nolan of the Biochemistry, Microbiology and Nutrition Department, New England University, Armidale, Australia, Associate Professor Dr. Qium Balcells of Departmento de Produccion Animal y Ciencia de los Alimentos, Facultad de Veterinaria, Universidad de Zaragoza, Miguel Servet 177, Spain, for their kind assistance, advices and guidance in this study.

Appreciation is extended to Dr. Nashariyah Mat and her staff at the of Unit Tenaga Nuklear, Kompleks PUSPATI, Bangi, Kajang, Malaysia, for their assistance on the preparation of samples for [^{14}C]-counting and to Dr. Chinoma for her assistance on the preparation of animals for catherisation.

I also wish to thank the staff of the Institute Bioscience, Mdm Haw Ah Kam, Mr. Khairul Kamar Bakri, Mr Jivanathan Arumugam, Mr. Nagayah Muniandy and Mr. Paimon Logimun and the staff of the Department of Animal Science, Mr. Ibrahim Mohsin, Mr. Brakari Abd Rahman and Mr. Saparin Denim, for their technical support and kind assistance.

To members of my office, Dr. Sunpeth, Dr. Rangsun, Dr. Kitiya, Dr. Sirima, Usa, Wanvipha, Ratree, Tan, Kriangsak, Wisut, Sangworn and friends, Mongkorn, Suthipong, Opart, Bodee, Anut, Benjamaporn, Atikha and Pensri, I wish to express my deepest gratitude for their encouragement and support.

Special thanks are due to my postgraduate friends at the Digestive Microbiology Lab, Institute of Bioscience, Sideig, Darlis, Latifah, Kala, Chin Chin, Wan and Chin Mei, for their friendship, assistance, encouragement and support.

Finally, I wish to express my deepest gratitude to my family, mom, wife, son, sisters and brothers for their love encouragement and support throughout the course of my study.

To them, I dedicate this thesis.

I certify that an Examination Committee met 21st November 2005 to conduct the final examination of Thongsuk Jetana on his Doctor of Philosophy thesis entitled "Urinary Purine Derivatives as Index for Estimation of Ruminal Microbial Nitrogen Production in Sheep and Goat" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recomments that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

Abdul Rani Bahaman, PhD
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Chairman)

Nor Aripin Shamaan, PhD
Professor
Faculty of Biotechnology and Molecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Abdul Razak Alimon, PhD
Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Ørskov Egil Robert, PhD
Professor
International Feed Resources Units
Macaulay Land Use Research Institute
Craigiebuckler, Aberdeen, AB158 QH, UK
(External Examiner)

ZAKARIAH ABDUL RASHID, PhD
Professor/Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

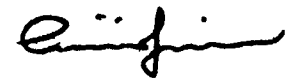
Date: 27 DEC 2005

This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirements for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

NORHANI BT. ABDULLAH, PhD
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

LIANG JUAN BOO, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

HO YIN WAN, PhD
Professor
Institute of Bioscience
Universiti Putra Malaysia
(Member)



AINI IDERIS, PhD
Professor/Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 12 JAN 2006

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.

Thongsuk Jetana

THONGSUK JETANA

Date: 20/11/2005

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURE

LIST OF ABBREVIATIONS

ADF	=	acid detergent fibre
AEP	=	aminoethyl-phosphnic acid
ARC	=	Agricultural Research Council
ATP	=	adenosine-5'-triphosphate
$BW^{0.75}$	=	metabolic body weight
Cal	=	calorie
CF	=	crude fibre
cm	=	centimetre
CMC	=	carboxymethy cellulose
CP	=	crude protein
d	=	day
DE	=	digestible energy
d.f.	=	degree of freedom
dl	=	decilitre
DM	=	dry matter
DMI	=	dry matter intake
DDMI	=	digestible dry matter intake
OM	=	organic matter
OMI	=	organic matter intake
DOMI	=	digestible organic matter intake
DOMR	=	digestible organic matter digested in the rumen
EE	=	ether extract (crude fat)
g	=	gram
GE	=	gross energy

GFR	=	glomerular filtration rate
GLM	=	General linear measurement
h	=	hour
ha	=	hectare
hd	=	head
HPLC	=	high performance liquid chromatography
IBC	=	isolated bacteria cells
i.e.	=	that is
i.d.	=	internal diameter
kg	=	kilogram
L	=	litre
LAB	=	liquid associated bacteria
m	=	metre
mM	=	millimoles of solute per litre of solution
ME	=	metabolisable energy
mg	=	milligram
min	=	minute
MJ	=	megajoule
ml	=	millilitre
mm	=	millimetre
N	=	nitrogen
NDF	=	neutral detergent fibre
ηm	=	nanometre
NRC	=	National Research Council (USA)
OPFC	=	oil palm frond plus concentrate