THE ROLE OF BYPASS PROTEIN IN IMPROVING THE INTAKE AND UTILISATION OF DIETARY NUTRIENTS BY RUMINANTS IN THE TROPICS

A thesis submitted to the University of New England for the degree of Doctor of Philosophy

by

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August 1997

Preface

I certify that the substance of this thesis has not already been submitted for any degree and is not currently being submitted for any other degree.

I certify that to the best of my knowledge any help received in preparing this thesis, and all sources used, have been acknowledged in this thesis.



Dahlanuddin August 1997

Acknowledgments

I sincerely thank my supervisors Associate Professor C. J. Thwaites and Associate Professor J. V. Nolar for their patient and friendly guidance. Without their continuous encouragement, it could have been very difficult to complete my study program on time. Their assistance and constructive criticism during the experimental work and the preparation of this thesis has been greatly appreciated. Academic supervision of Dr. M. K. Hill during the first 6 months of my PhD candidature is also appreciated. My sincere thanks go also to Professor J. B. Rowe and Dr. S. H. Bird for their useful suggestions in designing Experiments 6 and 7, and to Dr. R. S. Copland (Indonesia-Australia Eastern Universities Project) for his support during my home leave for Experiments 1 and 2.

I thank Mr. N. D. Baillie and Mr. Winston Hewitt for their invaluable technical and personal suppor. Thanks are expressed to Mr. Frank Ball, for his professional technical assistance during the infusion experiment and his assistance in the calculation and interpretation of data presented in Chapter 10. Thanks are also extended to Mr. Simco Stachiw, for his assistance in preparing the infusion experiment and for the analyses of ammonia and total nitrogen, Mr. Evan Thomson, for his assistance especially during the infusion experiment, and to Mrs. Carol Quinn and Mr. Peter Tyler, for their guidance for allantoin assays.

For their assistance ir feed preparation, sample collections and useful discussions throughout my study, I thank my fellow postgraduates, in particular Mr. James Hills, Mr. L. A. Zaenuri (Dept. of Physiology), Mr. Frans Umbu Datta and Mr. Marsetyo.

The present study would not have been possible without a study grant from the Australian Agency for International Development (AusAID), and financial support and excellent facilities provided by the Animal Science group at the University of New England.

Finally, this thesis is dedicated to my wife Nur and our son Danny, for their moral support, patience and understanding during the study.

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List of abbreviations

AAP = Amino Acid Profile

ADF = Acid Detergent Fibre

ADP = Adenosine DiPhosphate

ATP = Adenosine Triphosphate

BW = Body Weight

CM = Copra Meal

CP = Crude Protein

CSM = Cottonseed Meal

DAPA = Diaminopimelic acid

DM = Dry Matter

DMD = Dry Matter Digestibility

DMI = Dry Matter Intake

FADH = Flavin Adenine Dinuclec tide, reduced form

FCR = Feed Conversion Ratio

FI = Feed Intake

FM = Fish Meal

HIF = Heat Increment of Feeding

KS = Kapok Seed

LWC = liveweight Change

LWG = Liveweight Gain

M/D = Megajoules of ME per kg feed DM

ME = Metabolisable Energy

MEI = Metabolisable Energy Intake

MNS = Microbial Nitrogen Suppl /

MP = Microbial Protein

MUB = Molasses Urea Block

NADPH = Nicotinamide Adenine Dinucleotide Phosphate, reduced form

NDF = Neutral Detergent Fibre

NFE = Nitrogen Free Extract

NRC = National Research Counci

OMD = Organic Matter Digestibility

P/E ratio = Protein to Energy Rati

PD = Purine Derivatives

RDN =Rumen Degraded Nitrogen

RDP = Rumen Degraded Protein

RH = Relative Humidity

RNA =Ribo Nucleic Acid

RR = Respiration Rate

RT = Rectal Temperature

SC =Soybean Curd byproduct

T = Temperature

TCA-cycle = Tricarboxylic Acid cycle

UDP = Undegraded Dietary Prote n

VFA = Volatile Fatty Acids

WI = Water Intake

Summary

The overall objective of the studies presented in this thesis was to define nutritional strategies to improve productivity of ruminants under tropical conditions. Because productivity is a function of intake of nutrients and the efficiency of their utilisation, strategies to improve these two parameters have been studied. From a review of literature, it is apparent that there has been a controversy over factors possibly associated with the low efficiency of utilisation of metabolisable energy in ruminants fed roughage-based diets.

Two theories have been widely discussed. The first suggested that high acetate production on high-fibre, low-protein diets may create an imbalance between acetogenic and glucogenic substrates, resulting in inefficient use of acetate for production. Excess of acetate may then be oxidised and thus increase heat production which would eventually reduce the amount of energy used for productive purposes (see MacRae and Lobley, 1982: MacRae et al., 1985). In the tropics, where ambient temperature and humidity are h gh and ruminants are commonly fed high-fibre, lowprotein diets, additional heat production from any such 'wasteful' oxidation of acetate may contribute to heat stress and to depression of feed intake (Leng, 1990). This theory thus predicts that supplementation of low quality roughage with bypass or rumen undegraded dietary protein may improve the utilisation of acetate. Additional dietary amino acids and increased propionate production due to supplementation are expected to increase glucose production which in turn might overcome an insufficiency of NADPH for fatty acid synthesis. This theory has been used as the basis for recommending supple mentation in the tropics, but evidence to support this theory is limited. In fact, many studies have indicated that the contribution of heat from substrate cycling during acetate metabolism is negligible (see Crabtree et al., 1987, 1990).

The second theory, on the other hand, suggested that high acetate production does not increase heat product on, and that excess blood acetate if it did occur in practice, would be excreted in urine. The low efficiency of utilisation of metabolisable energy on roughage diets may be related to greater energy consumption for activities associated with eating and digestion. Thus, by reducing the particle size of the diets, energy expenditure for these activities may be reduced,

resulting in more energy being available for production (see Orskov and MacLeod, 1990, 1993). This theory currently appears to be more acceptable because it is based on more extensive studies in which extreme levels of acetate were applied and heat production was directly measured. However, all these experiments were conducted under thermoneutral conditions and thus studies to assess any possible effect of high ambient temperature were required.

To test these theories a series of experiments was conducted. Two preliminary experiments were carried out on Lombok, Indonesia, to study the effect of different protein-rich supplements on productivity of Kacang goats fed native grasses or rice straw basal diets. In Experiment 1, Kacang goats fed a basal diet of mixed native grasses supplemented with cop a meal, soybean curd byproduct, cracked kapok seed and fish meal at a rate equivalent to 21 g crude protein per day barely maintained liveweight. During weeks 1-5 of Experiment 2, when concentrates containing varying proportions of copra meal to cracked kapokseed (100:0, 10:90, 20:80, 30:70, 40:60) and a basal diet of rice straw were both offered ad libitum, almost all goats lost weight. When the goats were fed the above concentrates but the basal diet was changed to native grass (weeks 6-8), liveweight gain improved significantly to 112, 81, 30, 59 and 81 g/d for the respective concentrate treatments. However, the improvement in liveweight gain was probably a form of compensatory growth after the goats lost weight during the first 5 weeks of Experiment 2. It was concluded that dietary treatments during Experiments 1 and 2 did not satisfactorily improve the performance of Kacang goats. This lack of response of the goats to protein-rich supplements was probably related mostly to the low digestibility of the basal diets used, resulting in low DMI.

In subsequent experimens, deficiencies in the preliminary experiments were corrected by using animals of homogeneous genotype, supplementation of critical nutrients and by conducting the work in temperature and humidity controlled rooms. In particular, attention was paid to basal diets to ensure that they contained sufficient NH₃ and minerals for rumen function.

In Experiment 3, the effects of ambient temperature and CSM supplementation were studied in 16 Border Leicester x Merino crossbred lambs. Eight of these animals were housed at 17°C and the re nainder at 35°C (relative humidity was maintained at 60-70% in both environments) and animals in each ambient temperature were fed wheaten chaff (2.4% N, 55-59% DMD when supplemented with 2% urea and

minerals) ad libitum with or without 100 g/d CSM supplementation. DMI and LWG were significantly higher on CSM diet, but both were depressed significantly by high ambient temperature. While at low temperature LWG was significantly higher in lambs supplemented with CSM, at high temperature, the lambs supplemented with CSM did not perform any better than controls. It was concluded that the lack of response of the lambs to the additional amino acids from CSM at high temperature was probably due to the fact that the constant high ambient temperature imposed too much heat stress which could not be alleviated by supplementation with 100 g/d of CSM.

When 8 animals (from the high temperature treatment in Experiment 3) were subjected to a constant 35°C day-time ambient temperature but night-time temperature was reduced by Ω^{0} C every 3 days in Experiment 4, DMI increased significantly and progressively with reductions in night-time temperature, and DMI tended to be higher on the CSM diet than on the control diet. Liveweight gain was significantly higher on the CSM diet than on the control.

However, the results of Experiment 5 showed that the DMI of lambs fed oaten chaff (1.7% N, 51-57% DMD) ad libitum was not significantly improved by CSM supplementation either at constant 35°C or at 35°C (day)/20°C (night) temperatures. Liveweight gain, on the other hand, was significantly higher on the CSM diet than on the control diet. It is possible that the quality of the basal diet used (oaten chaff; 9%CP) was sufficiently high as to allow relatively high DMI (2.5-3% of liveweight), and that additional amino acids from CSM could have improved the balance between protein and energy in the absort ed nutrients, resulting in a higher growth rate.

When a lower quality roughage (barley straw: 1.5% N, 48% DMD when supplemented with 2% urea) was fed to Merino lambs (Experiment 6) housed at 36°C with lower relative humidity (40-50%), increasing levels (50, 100 and 150 g/d) of protein meal ("Norpro") significantly improved DMI, microbial N supply and LWG, which all appeared to have reached a plateau at 100 g/d supplementation. However, it was not clear whether the improvement in the performance of the lambs was due to additional amino acids, or rather to increased metabolisable energy intake. For this reason, Experiment 7 was conducted under similar environmental conditions to compare the performance of the same lambs when fed the same basal diet as that of Experiment 6 but supplemented with 100 g/d "Norpro" protein meal (A), 100 g/d barley grain (B), A + 100 g B/c (C) and A + 200 g B/d (D). DMI and LWG of lambs

on treatments A and B were very similar, indicating that the better performance of lambs in Experiment 6 was partly an energy response for which additional dietary bypass protein was not required. Increasing levels of grain in addition to protein meal tended to increase DMI, and s gnificantly increased microbial N supply and LWG. However the LWG achieved on treatments C and D were only 32 and 48 g/d respectively. The fact that the estimated ME intakes appeared to be lower than those required for higher growth rate indicated that the performance of sheep at such high protein intakes may be improved if more non-protein, metabolisable energy is made available.

In Experiment 8, to minimise energy expenditure for activities associated with eating and digestion of low quality roughage, and thus to improve energy availability for productive purposes, a basal diet similar to that used in Experiments 6 and 7, but was ground and pelleted. Sixteen similar sheep were fed *ad libitum* either chaffed or ground-pelleted barley straw basal diets, with or without 100 g/d CSM. DMI, microbial N supply and LWG were significantly improved both by grinding and CSM supplementation. CSM supplementation improved DMI by only 110 g/d when the basal diet was chaff. On the other hand, DMI was dramatically improved (by 364 g/d) by CSM supplementation when basal diet was ground and pelleted. The primary limitation to DMI in this experiment appeared to be the particle size of the basal diet, so that when the basal diet was ground, DMI was stimulated by provision of limiting nutrients such as RDN and amino acids. The LWG of sheep fed the pelleted diet supplemented with CSM was 1 3 g/d higher that that of sheep fed the chaff without CSM.

Experiment 9 was carried out to determine whether the responses to amino acids found in Experiment 8 were due, in part, to effects on oxidation of substrates or on the efficiency of utilisation of nutrients in host tissues, especially of acetate. The net flux of CO₂ and the proportion of acetate oxidised were determined by continuous infusions of ¹⁴C-sodium bicarbonate and ¹⁴C-sodium acetate, and the net flux of glucose was estimated using continuous infusion of ³H-glucose. The estimated energy expenditure (predicted from the net flux of CO₂) was significantly increased in sheep fed pellets with CSM, but the net fluxes did not differ significantly between other treatments. Glucose production, on the other hand, was significantly increased both by grinding and CSM supplementation. However, the increased supply of glucose (which is has been suggested improves the rate of fatty acid synthesis due to

increased supply of NADPH) did not reduce the rate of acetate oxidation. The relatively high estimate of the proportion (67 - 78%) of acetate oxidised, which may be under-estimated due to recycling of CO₂, suggested that acetate was utilised to meet the high energy requirements of the animals when housed at high ambient temperature. Thus, futile cycling associated with acetate metabolism was not likely to have occurred significantly in the present study, and if any related increase in heat production would have made or ly minimal contribution to the level of heat stress and thus to the depression in feed intake that occurs in ruminants under tropical conditions.

Results of the studies presented in this thesis indicate that bypass protein supplementation to low quality roughage (that is already adequately supplemented with RDN and minerals to eliminate rumen deficiencies), is unlikely to improve the performance of ruminants. On a very low digestibility basal diets such as barley straw, gut fill will most likely be the primary limitation to DMI once nutrient imbalances are corrected, for example by supplementation with rumen undegraded amino acids. Under tropical conditions, low productivity of ruminants is more likely to be related to low ME intake resulting from type of diet than to imbalances in absorbed nutrients.

In terms of priorities for improvements in feeding management of animals in the tropics, as the digestibility of most tropical forages is low, the rate of digestion should first be increased, for example by grinding or alkali treatment. Supplementation with nutrients will then be needed next to optimise rumen function. More rapid microbial fermentation and growth is then expected to improve ME availability in the rumen and to increase microbial amino acid supply to the intestines up to the point when rate of 'ermentation or rumen emptying again becomes the primary limitation to MEI. Additional soluble energy supplements can then be expected to support higher rate: of microbial growth. A combination of these dietary treatments may improve P/E ratio, reduce energy wastage during eating, rumination and digestion, increase the availability of ME and thus improve ruminant production. Additional bypass protein would only be beneficial if the requirement for protein relative to ME is higher than the rumen and minimal bypass can provide, for example in high producing animals during early growth, late pregnancy or early lactation.