METABOLISM IN COMPENSATORY GROWTH. IV. THE ARTERIAL BLOOD CONCENTRATIONS OF AMINO ACIDS AND ARTERIOVENOUS (AV) CONCENTRATION DIFFERENCES OF AMINO ACIDS ACROSS THE HIND-LIMB MUSCLES IN ANIMAL UNDERGOING COMPENSATORY GROWTH

PRAPTI MAHYUDDIN[1] and E. TELENI[2]

[1] Research Institute for Animal Production
P.O. Box 221, Bogor 16002, Indonesia
[2] Dept. Biomedical and Veterinary Science, James Cook University of North Queensland, Townsville, 4811 Australia

ABSTRAK

MAHYUDDIN, P. dan E. TELENI. 1998. Metabolisme dalam pertumbuhan kompensatori. IV. Konsentrasi asam amino dalam darah arteri dan perbedaan konsentrasi asam amino arteri-vena pada ternak yang mengalami pertumbuhan kompensatori. *Jurnal Ilmu Ternak dan Veteriner* 3 (2): 87-93.

Pengukuran konsentrasi asam amino dalam darah arteri dan perbedaan konsentrasi asam amino arteri-vena dari otot kaki dilakukan pada 4 titik kurva pertumbuhan, yaitu: sebelum pembatasan pemberian pakan (P1), 8 minggu setelah pembatasan pemberian pakan (P2), 3 minggu (P3) dan 15 minggu (P4) setelah ternak diberi pakan kembali secara *ad libitum*. Enam belas domba lepas sapih dibagi dalam 2 kelompok, Kelompok I dan II diberi pelet lucern (*Medicago sativa*) secara *ad libitum* selama 3 minggu; kemudian Kelompok I terus-menerus diberi pakan secara *ad libitum*, sedangkan Kelompok II diberi pakan yang sama sebanyak 1/2 kebutuhan pokoknya selama 8 minggu, kemudian diberi pakan secara *ad libitum* sampai akhir percobaan. Pembatasan pakan (P2) menurunkan konsentrasi asam amino berantai cabang (AABC) dan fenilalanin 43% dan histidin 30%. Pada periode P3 dan P4 konsentrasi AABC dalam Kelompok II naik lebih cepat daripada Kelompok I, tetapi pembatasan pakan dan pemberian pakan secara *ad libitum* tidak berpengaruh pada konsentrasi lisin, arginin, treonin dan asam amino yang non-esensial. Karena variasi yang besar antar ternak, perbedaan konsentrasi semua asam amino pada arteri-vena tidak berbeda pada ternak Kelompok I dan II pada semua periode (P1 sampai P4). Kenaikan konsentrasi asam amino AABC dalam peredaran darah dari ternak yang mengalami pertumbuhan kompensatori diduga karena sintesisnya lebih besar daripada penggunaannya. Karena itu, disarankan penambahan energi dalam pakan untuk menaikkan penggunaan asam amino untuk sintesis protein.

Kata kunci : Pertumbuhan kompensatori, asam amino, sintesis protein

ABSTRACT

MAHYUDDIN, P. and E. TELENI. 1998. Metabolism in compensatory growth. IV. The arterial concentration of amino acids and arteriovenous (AV) concentration differences of amino acids across the hind-limb muscles in animal undergoing compensatory growth. *Jurnal Ilmu Ternak dan Veteriner* 3 (2): 87-93.

The concentration of arterial amino acids and arterio-venous (AV) differences of amino acids were measured at 4 points of growth curve: before feed restriction (P1), 8 weeks after feed restriction (P2), 3 weeks (P3) and 15 weeks (P4) after resumption of *ad libitum* feeding. Sixteen lambs were divided into two groups (Group I and Group II) fed pelleted lucerne (*Medicago sativa*) *ad libitum* for 3 weeks; Group I was continuously fed *ad libitum*, whereas Group II was fed the same diet at half maintainance energy level for 8 weeks followed by *ad libitum* feeding until the end of the experiment. Restricted feeding caused arterial concentration of branch chain amino acids (BCAA), and phenylalanine reduced by 43% and histidin reduced by 30% respectively. At P3 and P4 the concentration of BCAA in Group II increased at a faster rate than that in Group I. The arterial concentration of lysine, arginine, threonine and non-essential amino acids were not affected by either restricted feeding or *ad libitum* following restricted feeding. Due to a large variation between animals, the (A-V) concentration differences of all amino acids analysed were not significantly different between Group I and II at all periods, from P1 to P4. The increase in arterial BCAA concentration in animals undergoing compensatory growth suggests that the synthesis of amino acids is higher than the utilisation. Therefore it was suggested that energy supplement should be added to the diet to improve the utilisation of amino acids for protein synthesis.

Key words : Compensatory growth, amino acids, protein synthesis

INTRODUCTION

In the previous report MAHYUDDIN and TELENI (1996) found that feed restriction in sheep resulted in reduced urea, glucose and CO_2 entry rates because of reduced N intake, available glucose precursor and metabolic rate. Whereas animals on *ad libitum*, following restricted feeding, showed a switch to anabolic mode which were indicated by increased urea, glucose and CO_2 entry rates. These results are consistent with the finding that animals on restricted feeding lost both body protein and body fat, whereas animals on compensatory growth prefer to deposit protein than fat (O'DONOVAN, 1984; MAHYUDDIN, 1995).

The rate of net protein deposition in muscle is determined as the difference between the rate of protein synthesis and the rate of degradation in the tissue; and that involves plasma amino acids. Since protein synthesis is expected to be higher than the degradation in the muscle of animals undergoing compensatory growth, it is anticipated that there would be a net up take of amino acids across the muscle. Nevertheless, amino acids uptake by the muscle might not be significant at 3 - 4 weeks of resumption of *ad libitum* feeding since during that period it was thought that a greater rate of protein deposition occur to replace the protein depleted in the digestive tract and the liver (STANGASSINGER and GIESECKE, 1986). When the size and metabolic capacities of the splanchnic organs are fully recovered, the direction of protein deposition might be chanelled towards deposition in the muscle. Therefore, the arteriovenous (AV) differences of amino acids across the muscle would probably be different when measured at different period of resumption of *ad libitum* feeding following feed restriction.

The work reported in this paper is the last part of a series of experiments aimed to investigate the metabolic changes at selected points in growth curve of animals undergoing compensatory growth.

MATERIALS AND METHODS

Animals and management

The animals and feeding management were the same as used in the previous experiment (MAHYUDDIN and TELENI, 1995). Sixteen Merino wethers were randomly divided into two groups of eight, Group I and Group II. Group I was fed lucern pellets (*Medicago sativa*) *ad libitum* for the whole experiment, while Group II was fed the same diet at half maintenance energy level for eight weeks, and at the end of 8 weeks they were fed *ad libitum* until the end of experiment.

Metabolism studies were conducted at 4 periods as described in Figure 1. For this purpose 4 animals in each group were selected at random. Each lamb was fitted with polyvinyl chloride catheter (1.0 mm id x 2.0 mm od, Dural Plastic, Auburn, NSW) placed in a femoral artery and a deep femoral vein, via the saphenous vein, as described by ODDY *et al.* (1984). These catheter allowed simultaneous collection of arterial and venous blood draining from the hind-limb muscle without disturbing the lambs.
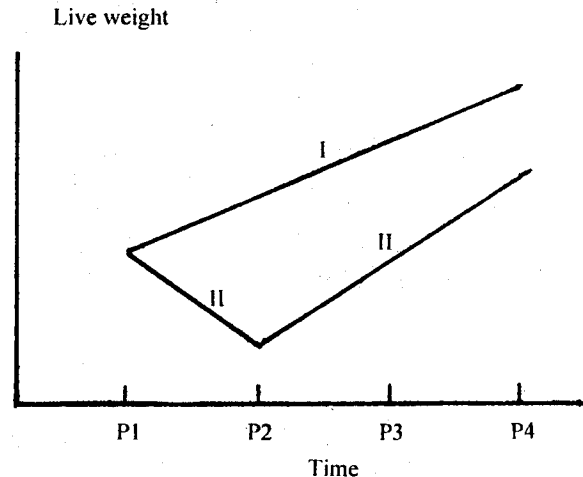

Figure 1. Growth curve of sheep

The measurement periods of the four metabolism study are indicated by:

P1 :   Eleven days immediately before animals in Group II were subjected to restricted feeding.

P2 :   Eleven days immediately after eight weeks of restricted feeding of animals in Group II.

P3 :   Eleven days immediately after three weeks of resumption of *ad libitum* feeding by animals in Group II.

P4:   Eleven days immediately after fifteen weeks of resumption of *ad libitum* feeding by animals in Group II.

Amino acid analysis

Blood samples were chilled immediately and plasma was prepared within 15 minutes of colllection. Plasma were assayed for free amino acids after deproteinisation with an equal amount of sulpho-salicylic acid (100 g/l). The measurement of arterial amino acid concentrations and A-V concentration difference of amino acids across the hind-limb muscle was done on the day when blood flow and glucose

entry rates were measured (MAHYUDDIN and TELENI, 1996).

Amino acid analysis involved the formation of ortho-phthalaldehyde derivatives and resolution of these on a reverse phase HPLC column (Analytical Instru-mens, Shandon, UK). The system used was broadly based on that described by JARRETT (1986).

Detection was with a Hitachi flourescence spectro-photometer (F 1000), set for exitation at 365 nm and emission 460 nm. The readings were computed using a Uni-X PC with Delta-Junior software (Digital Instru-ments Pty, Brisbane). The program stored results from 5 standard amino acids mixtures and calculated best fit lines for each amino acids using response relative to internal standard (amino caprylic acid).

Chromatograms were stored on disk. Because of some variation in retention times from run to run, each chromatogram was examined and identities assigned to each peak by manual observation before calculations were printed out.

Statistical analysis

Statistical analysis used was the same as in the previous experiment (MAHYUDDIN and TELENI, 1995). Briefly, a split plot analysis was done with contrast of individual treatments (period) and inter-actions (group x period) were made; the significance of these effects were tested using two-tailed t-test.

RESULTS AND DISCUSSION

Whole body

From the eight essential amino acids analysed (Table 1) the plasma concentration of the branch chained amino acids (BCAA), valine, isoleucine and leucine and phenylalanine were approximately 43% lower and histidine 30% lower in Group II than those in Group I at P2. The rate of increase of BCAA concentration in the period P2 to P3 was higher in Group II than that in Group I. At P4, the BCAA concentration in Group II was approximately 53% higher than in Group I. Neither underfeeding nor resumption of *ad libitum* feeding appeared to affect the arterial concentration of lysine, arginine, threonine and the non-essential amino acids.

The increase in BCAA concentration after resumption of *ad libitum* feeding of sheep in Group II (Table 1) most probably reflects the increased amount of amino acids that were absorbed from the small intestine. However, RIIS (1983) pointed out that the concentration of plasma essential amino acids are mainly determined by removal of amino acids from the

extracellular pool namely utilization for protein synthesis. In this study, the increased plasma concen-trations of some essential amino acids during the period of resumption of *ad libitum* feeding indicated that the rate of entry of dietary amino acids into the plasma pool was obviously greater than their utilisation rate. Thus even though an increased net protein deposition rate occurs during compensatory growth (MAHYUDDIN and TELENI, 1995) it appears that there might be scope for further increase in net deposition of protein.

The plasma profile of amino acids may indicate the partition of amino acids between protein synthesis, gluconeogenesis and oxidation. However, quantitative information about utilization of amino acids in different metabolic pathways requires the measurement of amino acids turnover. While the turnover amino acids was not measured in this study, speculations of amino acids utilization may be derived from previous data. MAHYUDDIN and TELENI (1995) revealed that there were approximately 143.8 g/d and 132 g/d dietary protein increment when the animals were on 3 weeks and 15 weeks resumption of *ad libitum* feeding following restricted feeding respectively. The increase in body gain contained about 42.8 g/d and 17.2 g/d protein (Table 4) or only 30% and 13% of the dietary increment over those periods. Therefore the increment in dietary protein would be associated with an increased utilization of amino acids for gluconeogenesis and oxidation. The substrate for gluconeogenesis and oxidation was presumably the amino acids which responded to the dietary change by increased plasma concentrations.

The increased utilization of amino acids for gluco-neogenesis during compensatory growth may be reflected in the increased contribution of amino acids to glucose entry rate as seen in Table 3. While data of amino acids contribution to CO_2 entry rate was not available, the increased amino acids utilization for oxidation may be suspected from the elevated production of CO_2 in the body during compensatory growth (Table 3). This may be true since during compensatory growth where supply and absorption of amino acids are expected to be high, the oxidation of amino acids would also be high as demonstrated by the work of ODDY *et al.* (1997). However, catabolism of essential amino acids either to CO_2 or to glucose is only a small percentage of amino acids turnover, being 5 - 20% and 1-3% respectively (MACRAE and REEDS, 1980).

Other possible metabolic pathway of amino acids is catabolism through urea production which can be reliably estimated from measurement of urea entry rate. The shortcoming of this measurement is that it gives too high values for the rate of amino acid catabolism

because part of urea is formed from ammonia which is absorbed from digestive tract. Table 3 shows that urea entry rate was significantly reduced during restricted feeding and increased during the 3-4 weeks after period of resumption of *ad libitum* feeding and stay steady

afterwards. When values of urea entry rates and N intake was plotted there was a positive correlation between urea entry rate and N intake (MAHYUDDIN and TELENI, 1996) indicating that there was N wastage.

Table 1. Arterial blood concentrations of amino acids in sheep, in normal (Group I) and interrupted (Group II) growth over different periods (P)

	Group	Periods			
		P1	P2	P3	P4
Essential amino acids (µM)					
Valine	I	381	324	273	221
	II	408	183*	304**	347***
Isoleucine	I	144	116	93	75
	II	144	67*	106**	114***
Leucine	I	205	174	146	114
	II	223	100*	155**	170***
Phenylalanine	I	71	63	61	49
	II	66	37*	61	68
Histidine	I	53	61	59	59
	II	54	43*	60	64
Lysine	I	252	252	247	248
	II	248	205	252	296
Arginine	I	179	103	67	113
	II	158	130	84	54
Threonine	I	216	203	145	149
	II	207	144	156	178
Non-essential amino acids (µM)					
Aspartate	I	28	23	16	18
	II	26	16	22	19
Serine	I	109	90	67	76
	II	102	91	82	78
Asparagine	I	39	37	34	27
	II	51	34	43	34
Glutamate	I	244	184	134	165
	II	189	113	136	171
Glutamine	I	128	66	70	74
	II	123	81	70	79
Glysine	I	515	425	345	319
	II	549	474	459	306
Alanine	I	162	145	96	110
	II	145	121	106	131

P1 is the period when both Group I and II were on *ad libitum* feeding
P2 is the period when Group II was on restricted feeding
P3 and P4 are periods of 3 and 15 weeks, respectively,
    of resumption of *ad libitum* feeding by Group II

* significant (P<0.05) between Group I and II at P2
** significant (P<0.05) between Group I and II on P2 - P3 increment
*** significant (P<0.05) between Group I and II at P4

Table 2. The arteriovenous (AV) concentration differences of amino acids across the hind-limb muscles of sheep in normal (Group I) and interrupted (Group II) growth over different periods (P)

	Group	Periods			
		P1	P2	P3	P4
Essential amino acids (μM):					
Valine	I	-42.6	20.8	14.4	-8.1
	II	32.9	6.6	-2.4	30.5
Isoleucine	I	-26.5	12.1	7.5	0.9
	II	18.3	2.8	5.3	11.1
Leucine	I	-5.7	15.3	10.4	-0.9
	II	25.6	2.8	6.6	20.3
Phenylalanine	I	4.6	-0.9	-4.5	-0.3
	II	5.6	-2.5	-3.3	1.7
Histidine	I	3.4	-0.8	-0.5	1.2
	II	2.7	-7.5	0.4	1.9
Lysine	I	-1.5	18.2	18.0	-0.8
	II	24.3	-8.2	1.1	24.3
Threonine	I	-28.1	13.1	-5.5	-4.8
	II	13.4	5.5	-4.7	5.8
Non-essential amino acids (μM):					
Aspartate	I	1.2	0.8	-0.02	-2.2
	II	3.4	0.7	1.5	-1.9
Serine	I	-6.0	6.6	2.0	-3.0
	II	7.1	4.9	1.9	-1.5
Asparagine	I	-1.6	3.8	1.5	0.6
	II	3.7	2.7	3.9	2.5
Glutamate	I	49.4	-38.6	1.4	-30.8
	II	-16.6	-8.5	-8.5	-33.5
Glutamine	I	-15.6	6.8	-28.7	-8.9
	II	-2.6	-0.4	-11.7	-13.8
Glycine	I	-35.5	-13.1	6.2	-46.2
	II	6.9	-12.0	-26.4	-58.5
Alanine	I	10.8	-8.7	-11.0	-19.5
	II	-0.4	-9.5	-5.9	-3.4

See Table 1 for P1, P2, P3 and P4
Negative values indicate net output

Catabolism of amino acids may be reduced by supplementing the diet with energy-yielding substrates thus facilitating the redirection of the acids towards protein synthesis. This has been proven by a number of experiments where infusion of propionate or glucose in lambs increases either N retention (ESKELAND *et al.*, 1973, 1974, MAHYUDDIN, 1997) or liveweight gain (LENG, 1978; JENKINS and THONNEY, 1988). However, an experiment conducted by VAN HOUTERTT *et al.*

(1993) indicated that increase in net glucose flux in the lambs offered propionate was not accompanied by increased liveweight gain. More studies should be done to clarify the differences in these results.

Hind-limb muscles

The metabolism of amino acids by the hind-limb muscle has been measured by multiplying concentration

differences (A-V differences) by rates of blood flow. As has been shown in the previous report (MAHYUDDIN and TELENI, 1996) that the rates of blood flow was not affected by the different periods of growth, thus the A-V concentration difference was the only factor to observe.

There was no apparent trend in A-V concentration differences of essential amino acids across the hind-limb muscles of animals in Group II (Table 2). Due to the high variation in values between animals, mean values of A-V concentration differences of all amino acids did not differ significantly between animals in Group I and those in Group II at any of the periods from P1 to P4. Similarly, the plasma insulin concentration was not affected by restricted or resumption of *ad libitum* feeding (Table 3). This is probably a reflection of the nonsignificant changes in A-V concentration differences of amino acids.

Table 3. The entry rate of urea, glucose and CO_2 and insulin concentration in sheep in normal (Group I) and interupted (Group II) growth over different periods (P) adopted from MAHYUDDIN and TELENI (1996)

	Group	Periods			
		P1	P2	P3	P4
Urea ER (g/d)	I	48.5	51.1	51.8	54.1
	II	47.2	13.4	52.6	49.8
Glucose ER (mmol/h)	I	29.4	30.0	24.0	29.8
	II	29.3	14.2	21.2	33.4
Amino acids to glucose (%)	I	30	34	45	38
	II	32	18	46	28
CO_2 ER (mmol/h)	I	813	644	718	650
	II	809	283	638	880
Insulin (mU/L)	I	16.3	27.0	29.6	31.0
	II	25.8	17.6	23.8	17.4

See Table 1 for P1, P2, P3 and P4

The difficulty of measuring any significant uptake of plasma amino acids by muscle was also observed by LINDSAY and BUTTERY (1980) which was thought to be caused by the matching rates between protein synthesis and protein degradation. Latter when [^{14}C]-alanine was infused (LINDSAY and BUTTERY, 1980) it was demonstrated that there was a marked extraction of alainine (25%). It was clear that there was in fact a marked uptake, matched by a corres-ponding output of amino acid.

HARRIS *et al.* (1992) studied with hot phenylalanine (not catabolized in muscle) and leucine meta-

bolism in muscle of animals with different intake (low and high), found that the net uptake of phenylalanine and leucine across the leg tissue was significantly related to the level of intake. Furthermore, there was a high correlation between arterial phenylalanine concentration and the net uptake.

Table 4. The protein intake and body protein in sheep in normal (Group I) and interrupted (Group II) growth over different periods (P), adopted from MAHYUDDIN and TELENI (1995)

	Group	Periods			
		P1	P2	P3	P4
Protein intake (g/d)	I	244.4	231.8	235	200.6
	II	236.8	78.7	222.5	210.6
Body protein (g)	I	3,730	4,540	4,720	5,370
	II	3,720	3,090	3,990	4,900

See Table 1 for P1, P2, P3 and P4

With respect to essential amino acids, LINDSAY and BUTTERY (1980) cited from BALLARD *et al.* (1972) indicated that while a number of amino acids were removed by the hindquaters in net amount, there was a large increase of alanine, glutamine and glycine together with small amounts of arginine.

If these results were true, then it is expected that during compensatory growth where feed intake was high, there would be a net uptake of essential amino acids, and output of non-essential amino acids across the hind-limb muscle.

CONCLUSION AND RECOMMENDATION

Feed restriction and resumption of *ad libitum* feeding respectively reduced and increased significantly the essential amino acids concentration particularly the branch chain amino acids. Increased essential amino acids in animals undergoing compensatory growth suggests that there was less protein deposition than anticipated. Protein deposition may be improved through additional energy to the diet. However, further studies should be done as there were still contradictory results on the effect of energy supplement on protein deposition.

REFERENCES

BALLARD, F. J., O. H. FILSELL, and J. G. JARRETT. 1972. Effects of carbohydrate availability on lipogenesis in

tissue from ruminant and non ruminant animals. *Biochem. J.* 226: 193-200.

ESKELAND, B., W. H. PFANDER, and R. L. PRESTON. 1973. Utilisation of volatile fatty acids and glucose for protein deposition in lambs. *Br. J. Nutr.* 29: 347 - 355.

ESKELAND, B., W. H. PFANDER, and R. L. PRESTON. 1974. Intravenous energy infusion in lambs. Effects on nitrogen retention, plasma free amino acids and plasma urea nitrogen. *Br. J. Nutr.* 31: 201 - 211.

HARRIS, P. M., P. A. SKENE, V. BUCHAN, E. MILNE, A. G. CALDER, S. E. ANDERSON, A. CONNELL, and G. E. LOBLEY. 1992. Effect of food intake on hind-limb and whole body protein metabolism in young growing sheep: Chronic studies based on arterio-venous techniques. *Br. J. Nutr.* 68: 389 - 407.

JARRETT, I. G. 1986. The separation of o-phthalaldehyde derivatives of amino acids by reversed phase chromatography on octylisilica columns. *Anal. Biochem.* 153: 189 - 198.

JENKINS, T. C. and M. L. THONNEY. 1988. Effect of propionate level in a volatile fatty acid salt mixture fed to lambs on weight gain, body composition and plasma metabolites. *J. Anim. Sci.* 66: 1028 - 1035.

LINDSAY, D. B. and P. J. BUTTERY. 1980. Metabolism in muscle. In: *Protein Deposition in Animals.* Butterworths. London.

LENG, R. A., S. ECONOMIDES, and F. M. BALL. 1978. The effect on growth of supplying glucose into duodenum of lambs on low-protein diets. *Proc. Aust. Soc. Anim. Prod.* 12: 134.

MACRAE, J. C. and P. J. REEDS. 1980. In: *Protein Deposition in Animals.* P. J. Buttery and D. B. Lindsay (eds). Butterworths. London.

MAHYUDDIN, P. 1995. Metabolism in compensatory growth. I. Growth pattern in compensatory growth. *Ilmu dan Peternakan* 8(2): 1 - 7.

MAHYUDDIN, P. 1997. Intravenous glucose infusion affects nitrogen retention in sheep. *J. Ilmu Ternak Vet.* 2 (4): 225-232.

MAHYUDDIN, P. and E. TELENI. 1995. Metabolism in compensatory growth. II. The intake, digestibility and N retention in animals undergoing compensatory growth. *Ilmu dan Peternakan* 8 (2): 8 - 14.

MAHYUDDIN, P. and E. TELENI. 1996. Metabolism in compensatory growth. III. The urea, glucose and CO_2 entry rates in animal undergoing compensatory growth. *J. Ilmu Ternak Vet.* 2 (1): 22 - 32.

O'DONOVAN, P. B. 1984. Compensatory growth in cattle and sheep. *Nutr.Abstr.Rev.* (Series B) 54: 389 - 410.

ODDY, V. H., J. M. GOODEN, and E. F. ANNISON. 1984. Partitioning of nutrients in Merino ewes. I. Contribution of skeletal muscle, the pregnant uterus and the lactating mammary gland to total energy expenditure. *Aust. J. Biol. Sci.* 37: 375 - 388.

ODDY, V. H., S. R. EDWARDS, H. M. WARREN, P. A. SPECK, P. K. NICHOLLS, and S.A. NEUTZE. 1997. Interrelationships between amino acid and glucose metabolism in lambs of different dietary history supplemented with rumen escape protein. *J. Agric. Sci. Cambridge* 128: 105 - 116.

RIIS, P. M. 1983. The Pools of Cellular Nutrients: Amino Acids. In: *Dynamic Biochemistry of Animal Production World Animal Science. A Basic Information.* P.M. Riis (Ed). p. 151- 172. Elsevier. Amsterdam.

STANGASSINGER, M. and D. GIESECKE. 1986. Splanchnic metabolism of glucose and related energy substrates. In: *Control of Digestion and Metabolism in Ruminants.* L.P. Milligan, W.L. Grovum and A. Dobson (eds). pp 347 - 366. Proc. Sixth International Symposium on Ruminant Physiology. Baniff Canada 1984. Reston Publishing Co: Reston, Va.

VAN HOUTERTT, M.F.J., J.V. NOLAN, and R.A. LENG. 1993. Protein, acetate and propionate for roughage-fed lambs. 2. Nutrient kinetics. *Anim. Prod.* 56: 369 - 378.