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PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATIONS

A Scientific Meeting was held at the University of Aberdeen on Wednesday–Thursday, 4–5 September 1996, when the following papers were presented.

All Abstracts are prepared as camera-ready material by the authors.

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Effect of protein degradability on protein splanchnic metabolism in dairy cows. By HÉLÈNE LAPIERRE[1], JEAN-PHILIPPE BLOUIN[2], GERALD E. LOBLEY[3], CHRIS K. REYNOLDS[4], PASCAL DUBREUIL[5] and JEAN-FRANÇOIS BERNIER[2], *[1]Agriculture and Agri-Food Canada, Lennoxville, Qc, Canada, [2]Université Laval, Ste-Foy, Qc, Canada, [3] Rowett Research Institute, Aberdeen AB21 9SB [4]Reading University, Reading RG6 6AT, [5]Université de Montréal, St-Hyacinthe, Qc, Canada*

The effect of ruminal protein degradability (PD) on protein splanchnic metabolism was measured in six Holstein cows (597 (SD 39) kg) during weeks 33-40 of lactation. At least 4 months before the experiment, the cows had been surgically prepared with arterial, portal and hepatic catheters for blood collection plus two mesenteric catheters to allow infusion of p-aminohippurate to determine blood flow (Huntington *et al.* 1989). Two diets, 60 *v.* 75% of PD, were tested according to a switch-back design, over two 35 d experimental periods. Isonitrogenous (160 g crude protein/kg) diets were given in twelve equal meals per d (average intake: 17.7 kg/d). On day 34 or 35, [1-^{13}C]leucine (99 atom %) was infused into a jugular vein (1.8 mmol/h for 6 h preceded by a priming dose of 1.8 mmol). Blood samples were collected every hour, from 2 to 6 h of infusion, to determine the concentrations of blood free leucine and CO_2, plasma 4-methyl-2-oxopentanoate (MOP) and their respective isotopic enrichments (IE). Milk and protein yields increased (P<0.10) on 60 *v.* 75% PD diet (17.7 *v.* 15.9 (SEM 0 .6) kg/d and 0.72 *v.* 0.65 (SEM 0.02) kg/d respectively), as well as total leucine irreversible loss (ILR: 135.8 *v.* 113.6 (SEM 6.1) mmol/h) and leucine oxidation (LO: 29.4 *v.* 18.1 mmol/h; Blouin *et al.* 1995).

Leucine kinetics (mmol/h) through the splanchnic tissue (portal-drained viscera: PDV; hepatic tissue: HEP; total splanchnic tissue: TSP) are presented in the Table. Leucine used for protein synthesis (LEU for PS) was calculated as the sum of net isotopic movements of leucine (LEUS), MOP (MOPS), and CO_2 (CO_2S) across the tissues, with the IE of blood leucine in the artery chosen as representative of the precursor pool. Leucine net appearance (LEUapp) was calculated as above but using mass transfers. Positive values indicate release and negative values uptake.

Tissue...	PDV			HEP			TSP		
Treatment...	60% PD	75% PD	SEM	60% PD	75% PD	SEM	60% PD	75% PD	SEM
LEU for PS	-31.5	-29.4	3.4	-22.8	-14.5	4.6	-54.3	-43.9	6.1
LEUS	-42.5§	-34.8§	3.5	-25.7	-19.3	4.0	-68.1*	-54.2*	5.1
MOPS	1.6	1.4	0.2	-1.8	-1.5	0.2	-0.2	-0.1	0.1
CO_2S	9.4*	4.1*	1.6	4.6	6.3	1.4	14.0	10.4	2.0
LEUapp	31.3§	25.3§	2.4	7.6	7.3	2.7	39.1	32.1	4.1

*: P<0.10; §: 0.10<P<0.20

Across the PDV, the LEUapp tended to be higher with the 60% PD diet but the difference between the rations was only 27 % of the increment in ILR. Across the PDV and TSP, LEUS increased as degradability decreased but leucine oxidaton also increased, although not significantly for TSP. Finally, this resulted in no effect of the diet on LEU for PS. Across the liver, leucine kinetics were not altered by the diets. The differences between diets in net LEUapp across the TSP, although not statistically significant, would only account for 32 % of the ILR increment and suggest that diet degradability may have effects on systemic protein metabolism. These results confirm the high metabolic activity of the splanchnic tissues, which together represent over 45% of total utilization and oxidation of leucine by the whole body.

Blouin, J.P., Bernier, J.F., Lobley, G.E., Reynolds, C.K., Dubreuil, P. & Lapierre, H. (1995). *Journal of Dairy Science* **78**, Suppl.1, 213.

Huntington. G.B, Reynolds, C.K. & Stroud, B.H. (1989). *Journal of Dairy Science* **72**, 1583-1595.

Effect of lysine deficiency on protein splanchnic metabolism in growing pigs. By NICOLE ROY[1], JONG-TSENG YEN[2], HÉLÈNE LAPIERRE[3], JOHN A. RATHMACHER[4], STEVEN L. NISSEN[4] and JEAN-FRANÇOIS BERNIER[1], [1]*Université Laval, Ste-Foy, QC, Canada,* [2]*USDA, ARS, USMARC, Clay Center, NE, USA,* [3] *Agriculture and Agri-Food Canada, Lennoxville, QC, Canada,* [4]*Iowa State University, Ames, IA, USA*

Growing barrows (32.9 (SD 1.4) kg) were used to determine the effect of lysine deficiency on growth and protein metabolism by portal-drained viscera (PDV) and liver (HEP). Chronic catheters were placed in the portal vein, hepatic vein and carotid artery for blood sampling and in the ileal vein to allow p-aminohippurate (PAH) infusion for plasma flow measurement. For a 5 week experimental period, pigs were fed on one of two diets: a lysine-deficient diet containing 3.6 g lysine/kg (L36: maize (490 g/kg), wheat (340 g/kg) and maize-gluten meal (100 g/kg)) and a control diet with 7.6 g lysine/kg (L76: L36 supplemented with L-lysine HCl), according to a completely randomized block design. Pigs were given their daily allowance (110 $g/kg^{0.75}$) in twenty-four equal meals, one served every hour. Feed intake (L36: 1551 *v.* L76: 1673 (SD 109) g/d) and average daily gain (L36: 0.45 *v.* L76: 0.66 (SD 0.06) kg/d) were lower ($P<0.10$) in pigs fed on the L36 diet (*n* 14) compared with those receiving the L76 diet (*n* 10). Lysine deficiency decreased ($P<0.10$) the weight of the carcass, PDV and HEP tissues. On day 15, [1-^{13}C]leucine (99 atom%) was infused in a jugular vein (11.07 μmol/kg per h for 6 h, preceded by a priming dose of 11.07 μmol/kg). Blood samples were taken at hourly intervals during the infusion period to measure PAH concentration and plasma concentration and isotopic enrichment (IE) of free leucine, 4-methyl-2-oxopentanoate (MOP) and CO_2.

Leucine irreversible loss rate for the whole body (LEU ILR) was calculated as the infusion rate of leucine divided by the IE of arterial leucine (L36: *n* 9 *v.* L76: *n* 7). Leucine used for protein synthesis (LEU for PS) was calculated as the sum of net isotopic movements of leucine (LEUS), MOP (MOPS), and CO_2 (CO_2S) across the tissues, with the IE of plasma leucine in the artery chosen as representative of the precursor pool. Leucine net appearance (LEUapp) was calculated as above but using mass transfers. Positive values indicate release and negative values uptake. Due to loss of patency of catheters, kinetics ± pooled SD through the PDV and HEP tissues were measured in L36, *n* 8, L76, *n* 5, and in L36, *n* 6, L76, *n* 3 respectively.

Variable (mmol/h)	PDV tissue			HEP tissue			TSP tissue		
	L36	L76	SD	L36	L76	SD	L36	L76	SD
LEUapp	4.28	4.65	2.26	-0.41	0.36	1.99	3.45	5.81	2.17†
LEU for PS	-4.47	-3.71	1.66	-5.68	-4.19	1.85	-10.04	-9.00	3.07
LEUS	-4.14	-3.83	1.31	-5.18	-3.47	1.72†	-8.80	-7.96	2.92
MOPS	-0.45	-0.15	0.63	-2.08	-1.07	0.98	-2.76	-1.75	0.71†
CO_2S	0.29	0.24	0.26	1.58	0.91	0.59†	1.87	1.21	0.57†

†: $0.10<P<0.20$

Lysine deficiency tended to decrease LEU ILR ($P<0.20$, L36: 14.05 *v.* L76: 16.80 (SD 3.75) mmol/h). LEU for PS in PDV, expressed as a proportion of LEU ILR, tended to increase ($P<0.20$, L36: 31.6 *v.* L76: 23.8 (SD 9.2) %) with lysine deficiency. LEUS in HEP, expressed as a proportion of LEU ILR, increased ($P<0.10$, L36: 38.2 *v.* L76: 22.7 (SD 10.9) %)) with lysine deficiency. Across HEP and TSP tissues, leucine oxidation, expressed as a proportion of LEU ILR, increased ($P<0.10$, L36: 11.5 *v.* L76: 5.7 (SD 4.2) % for HEP, $P<0.20$, L36: 13.7 *v.* 7.9 (SD 4.5) % for TSP) with lysine deficiency. The proportion of LEU ILR used by the HEP tissue for LEU for PS tended to be higher ($P<0.20$, L36: 41.2 *v.* L76: 25.4 (SD 12.4) %) with lysine deficiency. The results suggest that lysine deficiency decreased LEU ILR but increased both leucine used for protein synthesis and for oxidation by the splanchnic tissues when expressed relative to LEU ILR.

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Whole-body protein metabolism in cattle fed on grass silage and dried grass at different intakes. By H.M.R. Greathead[1], J.M. Dawson[1], V.A. Sessions[1], F.N. Tye[1], N.D. Scollan[2], A.B. McAllan[2] and P.J. Buttery[1], *[1]Department of Applied Biochemistry and Food Science, Faculty of Agricultural and Food Sciences, Sutton Bonington, Loughborough LE12 5RD, [2]Institute of Grassland and Environmental Research, Plas Gogerddan, Aberystwyth ST23 3EB*

Cattle fed on grass silage have a higher carcass fat:protein ratio than cattle fed on dried grass. In previous studies this has been shown to be due to an impaired protein accretion and an enhanced fat deposition. Comparative studies investigating whole-body protein metabolism in animals fed on these diets have not been reported.

Hereford x Friesian steers (initial live weight (LW) 94.2 kg; *n* 24) were fed on either grass silage (S) or dried grass (DG) at a range of intakes from 1.1x the maintenance (M) metabolizable energy (ME) requirement to *ad libitum* (1.55x M for S; 2.0x M for DG) for 12-14 weeks. Rates of whole-body protein metabolism were measured under steady-state conditions by continuous intravenous infusion of [1-^{13}C]leucine after which animals were slaughtered and carcass composition determined.

The composition of the S used was: 282.9 g toluene DM/kg fresh; pH 4.4; 33.5 g total N/kg DM; 108.2 g NH_3-N/kg total N; 10.4 MJ ME/kg DM estimated from modified acid detergent fibre (MADF). The composition of the DG used was: 28.4 g total N/kg DM; 10.8 MJ ME/kg DM estimated from MADF. Compared with animals fed on DG, animals fed on S had lower daily LW gains (mean 0.32 *v.* 0.38 kg/d, $P<0.05$) and lower daily carcass weight gains (mean 135.8 *v.* 198.5 g/d, $P<0.001$) at all equivalent levels of ME intake. Carcass protein contents were lower in S-fed animals compared with DG-fed animals (mean 19.3 *v.* 20.0%, $P<0.05$), but fat contents were higher (mean 8.6 *v.* 7.2%, $P<0.01$) resulting in higher carcass fat:protein ratios for animals fed on S compared with DG (mean 0.45 *v.* 0.36, $P<0.001$) at all equivalent levels of ME intake. There were no differences in carcass fat:protein ratio between animals fed on S and DG *ad libitum*. Compared with animals fed on DG, animals fed on S had lower leucine entry rates (mean 8.4 *v.* 10.3 μmol/min per kg$^{0.75}$, $P<0.001$) and lower rates of leucine oxidation (mean 1.4 *v.* 2.9 μmol/min per kg$^{0.75}$, $P<0.001$), resulting in animals fed on S having lower rates of whole-body protein synthesis than animals fed on DG at all equivalent levels of ME intake (mean 16.2 *v.* 17.3 g/kg$^{0.75}$ per d, $P>0.1$). Leucine entry rates, leucine oxidation rates and the rates of whole-body protein synthesis increased ($P<0.05$) with increasing ME intake. Compared with animals fed on DG, animals fed on S had lower plasma leucine concentrations (mean 93.6 *v.* 188.3 μM, $P<0.001$) and lower plasma KIC concentrations (mean 7.8 *v.* 16.3μM, $P<0.001$) at all equivalent levels of ME intake, however there were no differences in the ratios of leucine:KIC between the S and DG diets (mean 12.1 *v.* 11.6, $P>0.1$) suggesting no differences in the rates of transamination or the proportions of KIC oxidized between the two diets.

These results indicate that the performance of animals fed on S is inferior to those fed on DG, and confirm that animals fed on S have higher carcass fat:protein ratios than animals fed on DG at equivalent levels of ME intake. The results show that animals fed on S tend to have lower rates of whole-body protein synthesis than animals fed on DG. These results however do not indicate if their are any differences in the efficiency of utilization of the absorbed amino acids between animals fed on the two diets.

Preventing the insulin-induced fall in amino acids via variable infusion of an amino acid mixture increases the glucose response to insulin in sucking pigs. By DIANE WRAY-CAHEN, PHILIP R. BECKETT, HANH V. NGUYEN, PETER J. REEDS, DEBBIE ALLNUTT and TERESA A. DAVIS, *USDA/ARS Children's Nutrition Research Center, Baylor College of Medicine, Houston, TX USA*

The neonate has a high growth rate and the fractional rate of growth declines rapidly with age. Young animals utilize their nutrient intake efficiently to stimulate protein synthesis (PS) (Davis *et al.* 1996). These high rates of tissue accretion necessitate large energy inputs. Insulin (INS) is a key factor in the regulation and coordination of the post-absorptive use of nutrients, stimulating tissue glucose (GLC) and amino acid (AA) uptake and deposition. We performed INS-dose response (3-4000 μU INS/ml) studies in 7- and 26-d-old sucking pigs, using a hyperinsulinaemic-euglycaemic clamp technique with (*n* 10) or without (*n* 8) clamping AA. We determined: (1) the effect of maintaining near basal levels of AA under hyperinsulinaemic conditions on whole-body GLC disposal, (2) the effect of INS on whole-body AA disposal, and 3) the effect of stage of development on these variables. GLC alone or GLC and AA were maintained at basal fasting levels by variable infusions of glucose alone or glucose and an AA mixture (TrophAmine). GLC and AA disposal were estimated from the infusion rates of glucose (GIR) and AA (AAIR) respectively.

	Glucose infusion rate					AA infusion rate				
	R_{max} (mg/min per kg)		$\log(ED_{50})$ (μU/ml)		ED_{50}	R_{max} (μmol/min per kg)		$\log(ED_{50})$ (μU/ml)		ED_{50}
	Mean	SEM	Mean	SEM	(μU/ml)	Mean	SEM	Mean	SEM	(μU/ml)
7-d-old sucking pigs										
-AA	47.6	3.3	2.04	0.08	110					
+AA	54.7[ad]	2.5	1.49[bc]	0.07	31[bc]	49.0[d]	2.9	1.26[d]	0.08	18[d]
26-d-old sucking pigs										
-AA	46.3	4.0	2.13	0.10	133					
+AA	40.4	3.3	1.73[b]	0.12	54[b]	26.0	3.1	1.65	0.13	45

[a] Significantly different from -AA, $P<0.1$; [b] Significantly different from -AA, $P<0.05$
[c] Significantly different from 26-d-old pigs, $P<0.1$; [d] Significantly different from 26-d-old pigs, $P<0.05$

The Table shows that in 7-d-old pigs, clamping AA near basal levels increased the maximum GLC disposal response to INS. The INS-dose response curve for GIR was shifted to the left at both ages and therefore, clamping AA increased the sensitivity of GLC disposal to INS. In 7- compared with 26-d-old pigs, the maximum response for AAIR was 2-fold higher and the INS-dose response curve was shifted to the left. Plasma urea-N levels were not elevated by AA infusion, suggesting that the infused AA were not catabolized, but were entirely utilized for N accretion.

The results indicate that maintaining AA supply increases the GIR response to INS in young pigs. This is in contrast to evidence in adult human subjects, which shows that AA decrease the response of GIR to INS (Flakoll *et al.* 1991). However, INS stimulates PS in the neonatal pig (Wray-Cahen *et al.* 1996), but not in adult humans. The results of the present study, in combination with the findings in adults, suggest that the responses of GLC and AA metabolism to INS are dependent on stage of development and decline with age. The heightened sensitivity and responsiveness to INS for GLC disposal in the neonate may play an important role in supporting the energetic needs for its high rates of growth and protein synthesis.

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Wray-Cahen, D., Beckett, P.R., Burrin, D.G., Fiorotto, M.L., Wester, T.J., Reeds, P.J., Nguyen, H.V. & T.A. Davis. (1996). *Journal of Animal Science* **73**, Suppl.1, 148.

The effect of dietary protein on mammary nutrient uptake and milk production. By Z.K. RAJCZYK, I.J. LEAN and J.M.GOODEN. *Department of Animal Science, University of Sydney, Camden, NSW, Australia.*

The composition of milk, in particular the protein content and composition, has a marked effect on milk quality for manufacturing. Australian dairy farmers are currently being rewarded or penalized for the protein and fat content of their milk. Nutritional manipulation would appear to be the quickest way to alter milk composition, however attempts to increase milk content by dietary means are proving to be difficult, with variable results reported to date (DePeters & Cant, 1992). This paper presents the results of three studies investigating the effect of high protein (HP, 180 g/kg) and low protein (LP, 120 g/kg) diets on mammary uptake of nutrients and milk production and composition.

Multiparous, mid-lactation Friesian cows were used in these studies. They were paired for age, calving date and milk production, and assigned to either the HP or LP diet. The two diets were isoenergetic, but contained different levels of degradable and undegradable protein. The diets were similar between studies except that fishmeal was substituted for meatmeal in studies 2 and 3. Study 1 was a feeding trial only. In studies 2 and 3, 16 mm ultrasonic blood flow probes (Transonic Systems Inc., New York, USA) were surgically implanted around the right pudendal artery to measure blood flow to the right udder. The facial artery and milk vein were catheterized to measure arterio-venous differences of nutrients across the udder. In study 3, an additional catheter was inserted into the *porta hepatis* via the mesenteric vein to measure nutrient supply to the liver from the portal drained viscera. Milk yields were recorded at each milking, blood flow was measured continuously, and blood and milk samples were taken every 2-3 d. Data were statistically analyzed by ANOVA and unpaired *t* test.

The effects of diet on blood flow, milk yield and composition are shown in the Table. A consistent finding in HP cows in all studies was significantly elevated plasma β-hydroxybutyrate (portal, arterial and mammary uptake) ($P<0.1$) and plasma urea (portal and arterial) ($P<0.05$). This suggests ketone mobilization for increased production and wastage of some of the extra protein consumed. There was no difference between groups in blood α-amino Nitrogen (portal, arterial and uptake) despite the increased dietary protein intake. There was a trend to higher circulating methionine levels in HP cows in all three studies.

	Study 1		Study 2		Study 3	
Variable	HP	LP	HP	LP	HP	LP
n	6	6	3	3	2	3
Blood flow (BF) (litres/min)	-	-	4.5	3.9	6.0	4.5
BF:milk yield ratio	-	-	537.1	501.5	624.8	664.3
Milk yield (litres/d)	25.5***	19.8	25.1*	22.4	19.6	19.4
Protein (g/litre)	31.8*	32.7	33.5*	29.5	36.8	3.32
Protein yield (g/d)	808***	644	837***	661	716	650
Fat (g/litre)	44.3**	40.8	43.5*	36.3	47.7	43.2
Fat yield (g/d)	1129**	807	1085**	814	936	849

* ($P\leq0.1$), ** ($P<0.05$), ** ($P<0.001$)

The increase in arterial β-hydroxybutyrate concentrations and milk fat content reflect the impact that amino acid supply has on mammary metabolism and on the homeorhetic adaption to increased milk production. The increase in amino acid supply to the gland appeared to be mediated through increased blood flow rather than by an increase in the arterio-venous difference. Increased blood flow does not necessarily increase uptake of nutrients, as previously observed for the effect of lying on mammary blood flow and uptake (Rajczyk *et al.* 1995). While increased dietary protein increases blood flow and increases milk protein yields, the link between lactose production and protein production may limit the capacity of the diet to markedly increase milk protein percentage.

DePeters, E.J. & Cant, J.P. (1992). *Journal of Dairy Science* **75**, 2043-2070.
Rajczyk, Z.K., Sweeting, A., Lean, I.J. & Gooden, J.M. (1995). *Proceedings of the Nutrition Society of Australia* **19**, 119.

A technique for measuring arterio-venous difference, including blood flow, in the mammary gland of the sow. By NEIL J. GANNON, RONALD A. PARR, DOUGLAS J. KERTON and FRANK R. DUNSHEA, *Victorian Institute of Animal Science, Werribee, Victoria, Australia*

Efficient feeding of the lactating sow requires accurate information on the utilisation of nutrients, particularly amino acids, for milk production. In comparison with domestic ruminant species, limited information exists on metabolism of amino acids in the mammary gland of the sow during lactation, largely due to the absence of a suitable procedure for measuring arterio-venous (AV) difference of nutrients across the mammary gland. Trottier *et al.* (1995) described a technique for venous cannulation of the mammary gland in the lactating sow and, by also cannulating the carotid artery, were able to determine AV differences across the mammary gland. Mammary blood flow is an important variable to measure to quantify accurately the metabolism of nutrients during lactation, however mammary blood flow could not be measured by the technique of Trottier *et al.* (1995). The aim of the present study was to develop a surgical model in the lactating sow that would enable measurement of blood flow and AV difference of amino acids and other nutrients across the mammary gland.

The sow's udder has both caudal and cranial venous drainage which can complicate blood flow measurements. In the present study, cranial venous drainage from the sow's udder was encouraged in two sows by allowing only six thoracic glands (three on each side) to be functional during lactation. On day 3 post-partum, two sows had their litters reduced to six piglets by fostering. The excess functional mammary glands in the abdominal and inguinal regions were induced to regress by bandaging the teats which prevented piglet access. On day 7 post-partum, the two sows were surgically prepared with catheters as described below. In an approach similar to that of Trottier *et al.* (1995), a branch of the anterior mammary vein was located above the *plica lateralis*, between the first and second gland. A clear, polyvinyl chloride catheter (Dural Plastics, id 1.50 mm, od 2.50 mm, prepared before surgery with two cuffs about 10 mm apart positioned approximately 200 mm from the tip) was introduced into the vessel up to the first cuff so that the tip of the catheter was in the most cranial portion of the mammary vein. The first cuff was inserted into the vessel and a suture placed behind the cuff to occlude the vein and anchor the catheter in place. A second suture was passed through the underlying tissue and behind the second cuff to further anchor the catheter.

A second branch of the same mammary vein was exposed in a similar position to the first but located between the fourth and fifth glands. A shorter catheter than the first, with cuffs located at approximately 100 mm, was introduced into this second branch and anchored in place. Both mammary vein catheters were tunnelled subcutaneously and exteriorized just forward of the shoulder and enclosed in a pouch secured by elastic bandage.

A catheter for sampling arterial blood was introduced into the external iliac artery via the medial saphenous artery. The saphenous artery was palpated through the skin and a small incision made on the middle of the thigh to expose the artery. A catheter similar to the first mammary catheter was inserted approximately 200 mm into the saphenous artery and secured using the two-cuff approach described above. The arterial catheter was tunnelled subcutaneously about 100 mm dorsally, exteriorized and enclosed in a pouch secured by elastic bandage.

Indocyanine green (ICG) was used to measure mammary blood flow. An ICG solution (0.2 mg/ml) was infused (1 ml/min) via the posterior mammary catheter. Blood samples (5 ml) were taken simultaneously from the anterior mammary vein and arterial catheters at 30 min intervals for a period of 2 h on both day 14 and day 21 post-partum. The catheters remained patent for the duration of the study and each sow suckled a normal litter.

In conclusion, we believe the model described in this paper provides an excellent approach for measuring the qualitative and quantitative AV differences of nutrients across the mammary gland.

This work was supported in part by the Australian Pig Research and Development Corporation.

Trottier, N.L, Shipley, C.F. & Easter, R.A. (1995). *Journal of Animal Science* 73, 1390-1395.

Effects of dietary protein levels and porcine growth hormone administration on plasma concentrations of insulin-like growth factor binding proteins in growing pigs. By X.F. GUAN, J.M. BRAMELD and P.J. BUTTERY, *Department of Applied Biochemistry and Food Science, University of Nottingham, Sutton Bonington Campus, Loughborough, LE12 5RD*

The insulin-like growth factors (IGF) are present in the circulation bound to members of a family of high affinity IGF binding proteins (IGFBP). The IGFBP have been proposed to be essential to coordinate and regulate the biological activities of the IGF. Administration of exogenous growth hormone (GH) stimulates growth in growing animals. The GH anabolic effects are mediated primarily by IGF-1. Protein or feed deprivation causes growth retardation in growing animals and changes in the profiles of plasma IGFBP and IGF-1. However, effects of suboptimal dietary protein levels and the interaction of dietary protein level and GH administration on plasma IGFBP in growing pigs have not been investigated. The objective was to examine whether plasma concentrations of IGFBP were altered by porcine GH (pGH) administration and dietary protein level.

Forty-eight intact male Large White x Landrace pigs, with an average initial body weight of 42 kg, were randomly divided into eight treatment groups (four diets +/- GH). The diets differed only in crude protein levels (g/kg: diet 1 99; diet 2 131; diet 3 162; and diet 4 194), with constant digestible energy, and were restriction-fed for 3 weeks. The pigs were then injected intramuscularly with either pGH (50 µg/kg BW per d, kindly donated by Monsanto) or vehicle (sterile water) for the last 7 d, and were slaughtered 4 h after the final injection. Jugular blood samples were obtained immediately, the plasma separated, and then stored frozen at -40° before analysis. IGFBP profiles were quantified by image analysis after SDS-PAGE separation and Western Ligand Blotting with ^{125}I-labelled IGF-1. The plasma samples were assayed for IGF-1 concentration following initial removal of the IGFBP via acid-ethanol precipitation. Results of the absorbance obtained from the image analysis were subjected to two-way ANOVA.

Diet	1		2		3		4			Significance:		
GH	-	+	-	+	-	+	-	+	SED	GH	Diet	GHxDiet
IGFBP-3	12.0	21.4	22.1	27.8	22.2	40.6	27.3	33.3	6.67	**	*	ns
IGFBP-2	2.07	4.48	2.87	4.32	2.75	3.69	2.90	2.81	1.58	ns	ns	ns
IGFBP-1	1.04	1.29	0.88	1.97	1.44	3.42	1.88	3.79	0.99	*	+	ns
IGFBP-4	0.31	0.70	0.39	1.37	0.49	1.34	0.66	1.08	0.47	**	ns	ns

Values shown are means of 6 pigs.

SED: standard error of the difference of the means.

+ P<0.10; * P<0.05; ** P<0.01.

GH administration significantly increased plasma concentrations of IGF-1 (results not shown), IGFBP-3, -1 and -4, with no effect on IGFBP-2. Only IGFBP-3 was significantly increased by dietary protein level, although IGFBP-1 tended to increase with dietary protein, but this was not significant (P=0.065). No significant interaction was found between dietary protein level and pGH administration. Plasma IGFBP-3 and IGF-1 concentrations were significantly positively correlated (R 0.630).

It can be concluded that profiles of plasma IGFBP are differentially regulated by GH administration and dietary protein levels, with IGFBP-3 and -1 appearing to be more sensitive to dietary protein supply than the other IGFBP. These changes in circulating IGFBP might be involved in the effects on animal growth of dietary nutrient manipulation and pGH administration.

Increased body-weight gain of growth-hormone-treated newborn pigs is not due to increased rates of protein synthesis. By TIMOTHY J. WESTER, DOUGLAS G. BURRIN, TERESA A. DAVIS, MARTA L. FIOROTTO and XIAOYAN CHANG, *USDA/ARS Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, Houston, TX, 77030-2600, USA*

Neonatal growth has been generally considered to be independent of the effects of growth hormone (GH). However, there has been a paucity of studies performed using exogenous administration of GH in pituitary-intact neonates. In the present study, we examined effects of exogenous GH administration on growth, endocrine responses, and protein synthesis in newborn pigs. Newborn pigs were given saline or recombinant porcine GH (333 μg/ kg body-weight (BW); pGH was a gift from Monsanto Agric Co.) via i.m. injection three times daily for 7 d. Pigs were pair-fed porcine colostrum for the first 36 h of life and then a porcine milk-replacer for the next 6 d. The milk-replacer was fed to supply 15 g protein/kg BW per d and 544 kJ/kg BW per d. A serial blood sampling was performed after the first feeding and dosing at day 8 of life. At 1 h after a second dosing and feeding, *in vivo* fractional protein synthesis rates (K_s) of various tissues were measured by flooding-dose [^{3}H]Phe methodology (Burrin *et al.* 1995). Performance and plasma insulin-like growth factor I (IGF-I) concentration have also been reported previously (Wester *et al.* 1996).

Tissue K_s (%/d)	Control	pGH	Pooled SD
Longissimus	23.6	22.2	3.3
Semitendinosus	22.9	24.6	3.3
Soleus	22.0	23.8	3.2
Liver	76.6	84.3	9.4
Kidney	37.8	40.1	2.9

Measured after 7 d of pGH treatment, mean plasma GH was 30-fold higher and plasma IGF-I was 3-fold higher than controls ($P < 0.01$). Administration of pGH to newborn pigs resulted in 15% increases in body weight gain and feed efficiency ($P < 0.01$). There were also quantitative changes in the plasma concentrations of the IGF binding proteins (IGFBP) when measured by Western-ligand blotting after 7 d of treatment. The proportions of IGFBP-3 and -4 increased, and IGFBP-1 and -2 decreased with pGH treatment ($P < 0.05$). In addition, pigs treated with pGH were becoming insulin-resistant as judged by a 29% increase in plasma glucose area under the curve in response to feeding ($P < 0.02$). Quantitative dissection of soleus and semitendinosus muscle revealed no differences in mass between treatments. However, the 23% larger livers and 15% larger kidneys ($P < 0.01$) observed in pGH-treated pigs could account for only 14% of the increased body-weight gain in pigs treated with pGH. Despite increased tissue weights in pGH-treated pigs, K_s was not increased for liver or kidney. There was no difference in K_s between treatments for any other measured tissue, including skeletal muscle, spleen, and jejunum. In conclusion, newborn pigs are responsive to exogenous pGH as indicated by increased growth and alterations in endocrine status. However, we were unable to determine the exact component responsible for increased body-weight gain.

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The effects of ruminal degradable nitrogen intake and *in vitro* addition of ammonia and propionate on the metabolic fate of L-[1-^{14}C]alanine and L-[^{15}N]alanine in isolated ovine hepatocytes. By T. MUTSVANGWA, J. G. BUCHANAN-SMITH and B. W. McBRIDE, *Department of Animal and Poultry Science, University of Guelph, Guelph, Ontario, N1G 2W1, Canada*

For a wide range of diets, ruminants absorb as much as 65% of their dietary N intake as NH_3-N, which is transported to the liver via portal blood and efficiently detoxified to urea in the ornithine cycle (Reynolds, 1992). Ureagenesis consumes stoichiometric amounts of carbamoyl phosphate and aspartate, and N for the latter is probably derived from glutamate which, in turn, is derived from transamination of amino acids (Lobley *et al.* 1995). Therefore, NH_3-stimulated ureagenesis when an NH_3 load is presented to the liver might increase amino acid deamination and, consequently, penalize N retention (Reynolds, 1992). In general, ruminants absorb only small amounts of glucose from the digestive tract and gluconeogenesis, primarily from propionate in fed animals, supplies essentially all body glucose needs (Reynolds, 1992). Other metabolites (e.g. NH_3) might limit propionate conversion to glucose and, in turn, propionate may alter ureagenesis (Demigné *et al.* 1991).

Isolated hepatocytes prepared from sheep fed on a basal diet (bromegrass hay-maize, 50:50 w/w, as fed basis), with or without urea were used to determine the effects of added NH_3 (as NH_4Cl) and propionate on the partitioning of C from 1.25 mM L-[1-^{14}C]alanine between oxidation and gluconeogenesis, and the flux of ^{15}N from 1.25 mM L-[^{15}N]alanine to [^{14}N^{15}N]urea and [^{15}N^{15}N]urea. Hepatocyte suspensions were incubated with NH_4Cl (0, 0.31, 0.63 and 1.25 mM) and(or) propionate (0, 0.31, 0.63 and 1.25 mM), in the presence of either 1.25 mM L-[^{15}N]alanine, or 1.25 mM L-alanine and 18.5 kBq L-[1-^{14}C]alanine.

Feeding dietary urea did not affect [1-^{14}C]alanine oxidation to $^{14}CO_2$ ($P = 0.601$), or its conversion to [^{14}C]glucose ($P = 0.576$) by isolated hepatocytes. Increasing *in vitro* levels of both NH_4Cl and propionate between 0 and 1.25 mM reduced [1-^{14}C]alanine oxidation to $^{14}CO_2$ ($P < 0.001$). Increasing *in vitro* levels of NH_4Cl between 0 and 1.25 mM reduced [1-^{14}C]alanine conversion to [^{14}C]glucose in isolated hepatocytes ($P < 0.001$), whereas addition of propionate between 0 and 1.25 mM stimulated production of [^{14}C]glucose from [1-^{14}C]alanine ($P < 0.001$). Effects of feeding dietary urea on *in vitro* rates of total urea production by isolated hepatocytes ($P = 0.655$) and on ^{15}N enrichment (atom % excess) of [^{14}N^{15}N]urea ($P = 0.361$) and [^{15}N^{15}N]urea ($P = 0.244$) were not significant, but the relative productions of both labelled urea species increased ($P < 0.05$). Addition of NH_4Cl to the incubation medium increased the production of total urea, [^{14}N^{15}N]urea and [^{15}N^{15}N]urea by isolated hepatocytes ($P < 0.001$), but reduced ^{15}N enrichments of both labelled urea species ($P < 0.001$). Increasing *in vitro* propionate levels between 0 and 1.25 mM reduced total urea production ($P < 0.001$). Added propionate did not affect ^{15}N enrichment of [^{14}N^{15}N]urea and [^{15}N^{15}N]urea, but the relative productions of both labelled urea species were reduced at 1.25 mM propionate ($P < 0.001$).

Results demonstrate that increasing the NH_3 load *in vitro* stimulates urea synthesis, and this is accompanied by an increase in alanine deamination. This might have implications for N retention in ruminants consuming diets that promote considerable absorption of NH_3 from the digestive tract, such as those based on lucerne or grass silage.

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The incorporation of [5-^{15}N]glutamine into protein and nucleic acids in intestinal and other tissues in lambs. By J.J. GATE[1], D.S. PARKER[1] and G.E. LOBLEY[2], [1] *Department of Biological and Nutritional Sciences, University of Newcastle, Newcastle-upon-Tyne NE1 7RU and* [2] *Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB.*

Windmueller & Spaeth (1974) observed that the C skeleton of glutamine was utilized as an energy substrate by rat gastrointestinal tissue and suggested that the major fate of the 5-NH_2 group was release as NH_3 into the blood. Glutamine is known, however, to have a number of metabolic fates, including incorporation into proteins and acting as a precursor for nucleic acid synthesis where the 5-NH_2 moiety provides 50% of the N in purines and pyrimidines. The magnitudes of these latter fates have now been quantified in various ovine tissues.

Ten lambs (33-42 kg), fasted for 24 h, were given a 10 h intra-jugular vein continuous infusion of [5-^{15}N]glutamine (1 mmol/h). Pseudo-plateaux in arterial and mesenteric vein plasma [5-^{15}N]glutamine enrichments were established within 2 h. After 10 h the lambs were killed and samples of ileal mucosa and serosa, rumen, caecum, liver and hind limb muscle were taken. Extracts of protein, DNA and RNA were obtained from each sample. The ^{15}N enrichment of each fraction was determined using continuous flow isotope-ratio-mass-spectrometry. Total ^{15}N incorporation was calculated using published values for tissue composition.

	^{15}N incorporation (μmol ^{15}N/kg tissue)					
	Protein		DNA		RNA	
	Mean	SEM	Mean	SEM	Mean	SEM
Rumen	1094	156	9.9	2.0	49	12
Ileum mucosa	4124	664	83.2	30.5	149	36
Ileum serosa	1805	348	49.7	24.6	99	19
Hind gut	1197	215	37.7	14.3	51	12
liver	3212	645	34.5	6.2	183	63
Hind limb muscle	102	25	3.2	1.3	6	1

In general, the relative ^{15}N incorporation into protein reflected the fractional synthetic rates reported for sheep (Lobley *et al.* 1994) except for liver where higher rates were observed. This may be due to the presence of glutamine-rich proteins or alternative hepatic storage of the amido-N from glutamine. There was good concordance between the rates of ^{15}N incorporation into protein and RNA (20:1), demonstrating their linkage in the processes of cell growth and turnover. Rates of incorporation into DNA reflect cell proliferation and indicate that cellular half-lives are considerably shorter in post-rumen compared with rumen tissues of the gastrointestinal tract (GIT).

The incorporation of glutamine into intestinal proteins accounted for 73% of the gross flux of glutamine across the small intestine. Although incorporation into protein is the main route for glutamine utilization in GIT tissue a small but significant proportion is required for the synthesis *de novo* of nucleic acids which will complement the salvage of body purines and pyrimidines. The utilization of glutamine by GUT tissues may be maintained at high rates in order to provide substrate for cellular turnover and replacement .

The methodology used in the present experiment allows an estimation of the synthetic rates of protein, DNA and RNA using a single tracer.

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Effect of duodenal essential amino acid infusion on milk yield and composition in lactating dairy cows. By L.A. CROMPTON[1], C.K. REYNOLDS[2], M.A. LOMAX[1], B.J. BEQUETTE[3], F.R.C. BACKWELL[3], J.D. SUTTON[2], J.C. MACRAE[3] and D.E. BEEVER[2], *[1]School of Animal and Microbial Sciences, University of Reading, Whiteknights, PO Box 228, Reading RG6 6AJ, [2]Centre for Dairy Research, University of Reading, Arborfield Hall Farm, Arborfield, Reading RG2 9HX and [3]Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB21 9SB*

Previous reports from this research group have demonstrated that milk protein concentration is increased in response to vascular infusions of essential amino acids (EAA) in animals fed on low-crude-protein (CP) diets (140 g CP/kg DM) during mid-lactation (Reynolds *et al.* 1995) and high-CP diets (180 g CP/kg DM) during early lactation (Crompton *et al.* 1996). The present study examined the responses in milk protein content when the EAA were supplied into the small intestine.

Four Holstein/Friesian cows each fitted with permanent duodenal cannulas, were fed on a diet of grass silage-concentrate (40:60 w/w, DM basis; 160 g CP/kg DM), twice daily in mid-lactation (weeks 20-30). Animals received constant duodenal infusions (8 litres/d) of water for 7 d followed by EAA for a further 7 d, at a rate equivalent to the EAA in either 600 g milk protein/d (E600; 312 g EAA/d) or 900 g milk protein/d (E900; 468 g EAA/d) in a crossover design. Animals were milked twice daily, with milk samples taken for infrared milk analysis at each milking. The mean values for the last 6 d of the water infusion period and the final 3 d of EAA infusion for milk yield and composition are shown in the Table.

						$P<$†	
	Water	E600	Water	E900	SEM	Inf	Lev
Milk yield (kg/d)	23.6	24.5	24.3	25.0	0.2	0.005	0.55
Milk composition (g/kg)							
Fat	42.4	47.7	44.1	45.3	0.2	0.19	0.36
Protein	36.7	37.2	35.3	37.1	0.2	0.001	0.029
Lactose	47.7	46.4	47.6	46.6	0.04	0.030	0.72
Component yield (g/d)							
Fat	1027	1178	1059	1134	35	0.019	0.25
Protein	855	920	862	930	12	0.002	0.63
Lactose	1125	1141	1165	1170	18	0.58	0.66

†Probability values from analysis of variance testing effects of EAA infusion (Inf, water *v.* EAA at both levels) and level of EAA infusion (Lev, E600 *v.* E900).

The results demonstrate that the significant increase in milk protein content in response to vascular EAA infusion can only be repeated when the AA are supplied at the E900 rate, directly into the lumen of the duodenum in cows fed on a diet of 160 g CP/kg DM in mid-lactation.

The work reported here formed part of a collaborative project funded by a consortium of the MAFF, BBSRC, Purina-Mills, Nutreco, MDC and SOAFD.

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Effect of changes in insulin status during intravenous infusions of amino acids on milk protein secretion in dairy cows. By L.A. CROMPTON[1], M.A. LOMAX[1], J.A. METCALF[2], C.K. REYNOLDS[2], B.J. BEQUETTE[3], F.R.C. BACKWELL[3], G.E. LOBLEY[3], J.D.SUTTON[2], J.C. MACRAE[3] and D.E. BEEVER[2], *[1]School of Animal and Microbial Sciences, University of Reading, Whiteknights, PO Box 228, Reading RG6 6AJ, [2]Centre for Dairy Research, University of Reading, Arborfield Hall Farm, Arborfield, Reading RG2 9HX and [3]Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB21 9SB*

Previous reports have demonstrated that milk protein concentration can be repeatably increased in response to intravenous infusions of amino acids in dairy cattle (Metcalf *et al.* 1996). The present study was designed to examine the acute response to vascular infusions of amino acids when insulin status was manipulated.

Four Holstein/Friesian cows in week 34 of lactation and producing 16.4 kg milk/d were given hourly a 140 g crude protein/kg diet (grass silage-concentrate, 40:60, w/w) and were milked twice daily at 08.00 and 20.00 hours. The animals were given three 6 h jugular vein infusion treatments in a randomized block design. All treatments were split into three 2 h periods. In treatment 1 (T1), saline (S, 4.17 ml/min) was infused for 6 h. For T2 saline was infused for the first 2 h, followed by a mixture of amino acids based on the composition of milk protein (AA, 0.56 g/min) for a further 4 h. During the final 2 h of AA infusion, glucose was also infused (G, 9.74 mmol/min). T3 was an exact repeat of T2, except that somatostatin (SRIF, 0.1 μg/kg/min) was infused during the final 4 h (AA and AA+G). The animals were milked out using oxytocin immediately before the start and then every 2 h during the infusions and milk samples taken for infrared milk analysis. Blood samples were taken from the contralateral jugular vein every 30 min during the infusion and plasma stored for analysis. Results for milk yield and protein content and plasma glucose and insulin concentrations are shown in the Table.

	Milk protein content (g/kg)	Milk yield (g/2 h)	Milk protein yield (g/2 h)	Glucose (mM)	Insulin (ng/ml)
Saline	34.7	1424	49.1	3.37	0.68
AA	38.7**	1593	61.5*	3.41	0.93**
AA+G	37.8†	1738***	65.7**	6.85***	3.64***
Pooled SED	0.9	87	3.28	0.10	0.05
Saline	33.8	1322	44.5	3.10	0.63
AA+SRIF	37.2*	1459	54.2*	3.60	0.17***
AA+G+SRIF	33.5	1513	50.9	9.18***	0.15***
Pooled SED	0.8	138	4.18	0.11	0.06

Mean values for AA and AA+G infusions were significantly different from initial saline control values for each treatment; †$P<0.1$, *$P<0.05$, **$P<0.01$, ***$P<0.001$.

The results indicate that relative to the saline control, milk protein concentration and yield were increased within 2 h by an intravenous AA infusion, irrespective of wether insulin concentrations were altered by SRIF infusion. However, glucose infusion over the last 2 h only caused an increase in milk volume when insulin concentrations increased in the absence of SRIF.

The work reported here formed part of a collaborative project funded by a consortium of the MAFF, BBSRC, Purina-Mills, Nutreco, MDC and SOAFD.

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Effect of glucose and amino acid infusions on hindlimb protein turnover in undernourished lambs. By L.A. CROMPTON and M.A. LOMAX, *Growth Biochemistry Group, School of Animal and Microbial Sciences, The University of Reading, Whiteknights, PO Box 228, Reading RG6 6AJ*

Underfeeding lambs results in a net loss of protein across the hindlimb tissues, which can be restored to a net gain of protein within 5 h of initiating refeeding, primarily due to a rapid decrease in protein degradation (Crompton & Lomax, 1996). During refeeding there are increases in plasma glucose and insulin concentrations. Previous studies have identified insulin and amino acids as key nutrient signals in controlling the increase in protein synthesis in response to refeeding rats (Garlick & Grant, 1988). The aim of the present study was to determine whether the refeeding response in hindlimb protein metabolism, in particular the decrease in protein degradation, could be induced in underfed lambs by infusing glucose either alone or in combination with additional free amino acids.

Two groups of wether lambs (30-35 kg live weight) were fed on a diet of commercial barley-based concentrate and chopped hay (9:1, w/w, DM basis) twice daily to achieve a growth rate of 350 g/d. From 3 d before measurements of hindlimb metabolism the animals were only offered the forage portion of their ration twice daily. On day 3 of underfeeding, one group of four animals were infused into the jugular vein (0.5 ml/min) with saline for the first 4 h followed by glucose (18.7 μmol/kg/min) for a further 6 h. The remaining five animals were intravenously infused with saline for 4 h followed by glucose (18.7 μmol/kg/min) plus free amino acids (AA, 1.4 mg/kg/min; Synthamin 17, Travenol Laboratories, Thetford, Norfolk) for a further 6 h. Measurements of hindlimb protein turnover were made during the saline, glucose and glucose + AA infusions as described previously (Crompton & Lomax, 1993). The results for hindlimb protein turnover (%/d) are shown in the Table.

	Underfed (*n* 4)			Underfed (*n* 5)		
Protein	Saline	Glucose	Pooled SEM	Saline	Glucose + AA	Pooled SEM
Gain	-1.44	-1.69	0.45	-1.66	-0.25***	0.31
Synthesis	2.74	3.63	0.38	2.18	1.75†	0.40
Degradation	4.18	5.32	0.50	3.84	2.00**	0.42

Mean values for glucose + AA infusion were significantly different from saline control values; †$P<0.1$, **$P<0.01$, ***$P<0.001$.

Glucose infusion failed to reverse the net loss of protein across the hindlimb tissues that is observed after 3 d of undernourishment, however, both protein synthesis and degradation were increased by 30%. Infusion of glucose and amino acids into underfed lambs significantly reduced the loss of protein across hindlimb tissues due to a rapid and significant decrease in hindlimb protein degradation. Hindlimb protein synthesis was also decreased by 20% in response to glucose + AA infusion.

The results indicate that a combination of glucose/insulin and amino acids may be an important nutrient signal involved in the acute control of hindlimb protein degradation in response to refeeding in lambs.

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Effect of close arterial cimaterol infusion on hindlimb protein turnover in lambs. By L.A. CROMPTON, J. BROWN and M.A. LOMAX, *Growth Biochemistry Group, School of Animal and Microbial Sciences, The University of Reading, Whiteknights, PO Box 228, Reading RG6 6AJ*

Oral administration of β_2-adrenergic agonists to ruminants has been shown to increase protein deposition by primarily decreasing protein degradation (Bohorov *et al.* 1987). It is not known whether the stimulation of muscle accretion occurs via a direct action on muscle tissue or indirectly through the endocrine system or altered nutrient supply. The present study was designed to examine the local effects of the β_2-adrenergic agonist cimaterol on hindlimb tissue protein metabolism by infusing it directly into the hindlimb via the femoral artery.

Five wether lambs were fed on a diet of a medium quality coarsely chopped hay and a barley-based concentrate (1:7, w/w, DM basis) in two equal portions to achieve a growth rate of 350 g/d. Each lamb received close arterial infusions (0.07 ml/min), via femoral artery catheters, of cimaterol into one hindlimb and saline into the contralateral hindlimb for 5 d. The cimaterol was administered at two dose rates, 0.15 mg cimaterol/d (LC) and 2.6 mg cimaterol/d (HC). The hindlimbs receiving saline were termed LS and HS for the low and high doses of cimaterol respectively. Treatments were administered in a randomized block design and were randomized between limbs. On day 5 of the infusions, protein turnover was measured simultaneously in both hindlimbs using tyrosine metabolism as described previously (Crompton & Lomax, 1993). The fractional rates of hindlimb protein turnover (%/d) for saline and cimaterol infused limbs are shown in the Table.

	Treatment					
Protein	LS (*n* 5)	LC (*n* 5)	HS (*n* 4)	HC (*n* 5)	SED S *v.* C	SED L *v.* H
Gain	2.52	4.43***	3.28	2.66	0.35	0.61
Synthesis	4.98	6.53*	5.09	7.43†	0.52	1.30
Degradation	2.47	2.09	2.61	5.49*††	0.65	0.89

Mean values in the same row were significantly different from LS; *P<0.05, ***P<0.001 and from HS; $^{\dagger}P$<0.05, $^{\dagger\dagger}P$<0.01.

Protein gain across the LC infused limb was significantly increased by 76% compared with the LS limb due to a significant increase in protein synthesis and a decrease in protein degradation. Although the rate of protein gain was not significantly different between the HS and HC limbs, the cimaterol-infused limb had significantly higher fractional synthetic and degradative rates, indicating an increase in protein turnover in response to high localized doses of cimaterol.

The results suggest that β-adrenergic agonists act locally on muscle tissue, by directly stimulating protein synthesis. The lack of any significant effect of the high-dose cimaterol infusion on protein turnover in the contralateral HS limb suggests that different sensitivities and/or subtypes of β-adrenergic receptors may be involved in mediating this response.

The financial support of the BBSRC and a MAFF studentship (JB) are gratefully acknowledged.

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Effect of lysine deficiency on whole-body protein metabolism in growing pigs. By NICOLE ROY[1], HÉLÈNE LAPIERRE[2] and JEAN-FRANÇOIS BERNIER[1], [1] *Université Laval, Ste-Foy, QC, Canada,* [2] *Agriculture and Agri-Food Canada, Lennoxville, QC, Canada*

Seventeen growing barrows (24.3 (SD 1.3) kg) were used to determine the effect of lysine deficiency on growth, hormonal concentrations, N retention and whole-body protein metabolism. Pigs were assigned to one of three levels of dietary lysine: 3.6 (L36: maize (500 g/kg), wheat (340 g/kg) and maize-gluten meal (100 g/kg)), 5.6 and 7.6 g/kg (L56 and L76: L36 supplemented with respectively 2.5 and 5.0 g L-lysine HCl/kg), according to a completely randomized block design (day 0 of the experimental period). Experimental diets were given over an 18 d period. On day 5, two chronic catheters were placed in the distal vena cava. The shorter one, located near the right atrium, and the longer one, placed above the hepatic junction, were used respectively for isotope infusion and blood sampling. After surgery, pigs were given their daily allowance (110 g/kg$^{0.75}$) in twenty-four equal meals, one served every hour. On day 10, N balance was carried out for a 6 d period. On days 17 and 18, pigs were placed in respiratory chambers for measurement of CO_2 and leucine kinetics. On day 17, [^{13}C]sodium bicarbonate (99 atom%) was infused (1.66 μmol/kg per h for 6 h preceded by a priming dose of 2.35 μmol/kg) to measure the extent of CO_2 retention, which was used to correct the leucine oxidation. On day 18, [1-^{13}C]leucine (99 atom%) was infused (11.07 μmol/kg per h for 6 h preceded by a priming dose of 11.07 μmol) and samples were taken hourly during the infusion to determine isotopic enrichment of plasma free leucine and of expired CO_2.

Data are given for L36 (*n* 5), L56 (*n* 6) and L76 (*n* 6) ± pooled SD respectively. Average daily gain decreased linearly ($P<0.05$: 0.43, 0.51, 0.56 (SD 0.04) kg/d) with lysine deficiency. Feed intake was not affected ($P>0.10$) by treatments and averaged 1143 (SD 156) g/d. Insulin concentrations tended to decrease ($P<0.10$: 0.23, 0.23, 0.32 (SD 0.07) ng/ml) with lysine deficiency.Triiodothyronine concentrations were higher ($P<0.05$: 0.78, 0.66, 0.65 (SD 0.10) ng/ml) in pigs fed on the L36 diet compared with those receiving the L76 diet. Lower thyroxine concentrations were found ($P<0.10$: 74.4, 63.4, 78.4 (SD 10.5) ng/ml) in pigs fed with diet L56 compared with those fed with diets L36 and L76. Insulin-like growth factor-I ($P>0.10$: 260, 251, 287 (SD 47) ng/ml) and somatotropin ($P>0.10$: 13.2, 8.8, 7.3 (SD 5.5) ng/ml) concentrations were not affected by treatments.

	Diets				*P*=	
Variable (g/kg$^{0.75}$ per d)	L36 (*n* 5)	L56 (*n* 6)	L76 (*n* 4)	SD	Linear	Quadratic
Leucine ILR	3.69	4.30	4.52	0.44	0.04	0.44
Leucine oxidation	1.49	1.42	1.57	0.32	0.74	0.55
Protein synthesis	32.98	44.18	44.30	2.09	0.001	0.01
Protein degradation	23.71	33.97	33.62	2.72	0.01	0.01
Protein retention	9.27	10.22	10.68	1.38	0.21	0.75

Leucine irreversible loss rate (ILR) decreased by 18% with lysine deficiency. Bicarbonate recovery rate, which averaged 87 (SD 6) % was not affected ($P>0.10$) by the treatments. Total leucine oxidation was not affected by diets, as did fractional leucine oxidation ($P>0.10$: 39.6, 32.5, 34.7 (SD 4.6) %). Protein synthesis and degradation rates decreased with lysine deficiency, but values for L56 and L76 were similar. Lower protein retention observed with lysine deficiency could be related to a greater reduction of protein synthesis compared with protein degradation.

D-Aspartate transport by lactating rat mammary tissue. By I.D. MILLAR[1], D.T. CALVERT[1], M.A. LOMAX[2] and D.B. SHENNAN[1], [1]*Hannah Research Institute, Ayr KA6 5HL and* [2]*Department of Agriculture, University of Aberdeen AB24 5UA*

A knowledge of anionic amino acid transport by the lactating mammary gland is desirable given that L-glutamate is one of the most abundant amino acids in milk protein. Recently, it has been shown that the predominant, if not only, transport pathway for L-glutamate in the lactating rat mammary gland is a high affinity, Na^+-dependent process (Millar *et al.* 1996). This transport system appears to be situated in the blood-facing aspect of the mammary epithelium and therefore, is in a position to generate the large intra-to-extracellular glutamate concentration gradient which exists in mammary tissue (Shennan *et al.* 1994). It appears, on the basis of *cis*-inhibition studies that the mammary L-glutamate carrier also transports both L- and D-aspartate but not D-glutamate. In this respect, the mammary Na^+-dependent L-glutamate carrier is similar to that which has been described in other epithelia.

The aim of the present study was to confirm that the L-glutamate carrier accepts aspartate as a substrate. Thus, we have examined the transport of D-aspartate, using D-[^{3}H]aspartate as tracer, by lactating rat mammary tissue explants and the perfused lactating rat mammary epithelium. The transport of radiolabelled D-aspartate (influx and efflux) by mammary explants was measured using standard methods (Shennan, 1989). D-Aspartate transport by the perfused gland was assayed using a rapid, paired tracer dilution technique employing [^{14}C]sucrose as an extracellular marker. The mammary gland was perfused, via the superficial epigastric blood vessels with a physiological saline solution delivered with a flow and pressure profile similar to that found *in vivo* (Clegg & Calvert, 1988).

D-Aspartate uptake by mammary tissue explants was found to be a Na^+-dependent process: replacing extracellular Na^+ with choline ions almost abolished D-aspartate uptake. The fractional loss of D-aspartate from mammary tissue explants, preloaded with radiolabelled D-aspartate,was increased by reversing the trans-membrane Na^+ gradient. Thus, the fractional efflux (/min) was increased from 0.0091 (SE 0.0007) to 0.0376 (SE 0.0033) by transferring mammary explants from a buffer containing Na^+ to one where choline was the major cation. The clearance of D-aspartate (with respect to sucrose) by the perfused mammary gland was markedly reduced (> 90%) when Na^+ in the perfusate was replaced with N-methyl-D-glucamine. D-Aspartate clearance by the perfused gland was inhibited by the addition of L-aspartate and L-glutamate, but not D-glutamate, to the perfusate. D-Aspartate efflux from mammary tissue explants was *trans*-stimulated by L-glutamate in a dose-dependent fashion. Thus, extracellular L-glutamate at 50, 100, 200 and 500 μM respectively increased the fractional release of D-aspartate (above basal levels) by 132, 146, 257 and 667%. In contrast, extacellular D-glutamate did not stimulate D-aspartate efflux.

The results confirm that the mammary tissue L-glutamate transport system accepts D-aspartate as a substrate. D-Aspartate, on account of being non-metabolized, will be a useful tool to further examine the properties of the mammary anionic amino acid transport system. The finding that the mammary tissue anionic amino acid carrier can function in the exchange mode means that the substrate specificity of the transporter can readily be examined by testing the effect of extracellular amino acids on D-aspartate efflux.

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Temporal variation in plasma amino acid concentrations across the gut tissues of steers offered silage and silage-concentrate diets once daily. By A.R.G. WYLIE[1,2], C.L. THORP[3,4], D.J. KILPATRICK[1,2], R.W.J. STEEN[1,2,3] and J.D. McEVOY[1]. *[1]The Department of Agriculture for Northern Ireland, [2]The Queen's University of Belfast, Newforge Lane, Belfast BT9 5PX, [3]The Agricultural Research Institute of Northern Ireland, Hillsborough, County Down BT26 6DR and [4](current address) The University of Reading, Department of Agriculture, Earley Gate, Reading RG6 2AT.*

Amino acid (AA) appearance and metabolism across ruminant gut tissues varies between species and with both type and quantity of diet (Reynolds & Huntington, 1988; Seal *et al.*, 1992). Few studies have investigated temporal differences in individual AA concentrations arising from contrasting diets offered under practical feeding regimens of once or twice daily feeding.

Three Aberdeen Angus x Friesian steers (373 kg mean live weight), chronically catheterized in the portal (P) and hepatic (H) veins and an artery (A), were offered either a perennial ryegrass silage (S) *ad libitum* or an equal estimated digestible energy and N intake from silage (0.40) with a 9:1 rolled barley and soyabean meal mixture (0.60) placed on top (SC) so as to ensure full consumption of concentrates before silage. Diets were offered in a partially balanced changeover design with feeding at 09.00 hours daily. Supplements of fishmeal (300 g) and a mineral-vitamin mix (50 g) were given daily directly on top of the silage part of each diet.

Blood was sampled hourly (for 24 h) from each catheter and heparinized plasma was immediately prepared by high-speed centrifugation (11000 x g; 8 min; 3°). Plasma was denatured by perchloric acid addition and supernatant fractions were combined to provide five 4 h composites (1-4 h; 5-8 h; 9-12 h; 13-16 h; 17-20 h postfeeding), and a prefeed sample for ion exchange AA analysis (tryptophan not determined). Plasma AA patterns were compared in relation to time of feeding by ANOVA.

Mean concentrations (over 24 h) of each AA in each of H, P and A plasmas were unaffected ($P>0.05$) by diet and AA extraction coefficients across the PDV and liver were in line with reviewed data (Lescoat *et al.*, 1996). However, patterns of AA concentrations in each catheter differed in relation to time after feeding and in relation to peak magnitude and were best described by a general purpose mathematical relationship

$$y = a + \frac{(b + ct)}{(1 + dt + et^2)}$$

in the form of a ratio of polynomials representing a cubic curve with an asymmetric maximum (or minimum) falling to an asymptote and in which y is the AA concentration at time t after feeding. The Genstat statistical package was used to fit this relationship to the data. For each AA and each sampling site, individual curves were first fitted for the two diets and then compared by a model in which diet parameters were constrained to be equal and using ANOVA to compare the respective residual sums of squares.

In most cases the model provided a significant fit of the data ($P<0.05$ or better) despite considerable inherent variation. More significant differences ($P<0.05$ or better) were found between patterns of AA concentrations for S and SC diets in H plasmas (fourteen out of twenty AA) than in P or A plasmas (five and ten respectively). For S diets, plasma concentrations of the following AA increased post-prandially before returning to prefeed levels: asp, ser, glu, gln, tyr, his and arg (H); asp, thr, asn, glu, gln and pro (A); asp, glu and gln (P) while plasma concentrations of the following AA decreased post-prandially: gly, val, ile, orn and lys (H); gly, ala, val, orn, lys, his and arg (A); gly, ala, val, met, ile, tyr, phe, orn, lys, his and arg (P). For SC diets, concentrations of most AA in all plasmas decreased post-prandially before returning to prefeed levels. Exceptions were glu and gln (H); glu and gln (P) and glu and tyr (A).

The data indicate underlying differences in the timing of delivery of specific amino acids to the extra-hepatic tissues of ruminants from silage and mixed silage-concentrate diets despite supplementation with rumen undegradable protein sources such as fishmeal.

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The insulin response to exogenous glucose is decreased by a period of flushing in feed restricted anoestrus post-partum Charolais beef cows. By C. PONSART[1], B. KHIREDDINE[1], A.A. PONTER[1], B. GRIMARD[1,2], P. HUMBLOT[2], D. SAUVANT[3] and J.P. MIALOT[1], [1]*Laboratoire d'Epidemiologie et de Gestion de la Santé Animale, ENVA, 7 ave du général de Gaulle, 94704 Maisons-Alfort, France,* [2]*UNCEIA, Services techniques, 13 rue Jouet, BP65, 94703 Maisons-Alfort, France,* [3]*INA-PG, 16 rue Claude Bernard, 75231 Paris cedex 05, France*

In suckled post-partum beef cows ovarian activity is delayed by insufficient energy intake, however a period of flushing can improve reproductive success (Kabandana *et al.* 1993). Metabolic signals probably provide the link between energy status and reproductive function, but the mechanisms are complex and involve not only changes in circulating hormones such as insulin (INS), and metabolites such as glucose (GLU) and non-esterified fatty acids (NEFA) but also changes in the responsiveness of tissues at the cellular level to hormones (Vernon & Sasaki, 1991).

The present experiment was therefore designed to study the effects of a period of flushing after energy restriction on the dynamic metabolic reaction to exogenous glucose. Eighteen multiparous Charolais cows were fed at 70% of energy requirements from calving with (flushing (F), *n* 9) or without (no flushing (NF), *n* 9) an energy supplement of 2 kg triticale/d starting at 32 d postpartum. At 42 d post-partum, each cow was injected intravenously during 20 min with 1 mmol glucose/kg live weight. Blood samples were taken from the other jugular vein at -5, 0, 5, 10, 15, 20, 25, 30, 35, 40, 50, 60, 75, 90 and 120 min from the start of the infusion to measure plasma GLU, INS and NEFA. Statistical analysis was performed using the General Linear Model procedure to analyse the profiles of plasma insulin and blood metabolites after exogenous glucose injection and to test the effects of flushing.

Basal GLU, INS and NEFA levels were not different in the two groups (group F *v.* group NF, least square mean GLU 3.43 (SE 0.2) mmol/l *v.* 3.51 (SE 0.2) mmol/l, INS 22.8 (SE 1.2) pmol/l *v.* 23.4 (SE 1.1) pmol/l, NEFA 236 (SE 27) μeq/l *v.* 242 (SE 27) μeq/l). As expected plasma GLU concentrations increased significantly with time ($P<0.001$) and peaked at 20 min (11 (SE 0.83) mmol/l). Then, GLU concentrations decreased significantly during the following periods : 25-30, 30-35, 35-40, 40-50, 50-60, 60-75, 75-90 and 90-120 min after the start of the injection. At all time points studied for the GLU profile there were no differences observed between the two groups of cows. On the contrary the profile of plasma INS concentrations was significantly influenced by flushing ($P<0.001$). The exogenous GLU supply resulted in a significant increase in INS concentrations during the period 0-5, 5-10 and 10-15 min, but F animals had lower concentrations than NF animals at times 5, 10 and 20 min with the peak at 20 min (161.4 (SE 38.5) pmol/l *v.* 273.3 (SE 36.3) pmol/l). This difference between the two groups remained significant until 75 min after the start of the injection. Plasma NEFA concentrations decreased significantly with time after the start of the GLU injection ($P<0.001$), however there was no difference between dietary groups.

In the present experiment we have shown that a short period of flushing results in a lower INS response to exogenous GLU. This result supports the finding that energy level influences tissue INS sensitivity (Metcalf & Weekes, 1990). This may be part of the mechanism by which flushing improves reproductive success.

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Peptide absorption across the ovine gastrointestinal tract: a methodological comparison. By LAURENCE BERNARD[1], DANA L. WILSON[2], VIVIEN BUCHAN[2] and F.R. COLETTE BACKWELL[2], [1]*Station de Recherches sur la Nutrition des Herbivores - INRA de Theix, France,* [2]*Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB.*

The extent of gastrointestinal peptide absorption in ruminants is currently the subject of much controversy (Seal & Parker, 1991, 1996; Koeln *et al.* 1993; Bernard & Rémond, 1995; Hipólito-Reis *et al.* 1995) with differences in observed magnitude of peptide concentration and/or absorption being attributed to experimental methodology, dietary regimen or species. In the present study the objective was to compare two techniques developed independently (Bernard & Rémond, 1995; Hipólito-Reis *et al.* 1995) for determination of free and peptide amino acid (AA) concentrations in blood. Arterial and mesenteric blood collected from sheep were prepared in two ways following addition of internal standard: (1) deproteinization with perchloric acid (1 ml 1 M-PCA/ml blood), followed by gel-filtration on a Sephadex G-15 column (Hipólito-Reis *et al.* 1995); (2) deproteinization with sulphosalicylic acid (SSA, 400 g/l, 0.1 ml/ml blood) followed by ultrafiltration through a 3000 Da cut-off filter (Centricon-3, Amicon, 5000 *g*, 3 h) (Bernard & Rémond, 1995). AA concentration was determined before (free AA, FAA) and after (total AA, TAA) hydrolysis.

		Concentration (μmol/g blood) (n 5)										
		Arterial (A)				Mesenteric (M)				A-M		
		FAA		TAA		FAA		TAA		FAA	TAA	PAA
		Mean	SD	Mean	SD	Mean	SD	Mean	SD			
Sheep 1	PCA+G-15	2.187	0.157	3.294	0.389	4.542	0.384	5.715	0.045	-2.355	-2.421	-0.066
	SSA+UF	2.075	0.068	3.431	0.415	4.480	0.150	5.865	0.714	-2.405	-2.434	-0.029
Sheep 2	PCA+G-15	2.160	0.088	3.472	0.080	3.287	0.155	4.810	0.258	-1.127	-1.338	-0.211
	SSA+UF	2.059	0.029	3.835	0.266	3.069	0.101	4.875	0.310	-1.010	-1.040	-0.030

PAA, peptide AA = TAA-FAA.
Data are expressed for fifteen AA (Asp, Glu, Ser, Gly, His, Thr, Ala, Arg, Pro, Tyr, Val, Ileu, Leu, Phe, Lys).

There were no significant differences in FAA or TAA ($P>0.05$) determined by either method, neither was there any significant ($P>0.05$) PAA uptake (absorption) across the mesenteric bed (negative A-M of PAA would suggest uptake). This observation is in agreement with a similar study on sheep fed on the same diet (pelleted lucerne; Hipólito-Reis, 1996) but in conflict with a study on sheep fed on an orchard-grass hay and soya bean meal (80:20), where there appeared to be absorption of peptides in the mesenteric drained viscera (Bernard & Rémond, unpublished results). In light of these observations, we now propose to study the effect of dietary regime on peptide absorption across the ovine gastrointestinal tract.

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Response of isolated mohair secondary follicles to epidermal growth factor *in vitro*. By M. SOURI, H. GALBRAITH and J. R. SCAIFE, *Department of Agriculture, MacRobert Building, 581 King Street, Aberdeen AB24 5UA.*

Factors controlling the growth characteristics of caprine hair are poorly understood. Epidermal growth factor (EGF) is recognized to alter morphology of human, mouse and sheep hair follicles (Philpott & Kealey, 1994). The present study investigated the effects, on anagen mohair secondary hair follicles, (approximately 90/treatment) of exposure to 0, 1.0 or 10.0 μg/l EGF in supplemented Williams E medium. Hair follicles were isolated from small samples of skin from two 18-month-old Angora goats (Ibraheem *et al.* 1993) cultured *in vitro*, and measurements made on hair shaft elongation and viability of individual follicles, for 48 h incubation. Follicles were then removed, washed and placed in fresh medium containing EGF as before and in addition [U-^{14}C]leucine (specific activity 66.6 MBq/mmol) for 3 h. The uptake of [U-^{14}C]leucine was measured in the percholoric acid - insoluble protein fraction of the supernatant fraction from homogenized, centrifuged follicles. Total DNA was assayed in the supernatant fraction.

	EGF (μg/l)			
	0	1.0	10.0	SEM
0-24 h				
Elongation (mm)	0.19a	0.25b	0.17a	0.015
Proportion viable	0.67	0.64	0.76	0.03
24-48 h				
Elongation (mm)	0.19a	0.24b	0.17a	0.015
Proportion viable	0.66	0.64	0.76	0.02
0-48 h				
Elongation (mm)	0.38a	0.49b	0.34a	0.03
[U-^{14}C]leucine uptake (pmol/follicle per3 h)	3.12a	5.29b	4.86ab	0.54
DNA (ng/follicle)	87a	130b	71a	9.11
[U-^{14}C]leucine uptake (pmol/μg DNA)	36.0a	42.8ab	74.2b	9.13

abMean values within a row with unlike letters were significantly different ($P < 0.05$).

Responses to EGF were dose-dependent. At 1.0 μg/l EGF increased hair shaft elongation, [U-^{14}C]-leucine uptake and DNA concentration per follicle suggesting a stimulation of both mitotic activity and protein deposition. At 10.0 μg/l, while overall protein deposition was not enhanced, there was evidence of an increase in protein deposition when measured in relation to total DNA present per follicle.

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Nitrogen retention in Holstein steers offered isoenergetic rations containing increasing amounts of a blend of protein sources of low ruminal degradability. By T.F. ROBINSON[1], A.P. MOLONEY[2], D.H. BEERMANN[1], K.D. FINNERTY[1] and D.G. FOX[1], *[1]Cornell University, Ithaca, NY 14853, USA, [2]Teagasc, Grange Research Centre, Dunsany, Co. Meath, Ireland*

Abomasal infusion studies have demonstrated that an increase in post-ruminal amino acid supply above that achieved by conventional diets results in an increase in N utilization by young steers (Robinson *et al.* 1995). To maximize protein accretion at a particular energy supply, adequate amounts of amino acids in the appropriate balance must be available for absorption from the small intestine. The objective of the present study was to determine the effect of providing young steers with increasing levels of a "balanced" array of amino acids at the small intestine on the efficiency and rate of N retention.

The Cornell net carbohydrate and protein system (CNCPS) model (O'Connor *et al.* 1993) was used to formulate a maize-based diet that would meet the rumen requirements for a 250 kg Holstein steer treated with an oestrogenic implant, fed an ionophore and gaining 1 kg body weight/d. The CNCPS was then used to formulate a low-ruminally-degradable protein supplement from blood meal, hydrolysed feather meal, fish meal, and meat-and-bone meal that balanced the amino acid pool at the small intestine of such steers. Six Holstein steers (276 kg) were fed hourly (95% *ad libitum* intake) on a 90:10 concentrate-forage isoenergetic diet that contained 0, 26, 52, 78 or 104 g of the protein supplement/kg DM. The treatments were randomly administered to each steer with each treatment period lasting 15 d, days 1-7 for diet adjustment, days 8-14 for N balance measurement and day 15 for blood sampling. Data were analysed by ANOVA.

	Protein supplement (g/kg ration DM)					
	0	26	52	78	104	Pooled SE[1]
DM intake (kg/d)	4.62	4.90	4.86	5.28	4.84	0.21
Nitrogen balance (g/d)						
Intake	80.1	95.3	101.9	117.0	118.0	4.65*
Faecal loss	28.1	30.8	31.0	36.7	32.9	1.58*
Urinary loss	33.1	34.0	38.2	42.6	42.4	3.56
Retention	18.8	30.6	32.7	37.6	42.7	5.03*
Retention (g/kg absorbed)	353	452	452	465	489	60.8
Plasma urea N (mg/l)	45	49	49	44	54	9.1

[1]Significance of F test, * = P<0.005

The Table shows that feeding this blend of protein sources at up to 100 g/kg DM increased N retention by 127% and increased the efficiency of N use by 38% without an effect on DM intake. That plasma urea N concentrations were unaffected despite the ration crude protein concentration increasing from 114 to 162 g/kg DM supports the observed increase in the biological value of dietary protein. These findings demonstrate that formulating rations to increase the quantity of a balanced amino acid mixture reaching the small intestine results in an increase in the efficiency of protein growth.

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Exogenous xylanase (EC 3.2.1.8) improves digestion of protein, fat and energy and utilization of protein in rye-based diets for broilers. By S.S.P. SILVA and RON SMITHARD, *Department of Biological and Nutritional Sciences, University of Newcastle upon Tyne, NE1 7RU*

Exogenous enzymes are used in commercial broiler diets to minimize the adverse effects of non-starch polysaccharides. In an experiment to study effects of xylanase in broiler diets, a diet that included rye (600 g/kg) and TiO_2 (2 g/kg diet) as indigestible marker was given, either with (Plus enzyme) a commercial enzyme preparation (1 g Avizyme/kg diet; Finnfeeds International Ltd, Marlborough, Wilts) or without added enzyme (Control) to individually housed chickens from age 7 d to age 24 d. The enzyme preparation contained at least 2500 units xylanase from *Trichoderma longibrachiatum* and 800 units protease (*EC* 3.4.24.28) from *Bacillus subtilis*/g preparation. In the second week of feeding on the experimental diets total collections of excreta were made for the determination of digestibilities. At the end of the experiment, samples of jejunal tissue were collected for measurement of crypt-cell proliferation (CCP) and jejunal digesta were collected for measurement of fluid viscosity and xylanase activity.

Diet	Viscosity (cP)	Xylanase (arbitrary units)	CCP (cells/2 h)	Apparent digestibility (%) N	Lipid	Energy	ABV
Plus enzyme	103	0.039	33.1	69.2	77.3	71.6	72.9
Control	697	0.015	38.2	55.1	36.7	56.0	64.7
SEM	47	0.009	2.8	1.3	3.7	1.6	2.6
P	<0.001	0.017	0.204	<0.001	<0.001	<0.001	0.034

ABV, apparent biological value.

The birds that received the exogenous enzymes grew much faster (body weight at age 24 d, Control 796 (SD 55) g; Plus enzyme 873 (SD 80) g, $P<0.001$) and used their feed more efficiently (feed:gain between day 10 and day 24, Control 2.24 (SD 0.29); Plus enzyme 1.77 (SD 0.15) , $P<0.001$) than the Control birds. The inclusion of the enzymes markedly reduced the viscosity of the jejunal fluid (Table) and some xylanase, measured using Remazol Brilliant Blue R-D-xylan as the substrate, clearly remained active in the small intestine. In previous experiments, in which the enzymes were included at much higher levels (Silva & Smithard, 1996), significantly reduced CCP in birds that received the added enzyme may have contributed to improved performance. In the present experiment the lack of an effect on CCP suggests that any sparing of nutrients from decreased CCP would be small. Improved growth performance in the birds that received the exogenous enzyme in this experiment can be ascribed largely to improved digestion of N and energy. In addition to increased digestion, the N that was digested appears to have been more efficiently utilized. Although the feed contained only about 109 g lipid/kg, the big increase in digestibility of fat would have resulted in an increase of about 1.5 MJ metabolizable energy/kg diet.

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Influence of feeding acetylated peptides on fermentation and peptidase activities in the sheep rumen. By M.W. WITT, C.J. NEWBOLD and R.J. WALLACE, *Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB*

Acetylation of peptides inhibits their degradation by rumen micro-organisms *in vitro* (Wallace, 1992). The aim of the present experiment was to determine if adaptation of the rumen microbial population takes place when acetylated peptides are fed for a prolonged period, thus enabling the microbial population to break down the protected peptides. Three adult sheep, fitted with permanent rumen cannulas, received a maintenance diet of hay, barley, molasses, fish meal and a vitamins-minerals mix (500, 299.5, 100, 91 and 9.5 g/kg DM respectively). Meals (500 g), were given at 08.00 and 16.00 hours. The sheep received the basal diet alone (C) or with 20 g peptides (P; peptone 140, Gibco BRL) or 20 g peptides treated with acetic anhydride (A) added at each meal. The diets were given for 28 d in a 3×3 latin square experiment. Most measurements were made on samples of rumen fluid taken between 1.5 and 2.5 h after the morning feeding on four consecutive days during the last week of each period. Peptide concentrations were measured 1 h after feeding. Rumen VFA concentrations were unaffected, except for small increases in valerate and caproate ($P<0.05$) in the rumen fluid of animals receiving untreated peptides (P). NH_3 concentrations tended to be higher in sheep receiving untreated peptides (192, 242 and 215 (SED 19.3) mg/l for C, P and A respectively). There was a trend towards increased proteolytic activity with acetylated peptides (0.91, 0.96 and 1.14 (SED 0.074) mg [^{14}C]casein/mg protein per h) and a higher dipeptidase activity (31.7, 34.3 and 40.5 (SED 2.3) nmol Ala_2 hydrolysed/mg protein per h). However, hydrolysis of acetyl-Ala_2 remained low (2.4, 3.6 and 5.3 (SED 5.9) nmol acetyl-Ala_2 hydrolysed/mg protein per h), and ammonia production from acetylated peptides remained only 27% of that from untreated peptides (83, 103 and 70 (SED 20.7) *v.* 315, 289 and 260, (SED 94.9) nmol NH_3 produced/mg protein per h). Peptide concentrations in rumen fluid were higher ($P<0.05$) 1 h after feeding the diet containing acetylated peptides (58.5, 60.4 and 121.2 (SED 5.94) mg peptide-N/l). Thus it was concluded that the rumen microbial population did not adapt to utilize acetylated peptides after at least 21 d feeding, indicating that these peptides would be expected to pass from the rumen undegraded.

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Comparison of urinary purine derivative excretion with the flow of purine bases at the duodenum of lactating dairy cows. By R. J. DEWHURST[1], S. N. LEWIS[1], M. S. DHANOA[1], and R. T. EVANS[2], *Institute of Grassland and Environmental Research, [1]Plas Gogerddan, Aberystwyth SY23 3EB and [2]Trawscoed Research Farm, Aberystwyth SY23 4LL*

Purine bases (PB) in duodenal digesta and their derivatives (PD; allantoin plus uric acid) in urine have both been used as markers of microbial material leaving the rumen. The intra-gastric infusion studies of Chen and co-workers (e.g. Verbic *et al.* 1990, with steers) confirmed the usefulness of the urinary PD approach although there have been no direct comparisons of the two approaches in conventionally-fed lactating dairy cows.

Four multiparous lactating Holstein-Friesian cows (mean live weight 594 (SD 37.6) kg and mean milk yield 21.8 (SD 5.59) kg/d across all experimental periods), prepared with rumen and duodenal cannulas, were used in a 4 x 4 Latin Square experiment with 28 d periods. The animals were offered grass silage *ad libitum* with 5 kg/d (fresh weight) of one of four pelleted concentrates. The concentrates were formulated to be isoenergetic and isonitrogenous and were composed of the following ingredients with a common inclusion of mineral-vitamin premix (g/kg; air dry basis): (1) barley (674), extracted soyabean meal (237), white fishmeal (79) and protected fat (10); (2) unmolassed sugarbeet feed (627), extracted soyabean meal (253), white fishmeal (84) and protected fat (36); (3) barley (512), extracted rapeseed meal (462) and protected fat (26); and (4) unmolassed sugarbeet feed (459), extracted rapeseed meal (486) and protected fat (55).

Duodenal digesta was sampled continuously over days 18 and 19 of each period whilst total collections of urine (over 1.4 litres/d 2M-H_2SO_4) were made using externally applied urine separators over days 22-27 inclusive (fifteen collections were successful). Ytterbium acetate and Cr-EDTA were infused continually into the rumen from day 11 (priming dose) and used to calculate duodenal flow data. PB and PD were analysed according to the HPLC methods of Cozzi *et al.* (1993) and Dewhurst *et al.* (1996) respectively. Xanthine and hypoxanthine were not detected in urine whilst allantoin made up 0.908 (SD 0.0203) mol/mol of PD. The data were analysed by ANOVA (Genstat 5; Lawes Agricultural Trust, Rothamsted) and there were no significant effects of treatments on silage voluntary intake (kg oven dry matter/d), duodenal flow of PB (mmol/d) and urinary PD (mmol/d) which averaged 10.97 (SD 1.083), 382 (SD 69.6) and 321 (SD 58.0) respectively.

The relationship between duodenal PB (mmol/d) and urinary PD (mmol/d) was explored by fitting a functional relationship in the Maximum Likelihood Programme (MLP; Lawes Agricultural Trust, Rothamsted) assuming equal variation in PB and PD (equation). Although the variance ratios derived from ANOVA, or residual maximum likelihood (REML), point to a lower ratio (i.e. more variability with PB), it is safest to assume equal variances given the small sample size; in any case, these effects are within the error limits on the slope.

$$\text{Urinary PD} = 34\ (\text{SE } 76.7) + 0.754\ (\text{SE } 0.198) \times \text{duodenal PB}$$
$$(n\ 15) \qquad (r\ 0.64)$$

The duodenal flow technique has many limitations and cannot be regarded as a definitive standard against which to assess the urinary PD technique. Nonetheless, it is interesting to compare results of the present study with intra-gastric infusion results obtained with cattle (steers). The relationship obtained by Verbic *et al.* (1990) lies just above the upper 95% confidence limit of the equation. The slightly lower apparent recovery of PB as PD in the current work could reflect, for example, excretion of some PD in milk, loss of some PB in the duodenal sampling before urine collection or bias introduced by the dual-phase marker system. Both techniques (PB and PD) suggest that despite marked differences in composition, there were only small effects on rumen microbial yield when concentrates were offered at 5 kg/d alongside grass silage.

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Nitrogen balance and rumen microbial nitrogen supply measured in Zebu bulls given young or mature Napier grass (*Pennisetum purpureum*).

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The microbial protein (i.e. nitrogen, MN) synthesized in the rumen is the main source of protein to the host animal. This is particularly the case when poor-quality roughages are fed in tropical systems. The efficiency of MN synthesis when related to the organic matter fermented in the reticulo-rumen (DOMR) ranged from 15 to 60 g MN/kg DOMR (cited by Agricultural Research Council 1980). Measurement of MN yield estimated from the urinary excretion of purine derivatives (PD) is non-invasive, requiring only a total collection of urine. Six Zebu bulls of 317 (*SD* 14) kg were all individually given each of two Napier grass hays (*Pennisetum purpureum*) of 6 or 28 weeks maturity. A change-over design with periods of 5 weeks was used. The hays were chopped, supplemented with urea and $(NH_4)_2SO_4$, and offered *ad libitum* (20% refusal). Fresh hay was offered daily. Food intake, diet digestibility, N balance and PD (Allantoin and uric acid) excretion were measured during week 4 (7 d total collection of excreta).

Food intake and digestibility, and N retention were greater with young hay (Table). PD excretions were low, and converted to MN gave a negative value for mature hay with the model of Verbic *et al.* (1990). That of Osuji *et al.* (1995) which assumes a lower basal PD excretion (156 *v.* 385 μmol/$kg^{0.75}$ per d), gave low values which would not have provided sufficient protein-N for the retentions measured. The N retentions were equivalent to +0.56 and -0.19 kg/d wet tissue (0.3 protein DM) which compared with measured live-weight changes (Table). Theoretical MN yields needed to support the N balances observed were calculated using the factors of Agricultural and Food Research Council (1993). These gave 44 and 35 g MN/kg DOMR for the young and mature hays respectively. Protozoal predation within the rumen and/or overestimation of the animals' endogenous PD loss may have contributed to, but are not large enough to account for, the underestimation of MN supply. Therefore we think the main reason for low PD recovery is due to recycling of PD to the rumen (in saliva) and degradation by the rumen micro-organisms, giving recoveries in urine lower than the 0.85 assumed by Verbic, *et al* (1990) and Osuji *et al.* (1995).

Variable	Young hay	Mature hay	*SED*
DM degradability (a + b) (%)[a]	75.6	56.8	2.2
Digestible organic matter intake (DOMI) (kg/d)	3.85	1.40	0.15
Live wt change (kg/d)(35.d)	0.86	-0.66	0.39
N Balance (g/d)	28.0	- 8.0	2.6
PD excretion (mmol/d)	46.0	27.0	2.0
MN yield (Verbic) (g/kg DOMR)	6.9	-3.4	4.8
MN yield (Osuji) (g/kg DOMR)	13.0	15.1	3.3

a. When degradability after t hours is given by $a + b(1-exp^{-ct})$

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Estimation of gastrointestinal passage rates of different plant components in ruminants using isotopically-labelled plant wax hydrocarbons or sprayed even-chain n-alkanes. By R.W. MAYES, J. GIRALDEZ and C.S. LAMB, *Macaulay Land Use Research Institute, Craigiebuckler, Aberdeen AB15 8QH*

Herbage n-alkanes remain associated with particulate material within the ruminant digestive tract; furthermore, ruminal synthesis, measured as fixation of ^{14}C into faecal hydrocarbons, is negligible (Mayes *et al.* 1988). Thus ^{14}C-, or ^{3}H-labelled hydrocarbons in herbage, could be potential markers for estimating digesta passage rate. Passage rates of different dietary plant components should be obtainable by concurrently using both isotopic markers. An alternative approach may be to spray different plant components with different artificial n-alkanes which are normally absent from the diet.

In six adult Saanan goats, housed in metabolism cages and fed fresh perennial ryegrass, mean retention time estimates (MRT, Faichney, 1975) were obtained for leaf, stem and flowers of perennial ryegrass following oral doses of mixtures of the fresh plant parts, separately labelled with ^{14}C and ^{3}H and artificial alkanes (C_{36}, C_{38} and C_{40}). Ytterbium acetate, ^{51}Cr-mordanted hay fibre and CoEDTA were also used to obtain estimates of MRT. Faeces from seventeen subsequent total collections over 7 d, were analysed for ^{14}C- and ^{3}H-labelled hydrocarbons, n-alkanes and the conventional markers.

The partitioning of the various markers between particulate and liquid fractions of abomasal digesta was investigated in six grass-fed adult Scottish Blackface sheep. The animals were slaughtered 24 h after receiving doses of ^{14}C-labelled ryegrass, C_{38}-labelled grass, ytterbium acetate, ^{51}Cr-mordanted hay fibre and CoEDTA. Abomasal contents were centrifuged (28000 ***g*** for 25 min) and the precipitate and supernatant fractions analysed for the dosed markers and the natural dietary alkanes.

	Goats (*n* 6) Mean retention time (h)*				Sheep (*n* 6) Proportion in abomasal precipitate	
	Leaf	Stem	Flower	Overall	Mean	SE
[$^{14}C/^{3}H$]-Grass	39.4 (4)[a]	39.0 (4)[a]	39.7 (3)[a]	-	0.98†	0.006
[$C_{36}/C_{38}/C_{40}$]-Grass	35.1[b]	33.6[b]	32.5 (5)[b]	-	0.92‡	0.008
Natural grass alkanes	-	-	-	-	0.98	0.005
Ytterbium acetate	-	-	-	35.0[b]	0.87	0.023
^{51}Cr-Mordanted hay	-	-	-	48.6[c]	0.98	0.003
CoEDTA	-	-	-	32.6[b]	0.42	0.012

*Mean values; average SED (reml) = 1.26, range 1.10-1.53 (number of observations shown in parentheses, otherwise *n* 6).
[abc] Mean values with unlike superscript letters were significantly different ($P< 0.05$).
†^{14}C-labelled whole grass. ‡Whole grass sprayed with C_{38} alkane.

Assuming the behaviour of ^{14}C and ^{3}H markers to be the same, and sprayed alkane behaviour to be unaffected by chain length, the results in the Table show that the MRT of ryegrass plant parts were similar, but estimates using sprayed alkanes were significantly lower than those from isotopically-labelled grass. This may be due to the relative degree of association of the markers with the particulate phase of digesta, suggested from the results of the sheep study. Whereas ^{14}C-labelled hydrocarbons and natural alkanes of grass associated with particulate digesta to a similar extent as ^{51}Cr-mordanted hay, C_{38} alkane, sprayed on to grass, had a lower affinity, yet higher than that of ytterbium acetate.

These results suggest that dietary plants, labelled with ^{14}C and ^{3}H, have potential as markers for estimating rates of passage of individual dietary components through the ruminant digestive tract.

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