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PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATIONS

The Four Hundred and Fiftieth Meeting of the Nutrition Society was held in the Physics Lecture Theatre, Faraday Complex, University of Lancaster, Lancaster on Tuesday and Wednesday, 12/13 April 1988, when the following papers were read:

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Absorbtion of residual yolk and liver lipid changes in the newly hatched chick. By R. C. NOBLE and D. OGUNYEMI, *Hannah Research Institute, Ayr KA6 5HL* and *West of Scotland Agricultural College, Poultry Science Department, Auchincruive, Ayr KA6 5HW*

A large proportion of the yolk of the fertile egg remains unabsorbed at hatching and becomes incorporated into the body of the emergent chick as an extension of its small intestine. As a result, at least a quarter of the original yolk lipid remains available for absorbtion after hatching. The influence of the yolk lipid on embryonic tissue lipid patterns is extensive (Noble, 1986). Whether this influence continues over the early neonatal period is unknown.

By the 5th day after hatching about 90% of the lipid present in the remnant yolk at hatching has been absorbed (see Table). During this period, the composition of the yolk lipid displays a marked increase in its proportion of cholesteryl esters and decreases in the proportions of triacylglycerides and phosphoglycerides. There are increases in the proportions of the C_{20} and C_{22} polyunsaturated fatty acids in the phosphoglycerides with an associated reduction in the proportion of oleic acid. These major lipid and fatty acid synthetic processes that specifically accompany the absorption of lipid during the incubation period (Noble *et al.* 1984; Noble & Shand, 1985) are therefore retained during the early neonatal period. Growth of the liver after hatching is associated with a further accumulation of fat. The accumulation is, however, accompanied by extensive changes in its composition. The very high level of cholesterol esters (> 70% of the total lipid present) associated with the embryo liver is rapidly replaced by triacylglycerides. The accumulation of the triacylglycerides is also accompanied by a rapid change in their fatty acid composition away from that associated with embryonic development and differing markedly from that of the triacylglycerides being absorbed from the yolk.

Lipid composition changes (%) in the yolk and liver after hatching

(Mean values with their standard errors for four observations)

	Yolk				Liver			
	At hatch		5 d post-hatch		At hatch		5 d post-hatch	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Total lipid (g)	1·70	0·39	0·17**	0·04	0·47	0·06	1·03*	0·18
Composition(%):								
Cholesteryl ester	19·6	0·64	66.7***	2·51	69·2	5·56	8·50***	0·81
Triglyceride	60·2	0·79	17·6***	1·47	4·99	0·47	49·7***	3·10
Free cholesterol	6·25	0·56	6·53	0·38	6·67	1·59	13·4**	0·38
Phospholipid	10·4	1·12	4·23***	0·16	19·1	0·79	28·4*	3·08
Triglyceride:								
Arachidonic (%)	<0·50		<0·50		4·55	0·28	<0·50***	
Docosahexaenoic (%)	<0·50		<0·50		4·93	0·31	<0·50***	
Phospholipid:								
Arachidonic (%)	4·79	0·32	10·6***	0·44	22·9	1·16	7·16***	1·11
Docosahexaenoic (%)	0·71	0·09	4·46***	0·33	10·9	1·92	10·5	2·00

*P<0·05, **P<0·01, ***P<0·001

These changes are indicative of the rapid alteration for the role of the liver in the lipid metabolism of the newly hatched chick. Whereas during embryonic development the liver serves primarily as a depository for preformed yolk lipid components, immediately following hatching it demonstrates an extensive synthetic capacity that outweighs the large contribution to lipid accumulation within the chick still being made by the yolk.

Noble, R. C. (1986). *Proceedings of the Nutrition Society* **45**, 17–25.
Noble, R. C., Connor, K. & Smith, W. K. (1984). *Poultry Science* **63**, 558–564.
Noble, R. C. & Shand, J. H. (1985). *Lipids* **20**, 278–282.

Yolk lipid uptake by the chick embryo: an electron microscopic study with reference to low hatchability in young parent stock. By N. YAFEI and R. C. NOBLE, *Hannah Research Institute, Ayr KA6 5HL* and *West of Scotland Agricultural College, Poultry Science Department, Auchincruive, Ayr KA6 5HW*

The average 60 g chicken egg contains 6 g lipid confined almost exclusively to the yolk. This lipid, which constitutes one of the major nutrients of the embryo, is transferred from the yolk during the last 7 d of incubation and is associated with a rapid and unique accumulation of lipid within the liver (Noble, 1986). The lipid transfer is accomplished by the highly specialized yolk-sac membrane which increasingly encompasses the yolk from the 5th day of incubation onwards (Romanoff, 1960; Lambson, 1970).

In fertile eggs from young parent stock, low hatchability poses a considerable problem in the broiler industry. As a result of recent electron microscopic and analytical investigations (see Table) it has been shown that the problem is associated with a considerable reduction in yolk-lipid transfer and liver accumulation.

The distribution of lipid in the fertile egg

(Mean values with their standard errors for four observations)

		Young parent (23–24 weeks)			Mature parent (34 weeks)		
Embryo age (d)		Yolk (g)	Yolk-sac membrane (g)	Liver (mg)	Yolk (g)	Yolk-sac membrane (g)	Liver (mg)
15	Mean	4·24	0·39	13·7	3·53***	0·66***	20·7***
	SE	0·22	0·02	0·63	0·09	0·03	0·69
19	Mean	2·94	1·32	66·5	1·74***	1·48***	99·3***
	SE	0·11	0·09	0·73	0·09	0·08	0·92

***$P<0.001$.

In the normal embryo at days 15 and 19 of incubation, electron microscopy at 10–15 K shows an increasing accumulation, associated with transfer, of lipid in the yolk-sac membrane which is the result of a non-specific engulfment of the yolk-lipid droplets with no previous extracellular digestion. Consequentially, there is a large accumulation of lipid as droplets within the cytosol of the liver. By contrast, in fertile eggs from very young parent stock, engulfment and accumulation of yolk lipid by the yolk-sac membrane and subsequent cytosolic accumulation in the liver are very much reduced. Concomittant changes to cellular morphology and ultrastructure of the yolk-sac membrane and liver are also apparent. Under these circumstances, therefore, the reduced yolk-lipid uptake is denying access by the embryo to its major energy source and the essential nutrients that the yolk-lipid supplies.

Lambson, R. O. (1970). *American Journal of Anatomy* **129**, 1–20.
Noble, R. C. (1986). *Proceedings of the Nutrition Society* **45**, 17–25.
Romanoff, A. L. (1960). *The Avian Embryo*. New York: Macmillan.

Increased fluidity of the erythrocyte membrane in magnesium deficiency. By F. W. HEATON, *Department of Biological Sciences, University of Lancaster, Lancaster LA1 4YQ* and S. TONGYAI and Y. RAYSSIGUIER, *Laboratoire des Maladies Métaboliques, INRA Theix, 63122 Ceyrat, France*

Magnesium deficiency in rats has been found to increase the fluidity of the erythrocyte membrane and reduce the osmotic fragility of intact erythrocytes (Heaton *et al.* 1987). The present investigations were undertaken to examine the mechanism of this effect.

The fluidity of erythrocyte ghosts from weanling rats pair-fed on Mg-deficient and control diets for 8 d was determined by fluorescence polarization using diphenylhexatriene (DPH) and trimethylammonium-DPH (TMA-DPH) as probes. Measurements with DPH (which enters the centre of the membrane bilayer) showed that Mg deficiency increased the fluidity of ghosts by 15% and with TMA-DPH (which is restricted to the outer leaflet of the bilayer) it was increased by 14%. The similarity between these values implies that the change occurs predominantly in the outer leaflet of the membrane. Incubation of ghosts from either control or deficient rats with plasma from Mg-deficient animals for 20 h at 37° increased their fluidity, suggesting that the change is due to physico-chemical interaction between the membrane and blood plasma.

Effect of Mg on fluidity of erythrocyte membranes incubated in Tris buffer

	Anisotropy $(r_o/r-1)^{-1}$				
	Control (*n* 16)		Mg-deficient (*n* 16)		
Addition to buffer	Mean	SEM	Mean	SEM	Statistical significance
None	3·03	0·06	2·57	0·05	$P<0·001$
0·8 mM $MgCl_2$	3·53	0·10	3·18	0·12	$P<0·05$
0·8 mM $MgCl_2$ +1·0 mM EDTA	2·91	0·12	2·50	0·10	$P<0·05$

Analysis of membranes from similar deficient and control rats showed no difference in total lipid, protein or calcium contents, but the Mg concentration was significantly reduced in ghosts from Mg-deficient animals. The Table shows that when ghosts from Mg-deficient and control rats were incubated in Tris buffer with a physiological extracellular fluid concentration of 0·8 mM $MgCl_2$, anisotropy (which is the reciprocal of fluidity) increased in both cases, but this increase was completely prevented by the presence of EDTA. Although the increase in rigidity produced by Mg was proportionally greater with membranes from Mg-deficient than from control rats, a significant difference between the two still remained.

These results indicate that loss of Mg from the erythrocyte membrane may contribute to its increased fluidity during Mg deficiency, but other factors must also be involved.

Heaton, F. W., Tongyai, S., Motta, C., Rayssiguier, Y. & Gueux, E. (1987). *Nutrition Research* **7**, 655–663.

Lipid composition and erythrocyte membrane fluidity in magnesium deficiency. By Y. RAYSSIGUIER, S. TONGYAI and E. GUEUX, *Laboratoire des Maladies Métaboliques, INRA Theix, 63122 Ceyrat* and C. MOTTA, *Laboratoire de Biochimie, Hopital Hotel Dieu, 63000 Clermont-Ferrand, France* and F. W. HEATON, *Department of Biological Sciences, University of Lancaster, Lancaster LA1 4YQ*

The increased fluidity of the erythrocyte membrane in magnesium-deficient rats appears to be caused partly by the loss of membrane-bound Mg and partly by other mechanisms (Heaton *et al.* 1988). Because Mg deficiency alters the composition of lipoproteins in plasma (Rayssiguier, 1986), and plasma lipoproteins exchange rapidly with lipids in the erythrocyte membrane, we investigated the lipid composition of the membrane.

When weanling rats were pair-fed on Mg-deficient and control diets for 8 d, the deficient animals had increased levels of triglycerides and phospholipids in the plasma but there was no difference in cholesterol concentration. Erythrocyte membranes from the same deficient rats contained decreased concentrations of cholesterol and sphingomyelin (see Table), and the ratios of cholesterol:phospholipid and sphingomyelin:phosphatidylcholine were both significantly reduced. Cholesterol and sphingomyelin rigidify membranes and these ratios are widely recognized indices of the fluidity of membranes (Shinitzky, 1984). The decrease in their values must therefore contribute to the increased fluidity observed in Mg deficiency.

Lipid composition of erythrocyte membranes

	Cholesterol (mmol/g dry wt)		Cholesterol: phospholipid ratio		Sphingomyelin (% phospholipid)		Sphingomyelin: phosphatidylcholine ratio	
Group	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Control	0·223	0·004	0·84	0·01	10·65	0·74	0·22	0·01
Mg-deficient	0·203	0·003	0·76	0·01	8·40	0·68	0·18	0·01
Statistical significance	$P<0·001$		$P<0·001$		$P<0·05$		$P<0·01$	

n 16 for cholesterol and phospholipid, *n* 8 for sphingomyelin and phosphatidylcholine.

The fluidity of erythrocyte membranes from similar Mg-deficient and control rats, and liposomes prepared from the same batches of membranes, was assessed using the fluorescent probe, diphenylhexatriene. Mg deficiency increased the fluidity of both whole ghosts and liposomes by 15% indicating that the change is due entirely to the lipid bilayer region of the membrane and does not involve protein. The extent to which decreased Mg-binding to the phospholipid headgroups and alterations in lipid composition contribute to the increased fluidity of the membrane remains to be determined.

Heaton, F. W., Tongyai, S. & Rayssiguier, Y. (1988). *Proceedings of the Nutrition Society* **47**, 162A.
Rayssiguier, Y. (1986). *Magnesium Bulletin* **8**, 186–193.
Shinitzky, M. (1984). *Physiology of Membrane Fluidity*, vol. 1, pp. 1–51. Boca Raton, Florida: CRC Press.

Acute and chronic undernutrition and their effects on rat colonic secretion. By HELEN C. NZEGWU[1], M. M. C. PEREIRA[1], M. A. WARREN[2], R. J. LEVIN[1] and A. YOUNG[1], *Departments of [1]Physiology and [2]Anatomy, The University, Sheffield S10 2TN*

Total starvation for up to 72 h renders the rat proximal colon hypersecretory in response to cholinergic stimuli (Levin *et al.* 1987). Acute undernutrition and chronic undernutrition have also been shown to hypersensitize rat small intestine to secretory stimulants (Pereira *et al.* 1988; Young *et al.* 1988). Little is known, however, of the effects of restricted food intake on colonic secretion.

Four groups of rats were used: fed controls (*n* 6); 72-h starved rats (*n* 8); acutely undernourished (AU) rats given 33% of their normal dietary intake per day for 9 d (*n* 8); and chronically undernourished (CU) rats given 50% of their normal food intake for 21 d (*n* 8). Electrogenic ion movement across sheets of proximal mid-colon and distal colon was assessed as the short-circuit current (Isc) using standard techniques. Both basal and maximal changes in Isc above basal levels ($\triangle$Isc) in response to Bethanecol (1 mM), a muscarinic cholinergic agonist, were measured. The results were compared with fed controls using non-parametric ANOVA (Kruskal–Wallis) followed by Conover's multiple comparison *t* test.

In the proximal mid-colon the basal Isc was elevated only in the 72- h starved group (+23%, $P<0{\cdot}05$). Bethanecol stimulation induced greater secretion in all undernourished groups but to the greatest extent in the CU group (72 h: +47%, $P<0{\cdot}01$; AU: +33%, $P<0{\cdot}02$; CU: +71%, $P<0{\cdot}001$). The distal colon responded quite differently, the basal Isc was elevated in all undernourished groups but to the greatest extent in the AU (72 h: +58%, $P<0{\cdot}02$; AU: +174%, $P<0{\cdot}001$; CU: +90%, $P<0{\cdot}01$). The responses to Bethanecol followed an identical pattern (72 h: +42%, $P<0{\cdot}05$; AU: +97%, $P<0{\cdot}01$; CU: +38%, $P<0{\cdot}05$). The Bethanecol-induced currents were smaller in the distal colon compared with the proximal mid-colon in all four groups.

Thus hypersecretion can be elicited in both AU and CU groups following secretory stimulation in both areas of the colon, but AU has a greater effect in the distal colon, and CU in the proximal mid-colon.

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Levin, R. J., Nzegwu, H. C. & Young, A. (1987). *Journal of Physiology* **396**, 33p.
Pereira, M. M. C., Young, A., Warren, M. A. & Levin, R. J. (1988). *Gut* (In the Press).
Young, A., Nzegwu, H. C. & Levin, R. J. (1988). *Proceedings of the Nutrition Society* **47**, 127A.

Thermogenic effects of corticotrophin-releasing factor in genetically obese Zucker rats. By J. A. CARNIE[1], R. A. LEFEUVRE[2], E. A. LINTON[3], H. D. MCCARTHY[1] and N. J. ROTHWELL[2], [1]*Department of Biochemistry and Applied Molecular Biology, UMIST, Manchester,* [2]*Department of Physiological Sciences, University of Manchester, Manchester M13 9PT* and [3]*Department of Physiology and Biochemistry, University of Reading, Reading RG6 2AT*

Genetically obese Zucker rats exhibit reduced thermogenesis and impairment in the sympathetic activation of brown adipose tissue (BAT) which are fundamental to the development of obesity (Trayhurn, 1986). Obesity is inhibited and thermogenesis restored to normal by surgical adrenalectomy in obese Zucker rats (Marchington *et al.* 1983). These responses may be related to altered concentrations or actions of corticotrophin releasing-factor (CRF) which has potent thermogenic effects in normal rats (LeFeuvre *et al.* 1987).

Intracerebroventricular (icv) injections of rat CRF-41 (2–4 nmol) elicited rapid (peak response at 11·0 (SEM 1·5) min) increases (1·03 (SEM 0·13)°, *n* 12) in the temperature of the interscapular BAT depot of anaesthetized lean rats. Rectal temperatures showed smaller and delayed rises. CRF elicited small increases (0·5°) in two obese rats, but had no effect in a further ten obese animals (average response 0·09 (SEM 0·06)°, *n* 12; significantly different from lean rats, $P<0·001$).

In conscious lean Zucker or Sprague-Dawley rats, CRF (2 nmol icv) produced significant increases (25 (SEM 3) %) in oxygen consumption (V_{O_2}) and body temperature (1·1 (SEM 0·2)°) which was sustained for up to 2 h. Similar increases in V_{O_2} (26 (SEM 6)%) were observed in obese rats, but body temperature was only slightly (0·2 (SEM 0·3)°) increased. BAT activity, assessed from in vitro mitochondrial GDP binding, 90 min after injection of CRF was increased to a similar extent in lean (saline (9 g sodium chloride/l) 62 (SEM 5), CRF 106 (SEM 6) pmol/mg protein) and obese (saline 60 (SEM 6), CRF 90 (SEM 8) pmol/mg protein; *n* 6, 7) rats.

Concentrations of CRF in homogenates of combined hypothalamus and median eminence were estimated by radioimmunometric assay, and were almost identical in lean (3960 (SEM 100) pg) and obese (3920 (SEM 125) pg; *n* 5) animals.

These results reveal that obese rats can activate thermogenesis in response to central administration of CRF but show only modest changes in temperature. The possibility remains that CRF release in response to physiological stimuli may be altered in these mutants.

LeFeuvre, R. A., Rothwell, N. J. & Stock, M. J. (1987). *Neuropharmacology* **26**, 1217–1221.

Marchington, D., Rothwell, N. J., Stock, M. J. & York, D. A. (1983). *Journal of Nutrition* **113**, 1395–1402.

Trayhurn, P. (1986). In *Brown Adipose Tissue*, pp. 299–330 [P. Trayhurn and D. G. Nicholls, editors]. London: Edward Arnold.

Acute thermogenic responses to intramuscular typhoid vaccination in man. By A. COOPER[1], M. A. HORAN[2], R. A. LITTLE[3], N. J. ROTHWELL[1] and P. J. L. M. STRIJBOS[1,2], [1]*Department of Physiological Sciences, University of Manchester M13 9PT,* [2]*Department of Geriatric Medicine and* [3]*North Western Injury Research Centre, Hope Hospital, Salford M6 8HD*

Injection of typhoid vaccine in normal, healthy subjects induces acute fever. Ximenes *et al.* (1987) reported increases in metabolic rate within 6 h in about one-quarter of their subjects injected subcutaneously with typhoid vaccine. However, Ash *et al.* (1988) failed to observe any change in metabolic rate within this period in any of sixteen subjects injected subcutaneously with vaccine, and body temperature increased only after 12–16 h. We have now tested the effects of intramuscular administration of the same vaccine.

Male and female subjects (21–34 years of age), with no previous experience of typhoid vaccination, were fasted for at least 12 h and, after baseline measurements, were injected with either saline (9 g sodium chloride/l) or monovalent typhoid vaccine (Wellcome Laboratory, Beckenham, Kent; 0·5 ml) intramuscularly. White blood cell count increased in vaccinated subjects after about 3 h and reached a peak value (11·0 (SEM 1·0) $\times 10^6$ cells/ml) after 5 h, which was significantly ($P<0·01$, n 8) greater than after saline injection (7·6 (SEM 0·8) $\times 10^6$ cells/ml). Heart rate increased slightly in vaccinated subjects (saline 60 (SEM 4), vaccine 70 (SEM 4) beats/min), but arterial blood pressure remained constant. Oral temperature rose slightly during the 6 h post-injection period in both groups, but was significantly elevated in vaccinated subjects after 12 h, and reached a peak value after 16 h (saline 36·8 (SEM 0·1)°, vaccine 37·7 (SEM 0·3)°, $P<0·01$). The increase in oral temperature (1·3°) was greater than we have previously observed after subcutaneous injection (0·8°).

Respiratory quotient did not vary significantly after injection of either saline or vaccine, but oxygen consumption (V_{O_2}), carbon dioxide production and metabolic rate all increased significantly in vaccinated subjects. After 5 h, V_{O_2} was 15% greater than the pre-treatment value, and average post-injection values were 11% greater than those of saline-injected controls.

These results demonstrate that intramuscular injection of typhoid vaccine causes reproducible increases in metabolic rate in all subjects. We would therefore suggest that this protocol is suitable for future studies on the mechanisms and factors affecting fever in man, but subcutaneous administration of typhoid would be preferable to intramuscular injection for routine vaccination.

Ash, S., Griffin, G., Horan, M. A., Little, R. A., Matthews, S. L. B. & Rothwell, N. J. (1988). *Proceedings of the Nutrition Society* **47**, 139A.

Ximenes, R., Cox, M., Tomkins, A. M. & Collins, K. (1987). *Proceedings of the Nutrition Society* **46**, 16A.

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Nutritive value of oat grain: the importance of hull lignin. By J. B. ROWE and G. B. CROSBIE, *Department of Agriculture, Baron-Hay Court, South Perth, Western Australia 6151*

Whole oat (*Avena sativa* L.) grain is used throughout the world predominantly as a feed for sheep and horses. Variability in its nutritive value for ruminants is well-recognized and has, traditionally, been attributed to variation in protein, fibre content and bulk density (e.g. Margan *et al.* 1987). Recently, Crosbie *et al.* (1985) reported considerable variation in the lignin content of hulls of Western Australian oat cultivars and showed a negative correlation between in vitro digestibility and lignin content. Preliminary screening of samples from an extensive world-wide oat collection has indicated that approximately half the entries are of the high-lignin type. Differences in lignin content of the hull ranged from 9 to 61 g/kg and represented differences of 8–23 g/kg when considering the whole grain. The experiment reported here was designed to determine whether this apparently small difference could have a biologically meaningful effect on the digestibility of the whole grain.

Two oat grain diets were prepared by compositing 100 samples (approximately 2 kg each) of each of two cultivars (Murray and Mortlock), known to differ substantially in lignin content. Each sample was first cleaned to remove free hulls, free groats and all foreign material. The two diets were composited to make them almost identical with respect to hull:groat ratio (27·4:72·6), and crude fat (56 g/kg) and nitrogen content (21·6 g/kg). Urea (7·5 g/kg) and calcium carbonate (10 g/kg) were added to both diets. Twelve Merino wethers of similar live weight (40·5 (SE 1·2) kg) were housed in metabolism cages and fed on a ration of 750 g/d. Six animals received each diet for a period of 16 d, with a complete collection of faeces being made over the final 7 d. Samples of feed and faeces were analysed for N, ash, crude fat, neutral-detergent fibre (NDF), acid-detergent fibre (ADF), acid-detergent lignin (ADL) and gross energy. Two animals with variable feed intake during the collection period were excluded from the analyses. The results are summarized in the Table.

	Murray (*n* 4)	Mortlock (*n* 6)	SED	Significance of difference
Dry matter (DM) intake (g/d)	666	663	4·4	NS
Apparent digestion:				
Organic matter	0·83	0·72	0·018	$P<0·001$
NDF	0·63	0·31	0·050	$P<0·01$
ADF	0·57	0·28	0·054	$P<0·001$
N	0·80	0·78	0·015	NS
Fat	0·96	0·95	0·007	NS
Digestible energy (MJ/kg DM)	15·6	14·0	0·37	$P<0·01$

NS, not significant.

A decrease in lignin content of 15 g/kg (8 *v.* 23) increased organic matter digestibility by approximately 15% at the level of intake used in this experiment. This finding is particularly significant since differences between cultivars in hull lignin content have been shown (Crosbie *et al.* 1985) and considerable scope exists to select for the low-lignin character in oat breeding.

Crosbie, G. B., Tarr, A. W., Portmann, P. A. & Rowe, J. B. (1985). *Crop Science* **25**, 678–680.
Margan, D. E., Graham, N. McC. & Searle, T. W. (1987). *Australian Journal of Experimental Agriculture* **27**, 223–230.

Effect of diet and level of intake on the activities of acetyl-CoA synthetase and acetyl-CoA hydrolase in ovine adipose tissue. By N. D. SCOLLAN, J. R. BRISBANE and N. S. JESSOP, *Department of Agriculture, University of Edinburgh, Kings Buildings, West Mains Road, Edinburgh EH9 3JG*

Many tissues contain both acetyl-CoA synthetase (acetyl-CoA ligase; *EC* 6.2.1.1) and acetyl-CoA hydrolase (*EC* 3.1.2.1) activities. When both enzymes are present the potential exists for a substrate cycle between acetate and acetyl-CoA with the concomitant hydrolysis of ATP. This cycle has been demonstrated in rat hepatocytes where the rate of cycling was positively correlated with acetate concentration (Jessop *et al.* 1986). In ruminants the efficiency of utilization of metabolizable energy (ME) varies between diets. As it has been proposed that this is a consequence of inefficient utilization of acetate (e.g. MacRae & Lobley, 1982), the activities of the two enzymes were investigated in adipose tissue of sheep offered diets likely to result in different supplies of acetate as a nutrient.

Samples of perirenal adipose tissue were removed at slaughter from Blackface sheep (age approximately 30 weeks) and assayed for acetyl-CoA synthetase (Knowles *et al.* 1974) and acetyl-CoA hydrolase (Soling & Rescher, 1985). Animals had been receiving a fermentable, fibrous diet based on plain sugarbeet pulp (S) or a starchy diet based on barley (B), both of equivalent ME concentration, at one of three levels of intake, low, medium or high, calculated to achieve growth rates of approximately 30, 100 or 200 g/d respectively. Animals were given their respective diets for a period of 18 weeks.

		Level of feeding						Main effects	
	Diet	Low	*n*	Medium	*n*	High	*n*	Diet type	Level of feeding
Acetyl-CoA hydro-	S	16·0	3	16·2	4	6·7	4		
lase (nmol/min	B	13·2	3	4·4	3	6·3	5	**	*
per mg protein)	SE	2·2		3·2		0·9			
Acetyl-CoA syn-	S	12·9	3	13·5	4	7·3	4		
thetase (nmol/min	B	12·7	3	9·9	3	5·5	5	**	**
per mg protein)	SE	1·1		1·7		0·9			

*$P<0·05$, **$P<0·01$

The results show that the activities of both enzymes, and hence the activity of the substrate cycle, were greater for S than for B ($P<0·01$) and declined with level of feeding ($P<0·05$).

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Jessop, N. S., Smith, G. H. & Crabtree, B. (1986). *Biochemical Society Transactions* **14**, 146–147.
Knowles, S. E., Jarrett, I. G., Filsell, O. H. & Ballard, F. J. (1974). *Biochemical Journal* **142**, 401–411.
MacRae, J. C. & Lobley, G. E. (1982). *Livestock Production Science* **9**, 447–456.
Soling, H. D. & Rescher, C. (1985). *European Journal of Biochemistry* **147**, 111–117.

Substitution of barley and sugar-beet pulp for hay in young and adult wether sheep. By H. EAYRES, D. ANDERSON and J. D. OLDHAM, *Edinburgh School of Agriculture, West Mains Road, Edinburgh EH9 3JG*

While the potential performance of an animal determines its nutrient requirements, physical attributes of the diet may constrain food intake within that needed to achieve 'potential'. It has been suggested that relative energy deficit (Weston, 1982) might influence the retention of digesta in the rumen so it can be supposed that physical constraints to intake may be influenced by the physiological state of the animal. The amount of forage consumed by ruminants is influenced both by the amount and nature of the supplements offered. We wished to investigate the possibility that the influence of dietary supplements on forage intake ('substitution') might differ between sheep at different stages of maturity (age).

Fifteen young (Y) and fifteen 18-month-old (O) Suffolk × Greyface wethers (initial weights 21·6 (SE 2·17) kg and 46·5 (SE 2·42) kg respectively) were offered 150 g/d of a high-protein supplement (6·94 (SE 0·70) and 3·23 (SE 0·17) g/kg live weight (LW) for Y and O respectively) and *ad lib.* hay. Six sheep from each group were given either no further supplement or a medium level (9·32 (SE 1·78) g/kg LW) of a mixed supplement of barley (B) and sugar-beet pulp (S) (B/S) in a changeover design. The remaining eighteen sheep were arranged by age in two sets of three Latin squares with three 4-week periods. The treatments were three levels, low (L), medium (M) and high (H) (across squares) of three supplements (within squares) containing a high proportion of B, S or equal proportions of each (B/S).

Across all sheep, dry matter (DM) intake was directly proportional to LW and there was no difference between Y and O wethers in DM intake/kg LW. Hay DM intake fell as supplement allowance increased, such that DM intake was maintained (26·99, 27·23 and 27·33 g/kg LW for L, M and H respectively), i.e. the rate of substitution was approximately 1·0 on a DM basis. There were no effects of supplement composition on total DM intake (26·90, 27·29 and 27·37 g/kg LW for B, B/S and S respectively).

We conclude that, under these conditions, only the amount and not the nature of the supplemental DM affected forage intake and that differences in age/physiological state had no effect on substitution phenomena.

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Responses of dairy cows to variation in plane of nutrition and to source of metabolizable energy. By N. FRIGGENS[1], G. C. EMMANS[1], S. ROBERTSON[2], D. G. CHAMBERLAIN[2] and J. D. OLDHAM[1], [1]*Edinburgh School of Agriculture, West Mains Road, Edinburgh EH9 3JG* and [2]*Hannah Research Institute, Kirkhill Road, Ayr KA6 5HL*

An understanding of the response of dairy cows to variation in plane of nutrition is of central importance to any predictive system of dairy cow rationing. It is an area that has been defined as needing further examination (Broster & Thomas, 1981). An experiment was designed to measure responses and to test whether the source of energy-yielding nutrients used would affect the response.

Lactating Friesian cows (*n* 18, average 17 weeks post-partum) were used in a Latin-square design experiment with three periods of 4 weeks each. The concentrates contained cereals (C) or unmolassed sugar-beet pulp (S) (750 g/kg) or an equal mixture of the two (C/S). Rations consisted of hay and concentrates in the ratio 40:60 (dry matter (DM) basis) and each contained 147 g crude protein and 10·8 MJ metabolizable energy (ME)/kg DM. Each cow was offered three levels of feeding, low (L), medium (M) and high (H), within a Latin-square design and concentrate type was compared between squares. Level H was chosen to be sufficient to maintain current body state and predicted final milk yield. Levels M and L were 1·5 and 3 kg DM lower than H respectively.

Effect of feeding level on yields of milk and milk components

(Mean values for the last 3 weeks of each period, two missing values)

	Feeding level			
Yield/d	L	M	H	SED
Milk (kg)	13·1	14·7	16·4	0·21
Fat (g)	539·4	594·9	643·0	12·5
Protein (g)	441·0	502·4	560·4	7·8
Lactose (g)	575·3	662·5	746·0	9·9
Energy (MJ)	40·89	45·95	50·59	0·7

All effects highly significant ($P<0·001$).

The source of dietary energy did not affect performance or response; mean milk yields were 14·4, 14·6 and 14·3 (SED 1·4) kg/d for concentrates S, C/S and C respectively. Milk yield did not stabilize within each period, but after the first week of each period, the slope of declining milk yield did.

It is concluded that the source of ME had no significant effect on lactational response. In contrast, the level of feeding had a significant and linear effect on yield of all milk constituents and of milk energy.

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