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Effect of dietary protein concentration and ambient temperature on the energy, protein and water metabolism of the rat

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1. Groups of rats (*n* 8) were offered, to appetite, diets containing 10 (LP), 25 (MP) and 45 (HP) % of gross energy as protein energy from 45 d of age to slaughter 50 d later. At 53 d of age, half the rats on each diet were left at 22° while the other half were transferred to 6°. Water balances were measured daily, and digestibilities of energy and nitrogen and the metabolizability of the diets were measured for each rat over a 7 d period at some stage between the age of 74 and 95 d. The rats were slaughtered at day 95 and their carcasses were analysed for protein, lipid, energy and ash contents. Energy expenditure was calculated as the difference between metabolizable energy intake and energy retained.

2. Growth performance was best on the MP diet at both temperatures. At 22° the rate of gain of body-weight and of energy retention, although not of protein, were slightly reduced on the HP diet while overall performance was markedly inferior on the LP diet. At 6° the LP diet, while not so good as the MP diet, led to significantly better all-round growth performance than did the HP diet. Cold increased the energy expenditure of the rats by 50% (109–138 kJ/d); the increase was greater for the LP group than for the HP group.

3. Intrascapular brown adipose tissue hypertrophied in response to cold and to the LP diet.

4. Adrenal gland size was significantly increased by cold exposure and by increasing level of dietary protein concentration.

5. Urine volume was more closely related to the intake of protein than to that of energy. Urinary N concentration for the HP rats was approximately double that for those on the LP diet. Cold-exposed rats had a high water content in their fat-free carcasses, but there were no differences between the dietary treatments.

The interrelations between energy expenditure, food intake, dietary protein concentration and water intake are of considerable practical importance in the husbandry of small mammals. For example, mink (*Mustela vison*) fed on a high-protein diet show reduced energy retention at low environmental temperatures (Chwalibog *et al.* 1980). This problem may be exacerbated if the animals also face a shortage of drinking-water due to freezing.

It is well established that rats given diets low in protein eat less and grow more slowly, depositing less protein, than rats receiving an optimal protein intake (Radcliffe & Webster, 1976, 1978, 1979), possibly because they are neither able to store the excess dietary energy as body fat nor to dissipate it as heat (Meyer, 1958). The position with regard to supra-optimal protein intake is perhaps less-clearly defined, but there are suggestions that growth performance is impaired (Radcliffe & Webster, 1976, 1978, 1979).

The interactions of diet and ambient temperature make for hazardous prediction of an animal's water economy: low temperature leads to a decreased requirement for water for evaporative cooling (Degen & Young, 1981), but increased food, and particularly protein, intake increases the amount of water needed for urinary excretion of waste products (Bass, 1982). Hovell *et al.* (1977) reported that, in sows, urine output was unaffected by feeding level but was reduced in the cold, while Fregly (1968) found that rats exposed to 6° increased both their food intake and urine output on a constant water intake.

The present paper describes preliminary studies to examine some of the above interrelations in the laboratory rat.

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EXPERIMENTAL

Animals and diets

Male, hooded, Rowett Lister strain rats from a stock colony bred in specific-pathogen-free conditions, were fed on a commercial pelleted diet (Oxoid; Herbert C. Styles (Bewdley) Ltd, Bewdley, Worcs) from weaning at 19 d until 45 d of age (body-weight 155–175 g). They were then transferred to minimal disease conditions and caged individually. During the experiment they were given access, *ad lib.*, to one of three semi-synthetic diets containing 10, 25 and 45% of gross energy (GE) as protein energy. The three diets are subsequently referred to as low-protein (LP), medium-protein (MP) and high-protein (HP). The composition of the diets is given in Table 1. The diets are similar to those used by Radcliffe & Webster (1976) except that they contained more fat, because high-fat diets have been shown to increase resistance to cold in rats (Page & Babineau, 1953). Glycerol was added to the mixture to improve its consistency. Drinking-water (acidified to pH 2·5 with hydrochloric acid to prevent growth of pathogens) was always freely available.

Experimental design

Three randomly-selected groups of twelve rats each were weaned from the Oxoid to the experimental diets over a period of 7 d at 22° starting at 45 d of age. After this adaptation period, four rats from each diet-group were killed to obtain initial carcass composition. The remaining rats from each group were divided equally: four were left at 22° and the other four were transferred to an ambient temperature of 6°. A 12 h (08.00–20.00 hours) light–dark cycle was operated for all rats. The experimental period lasted for a further 43 d. Food intake and spillage and water intake were recorded daily.

Balance trials

Collections of urine and faeces were made in individual plastic metabolism cages (North Kent Plastic Cages Ltd, Dartford, Kent). Urine was collected in a 50 ml flask containing 2 ml 2 M-sulphuric acid as preservative. Daily collections were stored at −20°. Two groups of four rats on the same diet, but kept at different temperatures, were on balance at the same time. Thus rats fed on the three different diets participated in the balance trials on separate weeks, the times of which are indicated in Fig. 2 (see p. 368). Daily collections of urine from each rat were pooled for analysis. Due to the small quantity of faeces produced by individual rats over the week, daily collections of faeces from the four rats in each group were pooled.

Methods of analysis

The rats were killed by carbon dioxide inhalation. The contents of the digestive tract were removed by flushing with water, and empty-body-weight and the mass of the intrascapsular brown adipose tissue and adrenal glands were recorded. Carcasses were stored at −20° for analysis. They were chopped, freeze-dried and then ground in a Moulinette grinder (Moulinex, France). Faecal analysis was done on fresh material, while urine was dried under vacuum (0·05 bar) at 35°. Analyses were performed in duplicate or triplicate. GE content of all samples was determined by adiabatic bomb calorimetry, nitrogen content by macro-Kjeldahl method (Davidson *et al.* 1970) and lipid content by the method of Atkinson *et al.* (1972). Ash content of samples was measured after ignition at 550°.

Two-way analysis of variance was computed to evaluate the statistical significance of treatment effects.

Table 1. *Composition of the low-protein (LP), medium-protein (MP) and high-protein (HP) diets given to rats and their determined gross energy, crude protein (nitrogen × 6·25) and lipid contents*

Ingredients (g/kg)	Diets		
	LP	MP	HP
Casein	95	250	450
Cooking fat*	100	100	100
Maize oil	110	110	110
Glycerol	150	150	150
Sucrose	455	300	100
Mineral mixture*	38	38	38
Vitamin mixture*	50	50	50
Trance element mixture*	2	2	2
Gross energy (MJ/kg)	21·5	22·4	22·9
Crude protein (g/kg)	93·6	239	451
Lipid (g/kg)	208	206	217
Percentage of GE from:			
Protein	11	26	46
Lipid	39	38	36
Carbohydrate	52	39	21

* Radcliffe & Webster (1976).

RESULTS

Table 2 gives the body-weight and carcass composition values for each group of rats at slaughter, and Table 3 shows the mean daily gains over the 43 d period when the rats were exposed to either 22° or 6°.

The rats on the MP diet grew best and retained most energy at both temperatures. At 22° there was no difference in protein retention between the groups on the two higher levels of protein, while at 6° protein retention was at its highest for the MP group. In general, at 22° the performance of the LP group of rats was inferior, while at 6° it was similar to that of the HP group which was poorest. Energy expenditure, calculated as 'ME intake minus retained energy' was approximately 50% higher at 6° than at 22°; the extra energy expenditure occasioned by the cold ranged from 109 to 138 kJ/d, with no effect of dietary treatment. The increase in the ME intake in the cold was less than the increase in energy expenditure.

The digestibility trials carried out in the periods indicated in Fig. 2 (see p. 368) showed that losses of energy in both faeces and urine differed with diet and with ambient temperature, but that the differences mainly resulted from differences in food intake. The digestibility of the diets tended to rise, and the metabolizability to decrease with increasing level of dietary protein, and both metabolizability and digestibility were reduced at the lower ambient temperature. However, these differences were small: the overall range of metabolizability was 0·87–0·92 and that of digestibility 0·92–0·97.

Water intake and urinary volume increased as dietary protein increased and ambient temperature decreased (Table 4). The volume of urine excreted was more closely related to N than to energy intake. Faecal water losses were small under all conditions, but there was a pronounced reduction in body evaporative water loss at the lower temperature.

The cold-exposed rats were leaner (Table 2) and had a higher body-water content than those kept at 22°: this was in accord with the findings of Blaxter (1962) that body-water

Table 2. *Body composition at day 95 of rats offered low-protein (LP), medium-protein (MP) and high-protein (HP) diets† and kept either at 22° or 6° from 52 to 95 d of age*

(Mean values for four animals per treatment)

	Temperature (°)							Statistical significance of differences		
	22			6			SE of			
Diet...	LP	MP	HP	LP	MP	HP	difference	D	T	D×T
Body-wt (g)	304	409	379	294	349	288	14·5	***	***	**
Protein (g)	67·6	90·4	90·3	66·7	77·5	66·3	3·16	***	***	***
Lipid (g)	50·4	73·6	58·5	38·6	46·0	28·3	4·77	***	***	*
Energy (MJ)	3·52	5·05	4·34	2·94	3·44	2·57	0·19	***	***	***
Ash (g)	9·49	12·2	11·9	9·19	9·89	9·38	0·46	**	**	**
IBAT/EBW (mg/g)	2·11	1·22	1·04	3·54	3·51	2·33	0·31	***	***	NS
Adrenal glands (mg)	65·3	78·7	87·3	82·0	88·3	93·7	5·62	**	**	NS

D, diet; T, temperature; IBAT, intrascapular brown adipose tissue; EBW, empty-body-weight.
* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.
† For details, see p. 364 and Table 1.

Table 3. *Daily intake and retention values for rats offered low-protein (LP), medium-protein (MP) and high-protein (HP) diets† and kept at either 22° or 6° from 52 to 95 d of age*

(Mean values for four animals per treatment)

	Temperature (°)							Statistical significance of differences		
	22			6			SE of			
Diet...	LP	MP	HP	LP	MP	HP	difference	D	T	D×T
ME intake (kJ/d)	293	324	313	390	427	385	10·7	***	***	NS
Energy retained (kJ/d)	50·1	75·8	62·1	37·7	40·2	21·1	12·9	***	***	***
Heat produced‡ (kJ/d)	243	248	251	352	386	364	12·9	NS	***	NS
Nitrogen intake (mg/d)	220	610	1109	308	808	1390	27·3	***	***	***
N retained (mg/d)	98·6	175·7	177·3	100·6	134·7	88·8	10·6	***	***	***
Fat retained (mg/d)	915	1225	938	648	604	238	12·9	***	***	***
Body-wt gain (g/d)	2·93	4·84	4·32	2·85	3·66	2·21	0·29	***	***	***

D, diet; T, temperature; ME, metabolizable energy; NS, not significant.
*** $P < 0·001$.
† For details, see p. 364 and Table 1.
‡ ME intake − energy retained.

concentration was inversely related to fat content. The difference in body-water content was still apparent when its calculation was based on the fat-free body-weight.

Brown adipose tissue mass, expressed in relation to body-weight, was greater in the cold-exposed animals and decreased with increasing concentration of dietary protein.

The mass of the adrenal glands was greater for the rats reared in the cold, and it also increased with increasing protein concentration in the diet: both temperature- and diet-treatment effects were significant ($P < 0·001$).

The responses of the rats to the experimental regimens showed distinct temporal variation. Figs. 1 and 2 show the cumulative weight gains and the food intakes of the rats during the

Table 4. *Water metabolism of rats offered low-protein (LP), medium-protein (MP) and high-protein (HP) diets† and kept at 22° or 6°*

(Mean values for four animals per treatment)

	Temperature (°)							Statistical significance of differences		
	22			6			SE of			
Diet...	LP	MP	HP	LP	MP	HP	difference	D	T	D × T
Water intake (ml/d)	9·05	16·0	19·9	11·7	16·3	23·7	1·14	***	**	NS
Water loss (ml/d)										
Faeces‡	0·46	0·69	0·39	0·76	1·04	0·72	—	—	—	—
Urine	3·76	8·28	12·4	7·69	11·5	20·3	0·73	***	***	***
Evaporation§	4·83	7·03	7·11	2·97	3·76	2·68	1·08	NS	***	NS
Urine GE:nitrogen (kJ/g)	43·8	31·4	27·9	44·1	29·5	28·7	1·31	***	NS	NS
GE intake:urine volume (kJ/ml)	82·3	36·5	22·3	43·1	36·2	17·6	7·00	***	***	**
N intake:urine volume (mg/ml)	56·8	62·4	70·4	31·3	61·8	55·7	5·69	**	**	*
Urine N concentration (mg/ml)	27·1	41·5	53·5	20·6	41·7	44·5	3·77	***	*	NS
Percentage water in:										
Empty body	58·4	57·3	58·6	62·8	61·5	64·6	1·00	*	***	NS
Fat-free empty body	69·6	69·8	69·3	72·3	72·7	72·1	0·72	NS	***	NS

D, diet; T, temperature; GE, gross energy; NS, not significant.
* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.
† For details, see p. 364 and Table 1.
‡ Pooled samples from four rats.
§ Water intake–water loss in excreta.

experimental period. In the first week on experiment the rats on the LP diet ate considerably less than those on the other diets and were only able to maintain their body-weights, while the rats on the MP and HP diets showed appreciable weight gains. The reduction in intake had virtually disappeared by the second week, and thereafter the variability in the intakes of all the rats kept at 22° was reduced although the intake of the rats on the LP diet remained slightly lower throughout. At 6° the rats increased their intakes over the first 5 weeks but thereafter intake gradually declined, although never to the levels of the animals kept at the higher temperature. During the periods when the rats were kept in the metabolism cages their food intakes and weight gains were reduced by 20–25%.

DISCUSSION

Effect of diet and temperature on growth performance

The present results for the rats kept at 22° are in broad agreement with those of Radcliffe & Webster (1976, 1978), in so far that growth performance (either as body-weight gain or as protein accretion) was impaired on the LP diet, whereas body-weight gain, but not protein accretion, was slightly reduced on the HP diet as compared with the MP diet. At 6° the picture was altered; the MP diet was still superior in promoting both weight and protein gain but at the lower temperature the LP diet was clearly superior to the HP diet. These results suggest that when energy expenditure is low there is little handicap to an animal fed on a diet containing a large proportion of protein, but there is a penalty associated with a diet in which the protein:energy value is low. On the other hand, when energy expenditure

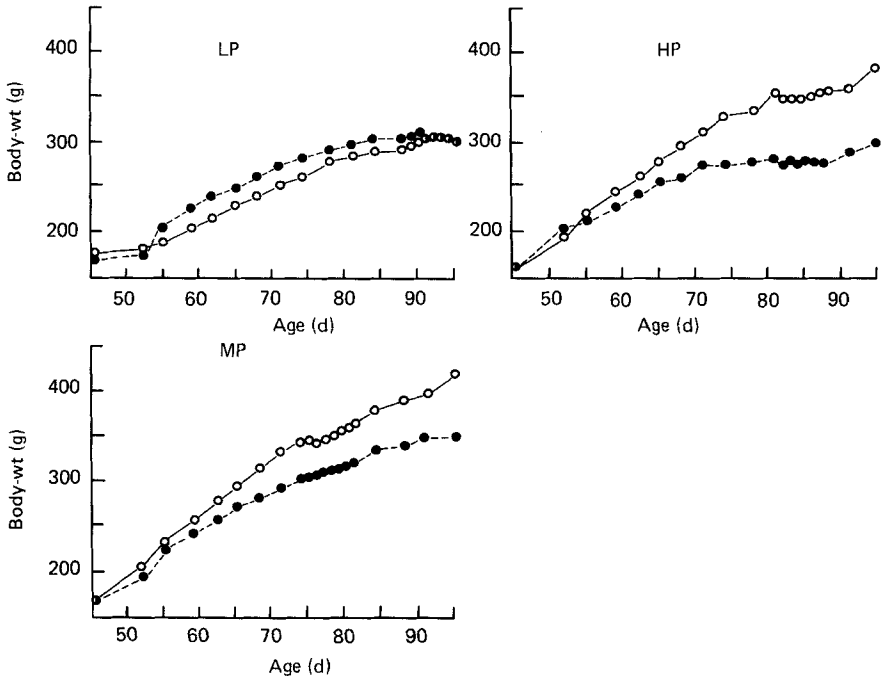

Fig. 1. Cumultative growth of rats offered to appetite diets containing 10 (LP), 25 (MP) or 45 (HP) % of gross energy as protein-energy from 45 to 95 d of age. At the age of 52 d half the rats in each diet-group were left at their previous temperature of 22° (○) while the other half were transferred to 6° (●). Balance periods of 7 d duration in the metabolism cages are marked by daily records of growth for each group of rats. Points represent mean values for four rats.

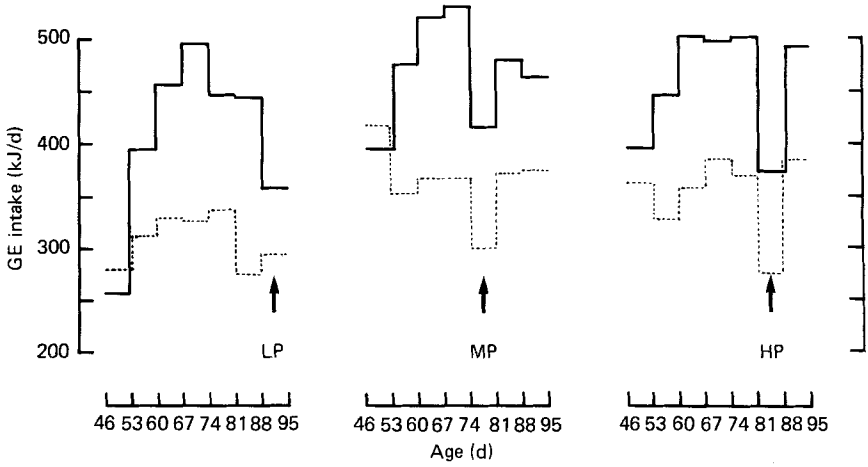

Fig. 2. Mean weekly intakes of gross energy (GE; kJ/d) for rats offered to appetite diets containing 10 (LP), 25 (MP) or 45 (HP) % of the GE as protein-energy and kept at 22° (——) or 6° (– – –). ↑, Balance periods in metabolism cages. Values are means for four rats.

is increased (e.g. in a cold environment) high values for dietary protein:energy may be disadvantageous and low values may become more favourable. These contradictions may be resolved by considering the performance of each group of rats in relation to those raised on the MP diet at 22°, i.e. assuming that these animals were in a near-ideal situation for the expression of their genetic potential for growth.

LP diet, 22°. Growth in these rats appeared to be limited by poor N retention, as a consequence of their restricted food intake. Their limited intake did not appear to be associated with any inability to dissipate additional dietary energy as heat, or to store additional energy as fat; the rats on the HP diet at 22° had a slightly higher heat production and an appreciably higher rate of fat deposition. It may be that the LP diet was less palatable than the others, although in face of the additional demands of the low temperature environment the other group of animals on the LP diet were able to increase their intake considerably, to produce somewhat better retentions of protein and fat.

HP diet, 22°. These rats had similar protein retentions to their counterparts on the MP diet, but a significantly reduced fat retention. It would appear that their growth was restricted by their energy intake. It is unlikely that intake was limited by physical capacity in that the rats kept at 6° ate about 5 g diet/d more than did those kept at 22°. Neither was intake limited by the rats' inability to metabolize non-retained N; the rats at 6° on the HP diet excreted 1300 mg/d, compared with 932 mg/d by the rats at 22°.

LP diet, 6°. Growth in these rats appeared to be limited by poor protein retention brought about by a less than maximum intake. As with the LP diet at the higher temperature, palatability may have been the first limiting step; the rats at 6° on the other diets ate more, so there was no physical limitation on intake. Nor was heat dissipation nor fat storage capacity limiting: the other groups at 6° produced more heat and all the rats kept at 22° deposited more fat.

MP diet, 6°. Growth appeared to be limited by intake in these rats. While they had the largest intake of all the groups they were obviously diverting part of their protein-energy intake to heat production. Limits on food intake could have been physical although intakes in excess of the 21 g/d by these rats have been reported by Rolls *et al.* (1980) and by Barr & McCracken (1984). Limits on intake were not created by any indices of N metabolism nor by fat retention.

HP diet, 6°. Growth in this group was restricted by poor N retention; a large proportion of their N intake must have been used as an energy source for the maintenance of body temperature. Intake was not limited physically, nor was heat production maximal. The capacity for N catabolism may have been limiting with 1300 mg/d difference between intake and retention. These rats had almost the same energy intake with more than four times the protein intake as did their counterparts kept at 6° but receiving the LP diet, yet the latter group's growth performance was superior, both as regards energy and N retentions; this difference can only be explained in terms of gross low efficiency of utilization of dietary protein-energy for thermogenesis.

It is, perhaps, tempting to suggest that the site of the increased heat production in the cold-exposed rats may lie in the intrascapular brown adipose tissue. There is no doubt that the rats maintained at 6° did have more of this type of tissue and a higher heat production than did the comparable rats kept at 22°. There were also significant dietary effects on the amount of brown adipose tissue, which was inversely related to dietary protein concentration at 22°, and considerably reduced in the HP group at 6°; Rothwell *et al.* (1983) reported that a low-protein, high-carbohydrate diet elicits a direct thermal response from brown adipose tissue. However, there were no significant dietary effects on heat production, and no evidence of any correlation between heat production and amount of brown adipose tissue at either temperature. Furthermore, Brockway & Lobley (1981) were unable to demonstrate

any thermogenic response to the injection of noradrenaline (an indicator of brown adipose tissue function) in rats of the same strain, and from the same stock colony, as those used in the present experiments. Nevertheless, the hypertrophy of the brown adipose tissue, in the face of reduced deposition of white adipose tissue, clearly indicates some metabolic significance.

Hypertrophy of the adrenal glands of rats kept in a cold environment has been reported by other workers (Heroux & Gridgeman, 1958). These authors have also shown that, in rats, the size of the adrenals is unrelated to body size and composition, so that the differences in the weights of the adrenals are probably a true reflection of the 'stress' of the cold environment. Increasing weight of adrenal glands was also associated with increasing levels of dietary protein concentration. With the exception of a weight-for-weight substitution of casein for sucrose, the diets consumed by the rats in the present study were identical although, of course, the quantity of diet consumed varied between groups. However, at either ambient temperature the range of weight of diet consumed was small (about 2 g/d) and, in any event, was maximal on the MP diets. It seems unlikely that any food item, apart from casein, could have been responsible for the adrenal hypertrophy, nor does it seem probable that the amount of chloride in the drinking-water could have imposed significant stress on the excretory system. The amounts of chloride consumed in the drinking-water were 1000 times less than those consumed in the diets. The effect of protein concentration on the adrenals has not been widely recognized, although Henriques *et al.* (1949) did report on the influence of dietary protein concentration on the corticotrophic action of an extract of anterior pituitary gland, with subsequent enlargement of the adrenals in rats fed on diets of up to 30% casein; this corticotrophic activity was absent when the rats received an LP diet. More recently, Slag *et al.* (1981) described an increased level of circulating cortisol in humans eating high-protein diets.

During the first week of the changeover from Oxoid to experimental diets, and while all the rats were still kept at 22°, the food intake of the rats on the LP diet was less than that of the other animals. This effect can probably be ascribed to a lower palatability of that diet; it was transitory, and of little overall significance in the energy-balance results.

Balance periods in metabolism cages produced distinct changes in the growth rate and food and water intakes of the rats. They appeared to lose appetite and accordingly their rate of weight gain was reduced. The stress of the change to the new environment seems inadequate as the only explanation because food intake was reduced for the whole 7-d period in the metabolism cages. A warmer microclimate in the cage may offer a further explanation. When cold-acclimated rats were returned to their previous warm environment they ate even less than they did before cold exposure (Fregly, 1968). The plastic metabolism cages used in the present study may have formed an enclosure which would effectively reduce convective heat loss as has been shown for the mink nest box (Harri & Korhanen, 1984) which has walls and a floor but no closed roof. Although exact measurements of the thermal protection provided by the metabolism cages are lacking in the present study, it is reasonable to assume that this type of protection is of value. Any environmental benefit of the cages for rats kept at 22° is unlikely to have been pronounced, but may not have been totally absent. It is perhaps noteworthy that the drop in ME intake while the rats were in the metabolism cages was less for those kept at 22° than for those at 6°. These results emphasize that energy-balance measurements based on values from a series of periods in metabolism cages may not reflect true values.

The effect of ambient temperature on digestibility was very small in the present experiments (0·95 at 22° and 0·94 at 6°); a similar result has been reported for mink (Chwalibog *et al.* 1980). These results are in contrast with those obtained with sows and ruminants where a reduction in digestibility was found at lower ambient temperatures, even

at a fixed level of food intake (Fuller & Cadenhead, 1969; Westra & Christopherson, 1975; Hovell *et al.* 1977). The absence of any major effect of dietary protein concentration on metabolizability was due in the most part to a decrease in faecal energy loss with increasing protein level which largely cancelled out the increase in urinary loss.

The effect of diet and temperature on water balance

These experiments confirm that HP diets require high volumes of urine for the dilution and excretion of nitrogenous end-products, and that the requirement for water for evaporative cooling is reduced at low temperature. These opposing requirements may well explain the variable results for water intake reported for cold-exposed animals (Fregly, 1968; Hovell *et al.* 1977; Degen & Young, 1981).

In the present study the water intakes of the rats were more closely related to the intakes of protein than to those of energy. However, water intake was not regulated simply to enable the elimination of urine of constant N concentration: despite an approximate two-fold increase in water intake and a three-fold increase in urine volume, the rats on the HP diets excreted urine containing twice as much N per unit volume as did those on the LP diet. Pullar & Webster (1977) found that the ratio, urinary energy:N was almost constant, independent of age or phenotype, in Zucker rats fed on similar diets. The present results show that the urine energy:N value declines with increasing dietary protein concentration but that it is unaffected by cold exposure.

The percentage of body water was higher in the cold-exposed rats than in those kept at 22°, but was unaffected by dietary treatment when considered in terms of the fat-free carcass. A higher percentage of body water has been reported for cold-exposed sheep (Degen & Young, 1981) but not for rats (Page & Babineau, 1953; Nakatsuka *et al.* 1983). In the latter experiments the duration of the cold exposure was either much shorter than that in the present study (Nakatsuka *et al.* 1983) or the values for cold-exposed rats which had gained no weight were compared with those for younger control rats which had been killed earlier (Page & Babineau, 1953). Rats may require at least 2 weeks' exposure to a cold environment to regain an equilibrium in tissue hydration following the initial dehydration which occurs on cold exposure (Fregly, 1968). Moreover, fat-free body water alters with age (Blaxter, 1962). For these reasons, the results of earlier experiments are not directly comparable with those of the present study.

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REFERENCES

Atkinson, T., Fowler, V. R., Garton, G. A. & Lough, A. K. (1972). *Analyst, London* **97**, 562–568.
Barr, H. G. & McCracken, K. J. (1984). *British Journal of Nutrition* **51**, 379–387.
Bass, J. M. (1982). *Animal Production* **35**, 293–294.
Blaxter, K. L. (1962). *The Energy Metabolism of Ruminants*. London: Hutchinson, Scientific and Technical.
Brockway, J. M. & Lobley, G. E. (1981). *Journal of Physiology* **314**, 85–89.
Chwalibog, A., Glem-Hansen, N., Henckel, S. & Thorbek, G. (1980). In *Energy Metabolism. Proceedings 8th Symposium Energy Metabolism, Cambridge, 1978*, pp. 283–286 [L. E. Mount, editor]. London: Butterworths.
Davidson, J., Mathieson, J. & Boyne, A. W. (1970). *Analyst, London* **95**, 181–193.
Degen, A. A. & Young, B. A. (1981). *Journal of Agricultural Science, Cambridge* **96**, 493–496.
Fregly, M. J. (1968). *Canadian Journal of Physiology and Pharmacology* **46**, 873–881.
Fuller, M. F. & Cadenhead, A. (1969). In *Energy Metabolism of Farm Animals, Proceedings 4th Symposium Energy Metabolism, Warsaw, Poland, 1967*, pp. 455–460 [K. L. Blaxter, J. Kielanowski and Grete Thorbek, editors]. Newcastle upon Tyne: Oriel Press Ltd.

Harri, M. & Korhanen, H. (1984). In *Proceedings of 3rd International Scientific Congress on Fur Bearing Animal Production, Versailles, France*, p. 21. Paris: INRA and ITAVI.
Henriques, S. B., Henriques, O. B. Selye, H. (1949). *Endocrinology* **45**, 153–158.
Heroux, O. & Gridgeman, N. T. (1958). *Canadian Journal of Biochemistry and Physiology* **36**, 209–216.
Hovell, F. D. DeB., Gordon, J. G. & MacPherson, R. M. (1977). *Journal of Agricultural Science, Cambridge* **89**, 523–533.
Meyer, J. H. (1958). *American Journal of Physiology* **193**, 488–494.
Nakatsuka, H., Shoji, Y. & Tsuda, T. (1983). *Comparative Biochemistry and Physiology* **74A**, 21–25.
Page, E. & Babineau, L.-M. (1953). *Canadian Journal of Medical Science* **31**, 22–40.
Pullar, J. D. & Webster, A. J. F. (1977). *British Journal of Nutrition* **37**, 355–363.
Radcliffe, J. D. & Webster, A. J. F. (1976). *British Journal of Nutrition* **36**, 457–469.
Radcliffe, J. D. & Webster, A. J. F. (1978). *British Journal of Nutrition* **39**, 483–492.
Radcliffe, J. D. & Webster, A. J. F. (1979). *British Journal of Nutrition* **41**, 111–124.
Rolls, B. J., Rowe, E. A. & Turner, R. C. (1980). *Journal of Physiology* **298**, 415–427.
Rothwell, N. J., Stock, M. J. & Warwick, B. B. (1983). *International Journal of Obesity* **7**, 263–276.
Slag, M. F., Ahmed, M., Gannon, M. C. & Nuttall, F. Q. (1981). *Metabolism* **30**, 1104–1108.
Westra, R. & Christopherson, R. J. (1975). *University of Alberta, Feeders Day Report* no. 54, p. 56.

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