The influence of protein nutrition in early life on growth and development of the pig

2. Effects on the cellularity of muscle and subcutaneous adipose tissue

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1. The effects of feeding either high-protein (HP) or low-protein (LP) diets between 1·8 and 15 kg live weight (LW) and a low-energy (LE) or high-energy (HE) intake subsequently on the cellularity of muscle and adipose tissue in pigs growing to 75 kg LW were investigated.

2. The effects of the nutritional treatments on muscle tissue were assessed from the weight and DNA content of the *m. adductor*. For adipose tissue the total DNA content and fat cell size of the subcutaneous adipose tissue contained in the left shoulder joint were determined.

3. Feeding the LP diets in early life reduced the weight and DNA content of the *m. adductor* ($P < 0·01$) and increased fat cell size ($P < 0·01$) at 15 kg LW.

4. Subsequent to 15 kg there was an almost linear increase in muscle DNA with increasing LW, and the difference between pigs from the initial protein treatments progressively diminished and was no longer apparent at 60 kg LW.

5. At 30 kg LW, pigs given the LP diets before 15 kg LW contained less DNA in the subcutaneous adipose tissue from the shoulder joint ($P < 0·01$) and had larger fat cells ($P < 0·05$) than pigs given the HP diets initially. However, adipose DNA and fat cell size increased with increasing LW and the differences resulting from the initial protein treatments progressively diminished. On the LE and HE treatments subsequent to 15 kg these differences were no longer evident at 45 and 60 kg respectively.

6. Pigs given the HE intake subsequent to 15 kg, contained less DNA in muscle tissue ($P < 0·05$) at 60 and 75 kg LW and had larger fat cells ($P < 0·05$) at 45, 60 and 75 kg LW, than pigs on the LE treatment.

Previous research at this centre (Campbell & Dunkin, 1980) indicated that protein deprivation over the live weight (LW) range 1·8–6·5 kg adversely affected the hyperplastic development of porcine muscle and subcutaneous adipose tissue and that these effects were still evident at 45 kg LW. The results of Gilbreath & Trout (1973) also suggest that protein restriction in early life reduces muscle DNA. However, for the pig there is little information on the extent to which cellular hyperplasia contributes to the post-natal development of muscle or adipose tissue. Thus, it is difficult to assess whether the adverse effects of protein deprivation in early life on the cellular development of either tissue are likely to be permanent or only transitory.

The experiment reported by Campbell & Dunkin (1983) provided the opportunity to investigate the effects of protein nutrition between 1·8 and 15 kg LW and subsequent energy intake on the cellularity of muscle and subcutaneous adipose tissue of pigs grown to 75 kg LW. Because pigs were killed at LW intervals of 15 kg between 15 and 75 kg, the experiment also provided information on the progressive changes in the cellularity of both tissues with increase in LW.

EXPERIMENTAL

General

Forty-two entire male piglets (Large White) were removed from their dams between 24 and 36 h after birth and as their individual LW reached 1·8 kg were randomly allocated to either

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Table 1. *Effects of dietary protein content between 1·8 and 15 kg live weight (LW) on cellularity of muscle and muscle and subcutaneous adipose tissue in pigs at 15 kg LW*

Dietary protein level† ...	High (HP)	Low (LP)	SEM	Statistical significance of difference HP *v.* LP
Adductor muscle				
Wt (g)	59·7	50·3	0·78	**
DNA (mg)	36·3	27·2	0·60	**
Subcutaneous adipose tissue				
Average fat cell diameter (μm)	34·1	49·1	1·8	**

** $P < 0·01$. † For details, see Campbell & Dunkin (1983).

Table 2. *Effect of dietary protein content between 1·8 and 15 kg live weight (LW) and subsequent LW on weight of the* m. adductor *in pigs at 30, 45, 60 and 75 kg LW*

(Values averaged for low-energy and high-energy treatments subsequent to 15 kg LW)

LW (kg)	Dietary protein level† (1·8–15 kg LW)	*m. adductor* wt‡ (g)
30	HP	117
	LP	95
45	HP	176
	LP	160
60	HP	266
	LP	257
75	HP	296
	LP	295
SEM (24 df)		4·8
Least significant difference ($P < 0·05$) between any two treatment means		13·1
Protein × LW interaction		*

HP, high-protein; LP, low-protein.
* $P < 0.05$.
† For details, see Campbell & Dunkin (1983).
‡ Four pigs per treatment mean.

a low-protein (LP) or high-protein (HP) treatment to 15 kg LW. At the latter weight three pigs from each treatment were killed. The remaining animals were allocated equally to a low-energy (LE) or a high-energy (HE) treatment to 75 kg LW. From each of the four treatment combinations, subsequent to 15 kg LW, two pigs chosen at random were killed at each of four LW (30, 45, 60, 75 kg). Two pigs from each of the pre-15 kg LW treatment groups died. Consequently, only sixteen pigs were allocated to the LE and HE treatments subsequent to 15 kg. Subsequent to 15 kg LW the results were analysed as a $2 \times 2 \times 4$ factorial design with two replicates per treatment. The respective factors were, level of dietary protein before 15 kg LW (HP and LP), level of energy intake after 15 kg LW (LE and HE) and LW at slaughter (30, 45, 60 and 75 kg). Details of the diets and management procedures have been described previously (Campbell & Dunkin, 1983).

Table 3. *Effects of dietary protein content between 1·8 and 15 kg live weight (LW), subsequent energy intake and LW on the DNA content of the* m. adductor *of pigs at 30, 45, 60 and 75 kg LW (two pigs per treatment)*

LW (kg) ...	Dietary protein level† 1·8–15	Energy intake† 15–75	30	45	60	75
	HP	LE	51·0	69·4	84·9	102
		HE	49·0	64·5	81·3	97·7
	LP	LE	44·2	67·3	83·5	105
		HE	40·0	59·2	81·6	98·4
Protein × energy interaction			*	*	NS	NS
SEM (16 df): 1·4						
Least significant difference ($P < 0·05$) between any two treatment means: 4·3 mg						

* $P < 0·05$; NS, not significant.
HP, high-protein; LP, low-protein; LE, low-energy; HE, high-energy.
† For details, see Campbell & Dunkin (1983).

Post-slaughter procedures

Immediately after slaughter the *m. adductor* was removed from the right hind leg, weighed and stored at −30° for subsequent DNA analysis.

A section of subcutaneous adipose tissue, measuring approximately 10 × 20 mm, was also removed from directly above the first thoracic vertebra and divided into two equal portions. One portion was prepared immediately for fat cell size determinations. The other was stored at −30° for subsequent DNA analysis. The left shoulder joint of pigs killed at 30, 45, 60 and 75 kg LW was removed by two transverse cuts passing between the atlas and occipital process and between the second and third thoracic vertebrae respectively. The subcutaneous adipose tissue contained in the shoulder joint was dissected out and weighed. The total DNA content of the dissected tissue was calculated as: weight of dissected tissue (g) × DNA concentration of tissue (mg/g).

Measurement of tissue cellularity

The DNA content of muscle and adipose tissue was determined using the method of Martin & Donohue (1972). The analysis of adipose tissue was preceded by extraction of lipid from the samples by shaking in cold chloroform – methanol (2:1, v/v) for 5–6 min. Average fat cell size was determined on duplicate samples by measuring the maximum diameters of 300 consecutive adipocytes using the collagenase technique of Smith *et al.* (1972).

RESULTS

1·8–15 kg LW

At 15 kg LW the *m. adductor* from pigs on the LP treatment was lighter ($P < 0·01$) and contained less DNA ($P < 0·01$) than that from pigs on the HP treatment. Pigs given the LP diets also contained larger fat cells ($P < 0·01$) than those given the HP diets (Table 1).

There was a significant ($P < 0·05$) interaction between the effects of protein nutrition before 15 kg LW and subsequent LW for the weight of the *m. adductor* (Table 2). Muscle weight increased in a linear fashion with increasing LW but the difference between the two initial protein groups declined progressively as LW increased.

For muscle DNA there was a significant interaction ($P < 0·05$) between the effects of

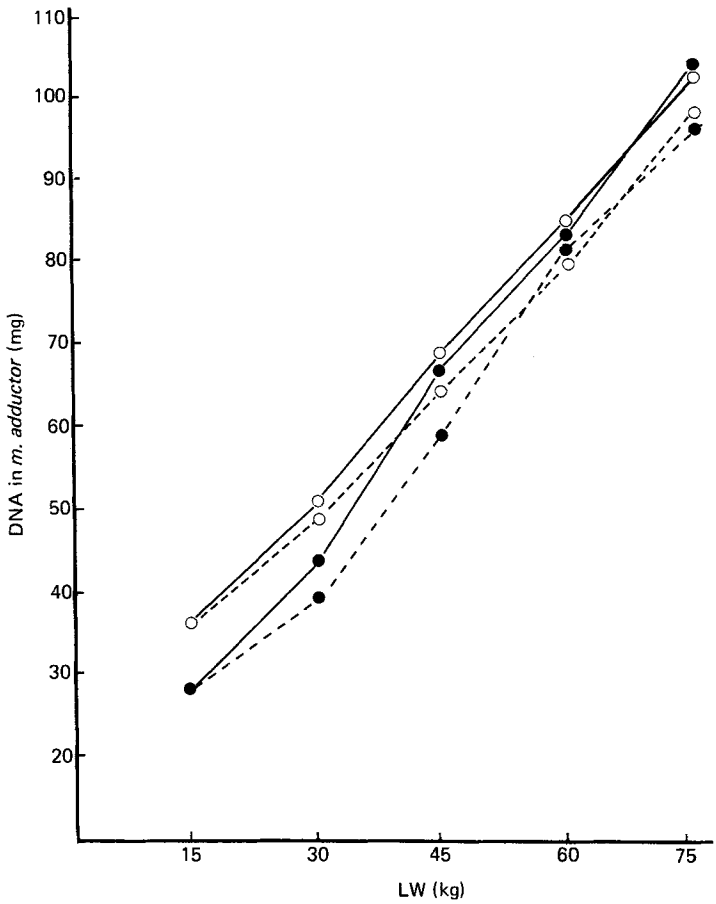

Fig. 1. Effects of feeding pigs a high-protein (○) or a low-protein (●) diet from 1·8 to15 kg live weight (LW) and a low-energy (----) or high-energy (——) intake subsequent to 15 kg LW on the DNA content of the *m. adductor*. For details of treatments, see Campbell & Dunkin (1983).

protein nutrition to 15 kg, subsequent energy intake and subsequent LW. The results are presented in Table 3 and shown diagrammatically in Fig. 1. Muscle DNA increased in a linear fashion with increasing LW and was lower ($P < 0{\cdot}05$) at 75 kg LW in pigs given the HE intake subsequent to 15 kg relative to those given the LE after 15 kg. However, within the LE treatment, pigs previously given the LP diets contained less muscle DNA at 30 kg than those previously given the HP diets but this difference was no longer evident at 45 kg. On the HE treatment, pigs previously given the LP diets contained less muscle DNA to 45 kg than pigs on all other treatments. Differences due to the initial protein treatments progressively diminished as LW increased and were no longer significant at 60 or 75 kg LW.

There was a significant interaction ($P < 0{\cdot}05$) between the effects of protein nutrition before 15 kg LW and subsequent LW both for the DNA content of subcutaneous adipose tissue and fat cell size (Table 4). Pigs given the LP diets contained less DNA at LW of 30 and 45 kg and had larger fat cells at 30, 45 and 60 kg than pigs given the HP diets before 15 kg. However, these differences gradually diminished and, at 75 kg, were no longer

Table 4. *Effects of dietary protein content between 1·8 and 15 kg live weight (LW) and subsequent LW on the cellularity of subcutaneous adipose tissue in pigs at 30, 45, 60 and 75 kg LW*

(Values averaged for low-energy and high-energy treatments subsequent to 15 kg LW)

LW (kg)	Dietary protein†	Subcutaneous fat in shoulder joint‡ Total DNA (mg)	Average fat cell diameter (μm)
30	HP	201	53·3
	LP	170	62·5
45	HP	243	64·5
	LP	219	68·0
60	HP	332	71·6
	LP	321	75·4
75	HP	365	83·7
	LP	376	85·6
SEM (24 df)		10·3	1·2
Least significant difference ($P < 0·05$) between any two means		21·0	3·4

HP, high-protein; LP, low-protein.
† For details, see Campbell & Dunkin (1983).
‡ Four pigs per treatment mean.

significant. The changes between 30 and 75 kg LW for adipose DNA and for fat cell size between 15 and 75 kg LW are shown diagrammatically in Figs. 2 and 3 respectively.

Raising energy intake subsequent to 15 kg increased average fat cell size ($P < 0·05$). The means for average fat cell diameter (μm), pooled for LW, were 73·5 and 66·2 for pigs on the HE and LE treatments respectively.

DISCUSSION

The results showed that the cellularity of muscle and subcutaneous adipose tissue was influenced by LW, protein nutrition before 15 kg LW and by subsequent energy intake. The results for DNA suggest that protein deprivation before 15 kg LW retarded the hyperplastic development of both tissues. Taken in conjunction with the results for body fat content (Campbell & Dunkin, 1983) the results indicate that at 15 kg LW the fattest pigs contained the least number of adipocytes and that the larger fat cells exhibited at 15, 30 and 45 kg by pigs previously given the LP diets were the net effect of these animals having to accommodate more fat into fewer cells than those given the HP diets before 15 kg. The same effect has been observed in a previous experiment at this centre (Campbell & Dunkin, 1980) in which pigs given a LP diet from 1·8 to 6·5 kg LW contained fewer but larger fat cells at both 6·5 and 45 kg LW than pigs given a HP diet before 6·5 kg LW. The results of both experiments demonstrate the elasticity in size of porcine adipocytes and suggest that fat cell number has little effect on the animal's propensity for fat development.

On the other hand, the lower level of muscle DNA exhibited at 15 kg LW by the protein-deprived pigs may have been the major factor responsible for these animals exhibiting a slower rate of protein deposition and LW gain on the HE treatment subsequent to 15 kg LW than pigs previously given the HP diets (Campbell & Dunkin, 1983). A similar

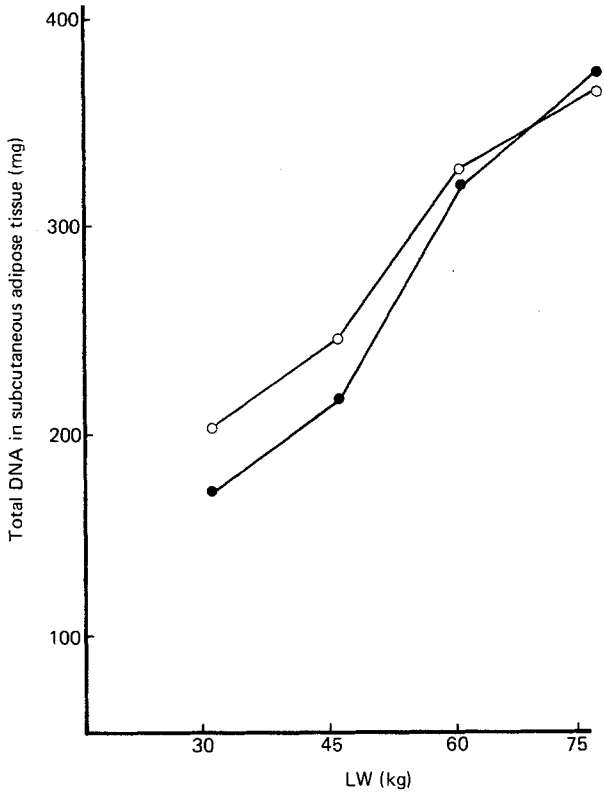

Fig. 2. Effects of feeding pigs a high-protein (○) or a low-protein (●) diet from 1·8 to 15 kg live weight (LW) on the DNA content of subcutaneous adipose tissue in the left shoulder joint subsequent to 15 kg LW. For details of diets, see Campbell & Dunkin (1983).

relationship between muscle DNA and subsequent protein deposition has been reported to occur in poultry (Moss, 1968). In a review of muscle development in meat animals, Allen *et al.* (1979) also concluded that the accretion of DNA in muscle tissue paralleled and possibly preceded protein deposition. The latter contention is further supported by the results of the present experiment, since the rapid increase in the rate of muscle DNA accretion exhibited between 45 and 60 kg by the formerly protein-deprived pigs on the HE treatment was associated with a rapid increase in the rates of protein deposition and LW gain (Campbell & Dunkin, 1983).

The results subsequent to 15 kg showed that the effects of protein deprivation in early life on the DNA content of muscle and subcutaneous adipose tissue were transitory, their persistence being dependent on energy intake in the later period. Furthermore, the almost linear increase in DNA in muscle and subcutaneous adipose tissue with increasing LW indicates that cellular hyperplasia contributes to the development of both tissues to at least 75 kg LW. It was also evident from the lower level of muscle DNA exhibited at 60 and 75 kg LW by pigs on the HE relative to those on the LE treatment that the vulnerability of muscle hyperplasia to nutritional stress is not limited to the early post-natal period; although this effect may have been due to the HE diet having a lower protein: energy value (11·1 g/MJ digestible energy (DE)) than the LE diet (14·4 g/MJ DE), rather than effect of energy intake *per se*. The combination of giving the LP diets up to 15 kg LW and the

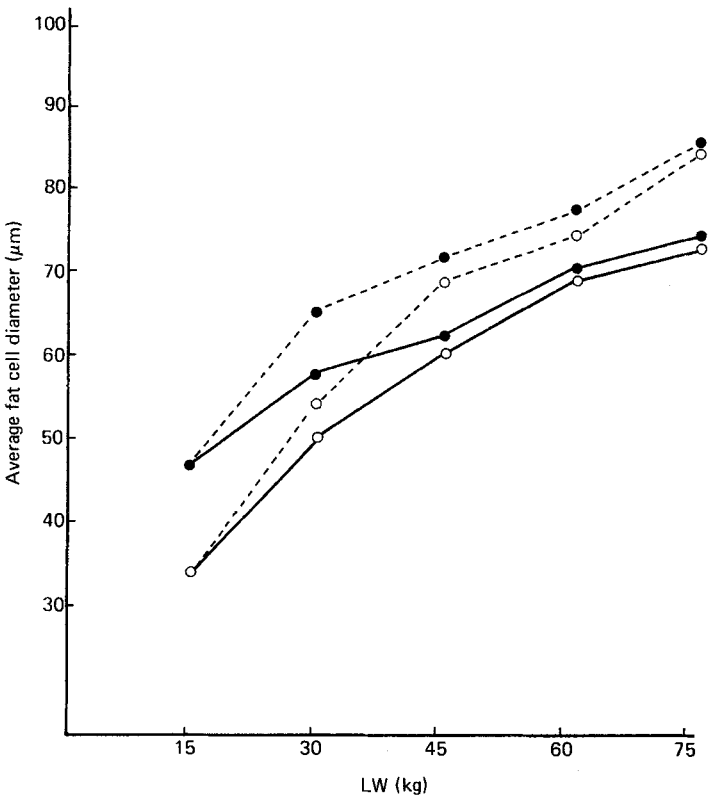

Fig. 3. Effects of feeding pigs a high-protein (○) or low-protein (●) diet from 1·8 to 15 kg live weight (LW) and a low energy (----) or high-energy (——) intake subsequent to 15 kg LW on fat cell diameter of subcutaneous adipose tissue between 15 and 75 kg LW. For details of treatments, see Campbell & Dunkin (1983).

diet with lower protein:energy value immediately after may have also been responsible for the very low level of muscle DNA exhibited at 30 kg by pigs on the LP–HE treatment. These results do not support the suggestion of Robinson (1969) and Lee *et al.* (1973*a*, *b*) that the post-natal development of porcine muscle and adipose tissue is characterized by cellular hypertrophy. However, our values for subcutaneous adipose tissue are in agreement with those of Hood & Allen (1977), Wood *et al.* (1978) and Kirtland & Gurr (1980).

The almost linear relationships between LW, and muscle and adipose DNA and fat cell size observed in the present experiment suggest that the effects of nutrition on tissue cellularity needs to be assessed in animals of constant weight. A similar conclusion was arrived at by Lee *et al.* (1973*a*, *b*) who reported that nutrition in early life appeared to have a marked effect on the cellularity of adipose tissue in pigs compared at constant age but that most of the differences disappeared when animals were compared at constant weight. Nevertheless, in the majority of experiments to study the influence of early nutrition on the cellularity of muscle (eg. Robinson, 1969; Gilbreath & Trout, 1973; Martin *et al.* 1974; Lodge *et al.* 1977) and adipose (eg. Martin *et al.* 1974) tissue, between-treatment comparisons have been made at constant age but at widely different body-weights. Thus, the conclusions drawn from these values may be erroneous and require critical re-evaluation.

Similarly, research with rats has suggested that pre-weaning feed restriction reduces fat cell number and that this, in turn, may prevent obesity in the adult (Hirsch & Han, 1969;

Hirsch & Knittle, 1970; Oscai *et al.* 1972, 1974; Bertrand & Masoro, 1977). However, since invariably these comparisons have been made between animals of the same age but of widely different body-weights both at infancy and adulthood, it is probable that these conclusions also may be misleading.

In conclusion, the results indicate that protein restriction during early development reduces DNA in both muscle and subcutaneous adipose tissue. These effects appear transient, their persistence being dependent on subsequent nutrition. However, although the reduction of adipose DNA appears to have little effect on performance or body composition, the results for growth performance (Campbell & Dunkin, 1983) suggest that the resultant decrease in muscle DNA limits the rate of protein accretion and consequent LW gain during subsequent development, and that these effects persist until muscle DNA is rehabilitated.

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