Genetic growth potential interacts with nutrition on the ability of mice to cope with *Heligmosomoides bakeri* infection

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SUMMARY

Artificial selection for improved productivity may reduce an animal's ability to cope with pathogens. Here, we used Roslin mice, uniquely divergently selected for high (ROH) and low (ROL) body weight, to assess interactive effects of differing growth potential and protein nutrition on host resilience and resistance. In a $2 \times 2 \times 6$ factorial design, ROH and ROL mice were either sham-infected or infected with 250 L_3 *Heligmosomoides bakeri* and fed diets with 30, 80, 130, 180, 230 and 280 g crude protein per kg. The infected ROL-30 treatment resulted in clinical disease and was discontinued. In the remaining ROL mice, infection and feeding treatments did not affect growth but infection reduced weight gain in ROH-30, ROH-80 and ROH-130 mice. Although infection resulted in temporarily reduced food intake (anorexia) in both mouse lines, mean food intake over the whole experiment was reduced in ROH mice only. ROH mice excreted more worm eggs and had higher worm burdens, with relatively fewer female worms, than ROL mice. However, these resistance traits were not sensitive to dietary protein. These results support the view that selection for high growth may reduce the ability to cope with pathogens, and that improved protein nutrition may to some extent ameliorate this penalty.

Key words: *Heligmosomoides bakeri*, protein nutrition, anorexia, resilience, resistance, growth potential.

INTRODUCTION

The phenotypic selection to improve performance traits, e.g. growth or milk yield, causes the co-selection of a generally unknown underlying genetic architecture (Dekkers and Hospital, 2002). As a consequence, selection for desired traits can have detrimental effects on other traits (Williams, 2005). For example, long-term selection for growth in mice has produced individuals with seemingly shorter lifespans and reduced fertility (Bünger *et al*. 2001). A review by Rauw *et al*. (1998) concluded that selection for high body weight in poultry decreased fertility and immunocompetence whilst also increasing the occurrence of defective eggs and chromosomal abnormalities. This review also suggested that selection for high milk yield in dairy herds increased the incidence of diseases such as ketosis or mastitis and general leg problems. The observation of these correlations may be due to various mechanisms.

It has been suggested that the genetic mechanism behind changes in correlated traits could be due to pleiotropy and/or genetic linkage and that consequently these would be seen under all environmental conditions (Rauw *et al*. 1998). Alternatively, observed losses in traits could be explained by a change in the prioritization of allocation of scarce resources. In selectively bred hosts, relatively more of the available scarce resources may be allocated towards the selected trait because of its increased nutrient demand, when compared to unselected counterparts. As a consequence, fewer resources would be allocated to other bodily functions, including immunity (Beilharz *et al*. 1993; Beilharz, 1998*a*, *b*; Glazier, 2002). In support of this view, protein scarcity has been shown to reduce immunity (resistance) to parasites to a greater extent in fast-growing and multiple-rearing hosts compared to their slower growing and single-rearing counterparts (reviewed by Houdijk *et al*. 2001; Houdijk and Athanasiadou, 2003).

During gastrointestinal (GI) parasitic infection, anorexia (or the reduction in voluntary food intake) is a common observation, which may be considered as a functional host response and substantially contributes to observed losses in production (Kyriazakis *et al*. 1998). It has been suggested that selected animals may have an altered degree of anorexia due to the observation that intensely selected animals show reduced resistance and the assumption that anorexia diminishes as immunity increases (Sandberg *et al*.

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Table 1. Chemical and analysed composition of the 6 experimental diets

Ingredients (% inclusion)	Dietary crude protein (g/kg)					
	30	80	130	180	230	280
Casein	3·0	8·86	14·7	20·6	26·4	32·3
Rice starch	57·0	51·1	45·1	39·2	33·2	27·26
Maltodextrin	13·2	13·2	13·2	13·2	13·2	13·2
Sucrose	10·0	10·0	10·0	10·0	10·0	10·0
Soya oil	7·0	7·0	7·0	7·0	7·0	7·0
Cellulose	5·0	5·0	5·0	5·0	5·0	5·0
Vitamins, minerals and amino acids	4·80	4·88	4·97	5·05	5·14	5·23
Analysed composition						
Dry matter (g/kg)	919	918	912	909	904	906
Crude protein (g/kg dry matter)	31·0	85·7	144	197	230	292
Acid detergent fibre (g/kg dry matter)	32·7	35·3	34·9	40·3	42·3	40·6
Ash (g/kg dry matter)	28·7	27·0	27·8	28·4	32·3	31·5

2006; Vagenas *et al*. 2007). Selection for high growth can be expected to increase nutrient demand and thus reduce disease resistance. However results have been inconsistent for the strength and direction of the genetic correlation between growth and resistance (Broughan and Wall, 2007). Studies in livestock species involving interactions between genetic selection for growth and immunity to GI nematodes are limited by the shortage of truly comparable breeds, as breeds have been selected often from different founder populations and have been selected for different and changing breeding goals in different environments. The use of a unique mouse model involving 2 lines derived from the same base population (implying an identical initial genetic makeup) divergently selected purely on the basis of high and low body weight at 42 days of age may enable the elucidation of further interactions between selecting for growth traits and immunity.

Using this mouse model our objectives were to assess the effects of selection for high body weight on the ability to cope with a pathogen challenge, on the degree of anorexia observed and the response to increasing protein nutrition during infection. We hypothesized that mice selected for high body weight experience a greater penalty to the resilience (performance under infection) and resistance to infection than their low body weight counterparts, that anorexia would be more severe in mice selected for high body weight, and that these penalties would be reduced at higher dietary protein contents.

MATERIALS AND METHODS

Animals and housing

A total of 72 Roslin High (ROH) and 72 Roslin Low (ROL), newly weaned male mice aged 21 to 23 days were housed in a room with an ambient temperature of 21 ± 1 °C and a 12 h light cycle (06.00 to 18.00 h). The 'Roslin lines' of mice were produced from a cross of 2 inbred lines (C57BL/6J × DBA/2J), which were subsequently divergently selected for high (ROH) or low (ROL) body weight (BW) at 42 days of age and afterwards inbred for over 38 generations (described in detail by Heath *et al*. 1995; Bünger *et al*. 2001). ROH mice reach body weights of 36 g and 41 g (at 42 and 70 days of age respectively) with ROL mice reaching 16 g and 19 g (at 42 and 70 days of age respectively) when offered a standard diet (see below) and kept under standard maintenance conditions. ROH and ROL mice entered the adaptation phase (see below) of the experiment with a mean (± S.E.) body weight of 16·7 ± 0·48 g and 6·66 ± 0·17 g, respectively. Mice were individually housed in solid-bottomed cages with fresh sawdust and bedding material provided weekly. The experimental details described below were approved by the Animal Experiment Committee of Scottish Agricultural College (ED AE 05/2007) and carried out under Home Office regulations (PPL 60/3626).

Diets

All mice were fed *ad libitum* a standard pelleted expanded breeding diet (Rat and Mouse No. 3, Special Diet Services, Witham, UK; digestible crude oil: 39 g/kg; digestible crude protein: 209 g/kg, starch: 273 g/kg; sugars: 112 g/kg; digestible energy: 12·1 MJ/kg) for 3 days after arrival. A total of 6 isoenergetic (Digestible Energy, 15 MJ/kg) pelleted experimental diets with a fixed amino acid to crude protein (CP) ratio were used at differing levels of CP; 30, 80, 130, 180, 230 and 280 g/kg (Table 1). These CP levels were chosen to range from scarce to more than adequate in gradually increasing increments (NRC, 1995), where most standard diets contain 180–200 g/kg, and to capture the low dietary protein contents used elsewhere in studies on nutritional

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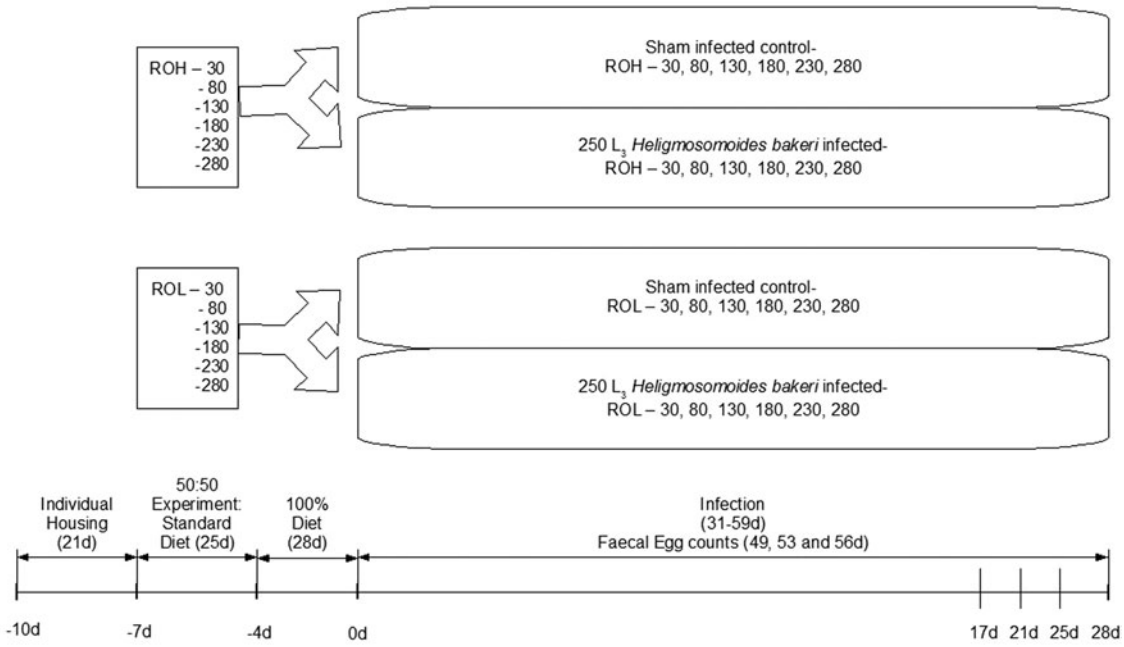

Fig. 1. Diagram of experimental design. Timeline in experimental days shown along the bottom and mean age of mice between brackets. Allocation of mice to diets occurred when 50 : 50 experimental : standard diet was offered, mice were then further allocated to infection groups within these diets at day 0 of the experiment.

sensitivity of parasitism in mice (Slater and Keymer, 1988; Boulay *et al*. 1998; Ing *et al*. 2000; Tu *et al*. 2007). As casein was used as the protein source, 15 g cysteine was added to each kg of casein to account for the relative scarcity of this amino acid. The 30 and 280 g/kg CP diets were formulated and the 80, 130, 180 and 230 g/kg CP diets were then produced using an appropriate mixture of the 30 and 280 g/kg CP diets (Table 1).

Infection protocol and experimental design

At day 0 of the experiment mice either received a single infection of 250 *Heligmosomoides bakeri* infective larvae (L_3) suspended in 0·2 ml of water (I) or a sham infection of 0·2 ml of water (C) via oral gavage (Houdijk and Bünger, 2007). The *H. bakeri*, formerly known as *Heligmosomoides polygyrus bakeri* and *Nematospiroides dubius* (Cable *et al*. 2006), were donated by Professor Jerzy Behnke, University of Nottingham, UK (see Jenkins and Behnke, 1977 for full origin details). The dose of *H. bakeri* was chosen to produce a subclinical level of infection that is known to affect the growth of the mice (Houdijk and Bünger, 2006, 2007). Previous studies using higher levels of infection with *H. bakeri* found that 400 L_3 caused a 10% mortality rate in heterozygous mice (Ehrenford, 1954*a*) whereas in NZB mice 100% mortality was achieved at 300 L_3 (Mitchell and Prowse, 1979). Although it is known that variation in the genetic background of mice used in infection studies substantially affects the response to larval dose (Liu, 1966; Mitchell and Prowse, 1979; Behnke *et al*. 2003), it is not known whether this is also the case for the ROH/ROL parent lines.

I and C mice of the ROL and ROH line were fed *ad libitum* on 1 of the 6 experimental diets (referred to as 30, 80, 130, 180, 230 and 280) with 6 replicates in each group, resulting in 24 treatment combinations. Figure 1 shows the experimental design and timetable. Mice entered the adaptation phase at approximately 3 weeks of age (day-10 of experiment). This consisted of a period where a 50 : 50 mix of experimental and standard diet was offered to acclimatize the mice to the experimental diet (day-7 until day-4) with infection occurring at day 0; between days-3 and 0 mice were only offered the experimental diets. Mice were humanely killed (aged between 49 and 53 days) on day 28 post-primary infection (p.i.), for the assessment of worm burdens, colon egg count and body fat percentage.

Sample measurements and collection

Body weight and food intake. Mice and food refusals were weighed twice weekly (Tuesday and Friday) throughout the experiment resulting in 8 experimental periods for food intake and 8 observations on post-infection body weight until day 28. On each of these days food refusals were weighed out and fresh food weighed in, around 30 g was offered to ROH and 15 g to ROL mice, this was sufficient for *ad libitum* feeding. Food intake was calculated per individual per day within each of the 8 experimental periods. Body weight data were used to calculate body weight gain over the post-infection (p.i.) period.

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Nematode egg counts. Mice were placed onto wire-bottomed cages overnight and faecal samples collected on days 17, 21 and 25 p.i. to assess faecal egg counts (eggs per g faeces). This was carried out using a modified flotation technique (Christie and Jackson, 1982). The total period of faecal collection (finish time – start time) was recorded to estimate faeces volume per 12 h, assuming even production of faeces during the collection period. This faeces volume was used to standardize egg output per 12 h (EO, eggs per 12 h) to eliminate variation between achieved collection times as well as to account for the dilution effect on faecal egg counts expected for ROH mice due to their larger volumes of faeces.

Colon contents and worm burden. Mice were humanely killed on day 28 via CO_2 inhalation and dissected to obtain the small intestine and the colon. The small intestine was weighed, opened up and placed in a gauze pouch suspended in Hanks' solution, this was then incubated at 37 °C for 3 h to collect the adult worms (Wahid and Behnke, 1992). A 5% formalin solution was added to the recovered worms, and the intestine and gauze checked for remaining worms. Male and female worms were separated and counted. The colon contents were weighed and a colon egg count (eggs per g) performed. The colon egg count was then multiplied by colon contents weight to account for dilution effects arising from the expected larger colon content volume of the ROH mice. Resultant data were therefore expressed as number of eggs in colon (EIC, number of eggs). The EIC was divided by the number of females counted to obtain an estimate for the *per capita* fecundity (PCF, eggs per female).

Fat percentage. The carcasses were then weighed and bagged for subsequent freeze-drying to allow prediction of fat percentage. To prepare the carcass for the freeze-drying process incisions were made in the back, tail and head of the animal to allow maximum water loss. The carcasses were then placed onto individual labelled trays and the freeze-drier turned on. After approximately 7 days (or weight loss ceased) at −70 °C the carcasses were removed and re-weighed. To calculate fat percentage the following equation was used (Hastings and Hill, 1989):

$$\text{Fat percentage} = [(\text{freeze-dried weight} \times 1{\cdot}13) / \text{carcass wet weight}) - 0{\cdot}302] \times 100$$

Statistical analysis

Due to the skewed nature of the data, EO, EIC and PCF were $\log_{10}(n+1)$ transformed. These are reported as back-transformed least-square means, accompanied by a lower and upper confidence interval, calculated from back-transforming least-square mean of transformed data (μ), μ − S.E. and μ + S.E. respectively. To adequately account for the relatively large differences in performance data between the mouse lines, arising from the difference in body weights *per se* between ROL and ROH mice, body weight and food intake data were also $\log_{10}(n+1)$ transformed before analysis (Falconer and MacKay, 1996). Repeated measures Restricted Maximum Likelihood (REML) was used to assess interactive effects of genetic growth potential, dietary protein content, infection status and time on body weight, food intake and EO. The full repeated measures model is as follows: parameter of interest = genetic potential (G) + crude protein content (CP) + infection status (I) + time (t) + all possible six 2-way interaction + all possible three 3-way interaction + the 4-way interaction (G.CP.I.t). REML analysis was also used to assess interactive effects between genetic growth potential, dietary protein content and infection status on average daily weight gain, average food intake and body fat percentage. The full REML model is as follows: parameter of interest = G + CP + I + all possible three 2-way interaction + the 3-way interaction (G.CP.I). Where significant, litter was included as a random effect. To account for a possible overestimation of effect size arising from the unbalanced nature of the data (see results), each *P*-value reported was calculated conservatively through including first all other terms into the final REML model. Interaction terms that did not approach significance at $P < 0{\cdot}05$ were omitted from the final REML models for each parameter. F-statistics reported are followed by the numerator then the denominator degrees of freedom in subscript. Effects with *P*-values less than 0·05 are considered significant whilst those with *P*-values between 0·05 and 0·10 are described as tendencies or trends. All statistical analyses were performed using Genstat 11 for Windows release 11.1, 2008 (VSN international, Hemel Hempstead, UK).

RESULTS

Loss of the infected ROL-30 group

ROL-30-I mice were found to show severe clinical signs of infection at day 7 p.i. such as starry coat, unsteadiness and disorientation. These mice were euthanized and this treatment group discontinued in accordance with previously defined end-points. Thus, the resulting dataset was characterized as an incomplete $2 \times 2 \times 6$ factorial design, which consequently was appropriately analysed through REML.

Body weight gain and food intake

Mean observed body weight and food intake are summarized in Tables 2 and 3, respectively, whilst mean performance data for each line × infection × feeding treatment are summarized in Table 4.

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Table 2. Summary of mean body weights (BW in g) and pooled standard errors (S.E.) over time and at each time-point[a,b]

Line	Infection	Protein nutrition	d0 BW	d3 BW	d7 BW	d10 BW	d14 BW	d17 BW	d21 BW	d25 BW	d28 BW	Pooled S.E.
ROH	C	30	23·96	24·14	24·56	24·97	25·71	26·17	26·64	26·71	27·64	0·89
		80	28·21	31·33	34·66	38·26	41·13	44·65	46·13	47·55	49·77	2·52
		130	28·82	32·11	34·89	38·19	41·38	45·02	46·75	48·21	50·20	1·51
		180	28·21	31·20	34·34	36·91	39·11	41·68	43·17	43·74	44·27	1·61
		230	31·36	34·70	38·39	41·33	43·91	46·76	48·69	48·95	50·48	2·44
		280	28·01	30·89	33·87	37·68	40·16	42·76	44·24	45·59	47·33	0·69
	I	30	23·07	22·97	22·37	22·87	23·26	24·14	24·14	23·34	22·42	1·05
		80	27·41	29·77	33·56	35·15	37·50	40·13	40·21	40·71	41·66	1·92
		130	29·60	31·86	34·92	37·96	39·57	42·10	44·02	45·00	47·12	0·92
		180	29·82	32·24	35·74	37·82	40·30	41·74	43·32	44·31	46·26	2·04
		230	28·02	31·42	34·40	36·34	38·29	40·52	41·10	41·65	43·10	1·23
		280	28·02	30·71	34·12	36·41	38·96	41·18	42·46	44·12	45·69	1·22
	Pooled S.E. for ROH		0·48	0·41	0·39	0·36	0·42	0·42	0·45	0·46	0·49	
ROL	C	30	11·38	11·95	12·60	13·39	13·70	14·31	14·80	15·18	15·52	0·50
		80	12·26	13·29	14·38	15·30	15·88	16·55	16·93	16·98	17·69	0·31
		130	10·21	11·81	13·28	13·99	15·09	15·61	16·21	16·46	17·12	0·45
		180	11·81	13·01	14·37	15·12	15·80	16·22	17·05	17·64	17·74	0·36
		230	11·13	12·21	13·63	14·30	15·07	15·42	15·84	16·46	16·60	0·65
		280	10·67	12·39	13·74	14·60	15·57	16·12	16·60	16·65	17·33	0·52
	I	30[c]	—	—	—	—	—	—	—	—	—	—
		80	10·79	12·10	13·16	13·82	14·67	15·27	15·82	16·56	16·95	0·52
		130	10·96	12·04	13·63	14·24	15·00	15·79	16·49	16·96	17·65	0·42
		180	11·77	12·82	13·98	14·62	15·33	15·98	16·35	17·12	17·69	0·33
		230	11·01	12·51	14·06	15·06	15·75	16·37	16·65	17·10	18·32	0·44
		280	12·08	12·91	14·60	14·95	15·55	16·48	17·03	17·59	18·42	0·61
	Pooled S.E. for ROL		0·15	0·13	0·13	0·12	0·12	0·14	0·13	0·14	0·14	

[a] Raw data shown.
[b] 'Roslin' mice divergently selected for high (ROH) and low (ROL) body weight were either sham-infected (C) or infected with 250L_3 *Heligmosomoides bakeri* (I). Experimental diets contained 30, 80, 130, 180, 230 or 280 g crude protein per kg.
[c] ROL-30-I showed clinical signs of infection and were discontinued.

Figure 2 shows the log-transformed mean of daily weight gain and food intake over the experiment. ROH mice, with the exception of ROH-30-I mice, gained more weight over the experiment than ROL mice (mean = 0·497 ± 0·202 g per day and 0·175 ± 0·005 g per day respectively; $F_{1,114} = 529{\cdot}55$, $P < 0{\cdot}001$). However, on average ROH-30-I mice did not gain weight across the experiment (mean = −0·02 ± 0·04 g per day).

A three-way interaction between genetic growth potential, protein nutrition and infection status was observed for body weight gain ($F_{4,114} = 3{\cdot}01$, $P = 0{\cdot}021$). This was due to ROH mice showing a reduction in weight gain on the 30 g CP per kg diet and also in response to infection on 30, 80 and 130 g CP per kg diets whilst ROL mice maintained a relatively stable weight gain regardless of experimental group. ROH mice gained more weight than ROL mice, with the exception of ROL-30-C gaining more than ROH-30-I ($F_{1,114} = 529{\cdot}55$, $P < 0{\cdot}001$). The random effect of litter was not significant ($P > 0{\cdot}10$).

Genetic growth potential interacted with dietary protein content and with infection status for average daily (log-transformed) food intake (Fig. 2). The interaction with dietary protein content resulted from decreased food intake of ROH-30 mice whereas ROL-30 mice increased their intake on this diet when compared to other diets ($F_{5,103} = 29{\cdot}44$, $P < 0{\cdot}001$). The interaction with infection status was reflected in a reduced intake following infection in ROH mice but not in ROL mice ($F_{1,99} = 6{\cdot}70$, $P = 0{\cdot}011$). Across feeding and infection treatments, ROH mice consumed more food than ROL mice ($F_{1,36} = 1188{\cdot}36$, $P < 0{\cdot}001$). The random effect of litter was significant (deviance ratio = 6·53, D.F. = 1, $P = 0{\cdot}01$).

Figure 3 shows the log-transformed mean of daily food intake over time. Time and infection status interacted for average daily food intake ($F_{7,811} = 3{\cdot}33$, $P = 0{\cdot}002$); infection caused a temporary decrease in voluntary intake between day 3 and day 14 p.i. This anorexia was not affected by genetic growth potential. However, the analysis of food intake over time showed two 3-way interactions. Firstly, infection status interacted with protein and time as evidenced by the variable presence of anorexia over the 6 levels of dietary CP ($F_{35,860} = 2{\cdot}6$, $P < 0{\cdot}001$). Anorexia was not observed for 3 of the remaining 11 mice line – feeding treatment combinations, i.e. ROH-130,

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Table 3. Summary of mean average daily food intakes (DFI in g) and pooled standard errors (S.E.) over time and at each time-period[a,b]

Line	Infection	Protein nutrition	d0-d3 DFI	d3-d7 DFI	d7-d10 DFI	d10-d14 DFI	d14-d17 DFI	d17-d21 DFI	d21-d25 DFI	d25-d28 DFI	Pooled S.E.
ROH	C	30	4·47	3·86	3·57	3·64	3·31	3·38	3·17	3·32	0·15
		80	5·25	5·19	5·52	5·27	5·52	5·04	4·93	4·90	0·09
		130	4·88	4·97	5·13	5·41	5·69	5·39	5·23	5·00	0·07
		180	4·88	5·03	4·84	5·19	4·87	4·66	4·51	3·98	0·09
		230	5·20	5·71	5·36	5·66	5·47	5·21	4·77	4·81	0·11
		280	4·70	4·97	5·23	5·17	5·06	5·08	4·62	4·51	0·08
	I	30	4·32	3·28	3·33	3·93	3·70	3·09	2·37	1·96	0·11
		80	4·94	4·71	4·39	4·62	4·92	4·48	4·21	3·87	0·09
		130	4·79	4·72	4·77	4·88	4·80	5·15	4·71	4·84	0·09
		180	4·73	4·91	4·57	4·72	4·70	4·71	4·60	4·71	0·08
		230	5·12	4·84	4·24	4·68	4·98	4·60	4·44	4·72	0·07
		280	4·73	4·79	4·41	4·79	4·81	4·74	4·62	4·65	0·07
	Pooled S.E. for ROH		0·03	0·02	0·02	0·03	0·02	0·03	0·03	0·03	
ROL	C	30	2·91	2·96	3·02	2·95	2·94	3·14	3·01	2·96	0·05
		80	2·64	2·65	2·60	2·49	2·54	2·69	2·51	2·49	0·03
		130	2·55	2·51	2·52	2·57	2·47	2·52	2·56	2·54	0·03
		180	2·67	2·67	2·51	2·50	2·48	2·74	2·58	2·62	0·03
		230	2·56	2·52	2·44	2·41	2·46	2·50	2·53	2·27	0·04
		280	2·55	2·44	2·50	2·48	2·38	2·49	2·41	2·43	0·04
	I	30[c]	—	—	—	—	—	—	—	—	—
		80	2·48	2·24	2·27	2·37	2·42	2·48	2·58	2·38	0·03
		130	2·46	2·36	2·32	2·45	2·58	2·54	2·60	2·59	0·03
		180	2·52	2·30	2·29	2·46	2·40	2·47	2·63	2·44	0·03
		230	2·66	2·47	2·47	2·57	2·56	2·69	2·54	2·68	0·04
		280	2·47	2·39	2·19	2·47	2·53	2·59	2·47	2·53	0·04
	Pooled S.E. for ROL		0·01	0·01	0·01	0·01	0·01	0·01	0·01	0·01	

[a] Raw data shown.
[b] 'Roslin' mice divergently selected for high (ROH) and low (ROL) body weight were either sham-infected (C) or infected with 250L_3 *Heligmosomoides bakeri* (I). Experimental diets contained 30, 80, 130, 180, 230 or 280 g crude protein per kg.
[c] ROL-30-I showed clinical signs of infection and were discontinued.

ROH-180 and ROL-230. Secondly, genetic growth potential interacted with protein and time as evidenced by a larger decline in intake over time on the 30 g/kg CP in ROH mice compared to ROL mice ($F_{35,860}=1·62$, $P=0·014$). In addition and in comparison with the other experimental diets, ROH-30 mice had a lower intake whilst for ROL-30 mice intake on this diet was the highest.

Egg output, eggs in colon and worm burden

Mean parasitism data for each line × infection × feeding treatment combination are summarized in Table 5. Figure 4 shows the back-transformed mean EO, EIC and mean total worm burden. The 12 h faecal production was higher in ROH mice than in ROL mice (0·55 *vs* 0·34 g; S.E.D: 0·025 g; $F_{1,54}=38·49$, $P<0·001$), which justified the need to account for potential dilution effects on faecal egg counts. Genetic growth potential and feeding treatment did not significantly interact with time for EO. Therefore, the mean EO averaged over days 17, 21 and 25 p.i. was analysed. Back-transformed EO are shown in Fig. 4. Genetic growth potential and dietary protein contents interacted for EO ($F_{4,29}=2·95$, $P=0·036$); EO tended to be consistently higher in ROH than in ROL mice ($F_{1,32}=2·94$, $P=0·096$) but was significantly higher on 80 g/kg CP diets only. Increasing dietary protein content also increased 12 h faecal production in both lines ($F_{5,27}=5·65$, $P<0·001$). The random effect of litter was significant (deviance ratio = 19·19, D.F. = 1, $P<0·001$).

Genetic growth potential and feeding treatment interacted for colon contents weight ($F_{5,54}=23·01$, $P<0·001$); increasing dietary protein content increased colon content weight in ROH mice but decreased colon contents weight in ROL mice. Across feeding treatments, weight of colon contents was higher in ROH mice than in ROL mice (0·21 *vs* 0·16 g; S.E.D. 0·014 g; $F_{1,54}=13·00$, $P=0·001$). As with faecal output, these observations justified accounting for effect of colon contents volume on worm egg concentrations. Genetic growth potential and feeding treatment did not interact for EIC. However, EIC was significantly higher in ROH mice than ROL mice ($F_{1,58}=5·97$, $P=0·018$; Fig. 4). The random effect of litter was not significant ($P>0·10$).

Genetic growth potential and feeding treatment did not interact for total worm counts (Fig. 4). However, genetic growth potential affected male

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Table 4. Summary showing means and pooled standard errors (S.E.) of performance data[a,b]

Line	Infection	Protein nutrition	Daily weight gain (g)	Daily food intake (g)	Fat (%)
ROH	C	30	0·13	3·59	18·63
		80	0·66	5·20	25·87
		130	0·64	5·21	22·39
		180	0·47	4·75	20·30
		230	0·56	5·27	25·26
		280	0·59	4·92	21·68
	I	30	−0·02	3·16	14·20
		80	0·42	4·51	16·50
		130	0·54	4·83	19·20
		180	0·50	4·71	18·80
		230	0·42	4·70	16·90
		280	0·54	4·68	19·20
		Pooled S.E.	0·15	0·04	1·50
ROL	C	30	0·13	2·99	16·00
		80	0·16	2·58	11·24
		130	0·19	2·53	11·82
		180	0·17	2·60	13·50
		230	0·16	2·46	12·66
		280	0·18	2·46	12·45
	I	30[c]	—	—	—
		80	0·17	2·40	11·30
		130	0·20	2·49	9·94
		180	0·17	2·44	10·70
		230	0·21	2·58	9·74
		280	0·20	2·46	8·95
		Pooled S.E.	0·06	0·02	0·92

[a] Data were log transformed for statistical analysis.
[b] 'Roslin' mice divergently selected for high (ROH) and low (ROL) body weight were either sham-infected (C) or infected with 250L_3 *Heligmosomoides bakeri* (I). Experimental diets contained 30, 80, 130, 180, 230 or 280 g crude protein per kg.
[c] ROL-30-I showed clinical signs of infection and were discontinued.

worm numbers, as ROH mice had significantly higher numbers of male worms than ROL mice (96 *vs* 68; S.E.D. 10·9; $F_{1,54}=6·10$, $P=0·017$). Figure 5 shows the sex composition of the worm burdens and *per capita* fecundity. Genetic growth potential significantly affected worm burden sex composition; ROL mice had a higher percentage of female worms than ROH mice (54·43 *vs* 49·72%; S.E.D. 1·889%; $F_{1,63}=6·21$, $P=0·021$). *Per capita* fecundity tended to be higher in ROH mice than in ROL mice ($F_{1,62}=3·37$, $P=0·071$) but was not affected by dietary CP contents. The random effect of litter was not significant for worm numbers or *per capita* fecundity ($P>0·10$).

Body fat percentage

A three-way interaction between genetic growth potential, dietary protein and infection status was significant for body fat percentage ($F_{4,109}=2·78$, $P=0·030$; Fig. 6). ROH mice had higher body fat percentage than ROL mice ($F_{1,35}=134·95$, $P<0·001$) and infection reduced body fat percentage in both ROH and ROL mice ($F_{1,92}=65·88$, $P<0·001$). However, body fat percentage seemed to increase with higher levels of dietary CP contents for infected ROH mice, whilst it decreased for infected ROL mice. ROL mice had their highest body fat percentage at 30 g CP per kg. The random effect of litter was significant (deviance ratio = 9·78, D.F. = 1, $P=0·002$).

DISCUSSION

The results of this study supported our hypothesis that selection for high body weight in mice imposes a greater penalty on resistance and resilience to parasite infection than selection for low body weight, and that improved protein nutrition could ameliorate the penalty on host resilience. However, in contrast to our other hypotheses, our results also showed that this difference in genetic growth potential did not affect anorexia, and that increased protein nutrition did not affect host resistance.

Body weight and feed intake

It was found that ROH mice had a greater reduction in average daily intake and daily weight gain, but a

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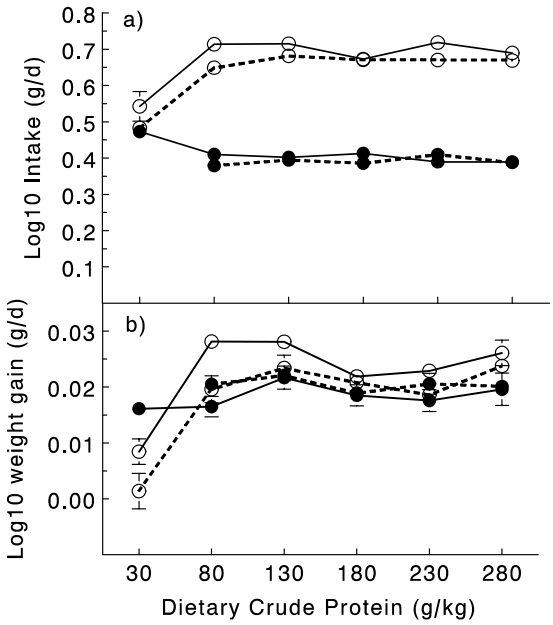

Fig. 2. (A) Log_{10} transformed daily food intake and (b) body weight gain of high (ROH – open circle) and low (ROL – closed circle) body weight mice averaged across 28 days of infection with *Heligmosomoides bakeri* (dashed line) or sham infection with water (solid line) at different levels of dietary CP.

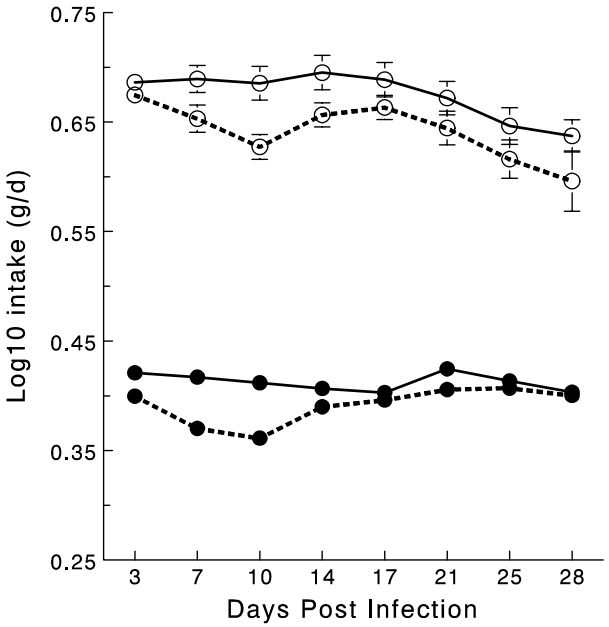

Fig. 3. Log_{10} transformed daily food intake of high (ROH – open circle) and low (ROL – closed circle) body weight mice following infection with *Heligmosomoides bakeri* (dashed line) or sham infection with water (solid line) over the 28-day experimental period.

similar reduction in body fat percentage in response to infection relative to ROL mice. Although this effect has not been addressed in divergently selected mouse lines, infections have been shown to produce reductions in both food intake and body weight gain in mice (Brailsford and Mapes, 1987; Tu *et al*. 2007). Moreover, Kristan and Hammond (2001, 2006) investigated the effect of *H. bakeri* infection on body fat in Swiss-Webster mice and found a reduction of 20% on average, which corresponds very well to the reduction found in the current study in both the ROH and ROL mice (an average of 21·5% and 21·8% respectively). This reduction of body fat appears to suggest that the two genetic lines utilized energy similarly in response to infection and suggests a possible role for energy nutrition during gastrointestinal nematode infections. Indeed, caloric restriction, via restrictive feeding protocols (causing mainly energy restriction but in addition also some degree of protein restriction), may increase susceptibility to parasite infection and also worm burdens and parasite fecundity (Koski *et al*. 1999; Kristan, 2008). However, the sham-infected animals showed a difference in their allocation rules, with ROH-80-280 mice having increased body fat compared to ROH-30 whilst ROL-30 mice have the highest body fat compared to ROL-80-280.

Increasing dietary CP contents to 130 g CP per kg and above resulted in increased host resilience in ROH mice, as illustrated by reduced penalty of infection on food intake and body weight gain. A preliminary study using ROH and ROL mice did find that feeding a 250 g CP per kg diet similarly reduced the penalty of infection on body weight gain in ROH mice when compared to ROH mice fed 50 g CP per kg (Houdijk and Bünger, 2007). This is consistent with our findings, and suggests that moderate protein nutrition using highly digestible protein sources (130 g CP per kg diet) may ameliorate losses in production during a primary infection with gastrointestinal nematode parasites.

Anorexia, characterized as a temporary reduction in food intake following infection, is a common outcome of exposure to pathogens, including gastrointestinal parasites (Kyriazakis *et al*. 1998; Mercer *et al*. 2000). Its biological relevance is evident from force-feeding studies. For example, Murray and Murray (1979) observed increased host mortality following force-feeding mice during a *Listeria monocytogenes* infection. However, the time-course of anorexia has not been described in detail during *H. bakeri* infection; to date, studies have only reported a decrease in food intake over the entire experimental period (Brailsford and Mapes, 1987; Shi *et al*. 1997; Boulay *et al*. 1998; Tu *et al*. 2007). This study, therefore, is the first to describe the time-course of anorexia during a primary infection of *H. bakeri*. Anorexia in both lines was found to occur between day 3 and day 14 post-primary infection. Although genetic growth potential was not found to affect anorexia overall, whether anorexia differed between lines depended on dietary protein content. ROH mice did not show anorexia at 130 or 180 g CP per kg whilst ROL mice did not show anorexia at

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Table 5. Summary showing means and pooled standard errors (S.E.) of parasitism data[a,b]

Line	Protein	Eggs in colon (*n*)	Egg output (*n*/12 h)	Total worms (*n*)	Female worms (*n*)	Male worms (*n*)	*Per capita* fecundity (*n*/female)
ROH	30	2032	9937	214	106	108	23·88
	80	7309	21 582	268	140	128	52·69
	130	4154	21 139	126	60	66	77·26
	180	7186	12 347	191	100	91	75·37
	230	6813	13 147	229	114	115	71·80
	280	6170	19 217	146	75	71	82·58
	Pooled S.E.	1104	4314	31	21	21	12·38
ROL	30[c]	—	—	—	—	—	—
	80	6028	5956	143	84	59	64·51
	130	2735	13 604	139	73	67	39·42
	180	4586	6587	129	68	62	69·99
	230	2808	6828	152	78	74	45·07
	280	2916	11 787	173	94	79	35·56
	Pooled S.E.	978	1306	15	12	12	17·36

[a] Egg count and fecundity data were log transformed for statistical analysis.
[b] 'Roslin' mice divergently selected for high (ROH) and low (ROL) body weight were fed either 30, 80, 130, 180, 230 or 280 g crude protein per kg diets.
[c] ROL-30-I showed clinical signs of infection and were discontinued.

230 g CP per kg only. This apparent lack of systematic effect of dietary CP content on anorexia in both lines may be due to variation in food intake at different levels of dietary CP in the sham-infected mice. Although a lack of systematic research in this area makes it difficult to reach a consensus on the effect of diet composition on anorexia (Kyriazakis, 2009), the data from this study would support the view that the degree of anorexia does not depend on dietary CP contents. The latter is consistent with earlier work done on *H. bakeri* (Brailsford and Mapes, 1987) but also on sheep infected with *T. colubriformis* (Kyriazakis *et al*. 1996) and *Haemonchus contortus* (Datta *et al*. 1998).

Egg output, eggs in colon and worm burden

Compared to ROL mice, infection in ROH mice produced greater 12 h egg output, total eggs in the colon contents and *per capita* fecundity. This suggests that ROH mice had an impaired resistance to *H. bakeri*, which in turn implies that selection for high body weight may produce a loss of immunity towards pathogen challenge. Since this reduced immunocompetence was observed at times of apparently adequate CP nutrition, it may not necessarily have arisen from reduced allocation of scarce protein to host immune functions. Influences of host genetics on *H. bakeri* infection have long been considered and some commonly used mouse strains have now been categorized as high or low responders based on LD_{50} experiments and also according to time taken to clear out the infection (Liu, 1966; Iraqi *et al*. 2003). Given that genetic differences in immunocompetence between the ROH and ROL mice exist they could potentially be the result of a true genetic correlation or drift. As selection for high production in lambs can also impair the ability to cope with pathogens, as lambs with a high growth potential had higher faecal egg counts than their low growth potential counterparts (Zaralis *et al*. 2008) it is suggested that it is more likely a genetic correlation than drift.

Consistent with our results, Slater and Keymer (1988) also did not find an effect of increased protein nutrition on worm burdens or egg counts during a primary infection. However, Boulay *et al*. (1998) did report a significant reduction in worm burdens in mice fed 240 g CP per kg when compared to 30 and 70 g CP per kg diets. These mice were older than those in the current study, which may have led to stronger immune response per se (Goff *et al*. 2001; Miller *et al*. 2005). In addition, it remains to be elucidated whether the differences between Boulay's findings and our own may have arisen from differences in feeding treatment regime, i.e. our study introduced the experimental diets 1 week before infection whilst in the study of Boulay *et al*. (1998), mice were fed the experimental diets several weeks before infection. During secondary infections, protein scarcity, arising from feeding 20 or 30 g CP per kg diets, decreased weight gain and increased worm burdens and egg counts when compared with diets with 70 or more g CP per kg (Slater and Keymer, 1988; Boulay *et al*. 1998). In addition, it was shown that switching food types from protein deficient (30 g/kg) to protein sufficient (240 g/kg) during primary or secondary infection with *H. bakeri* was also shown to rapidly restore body weights in mice and resulted in a reduction of worm burdens and faecal egg counts during secondary challenge to levels

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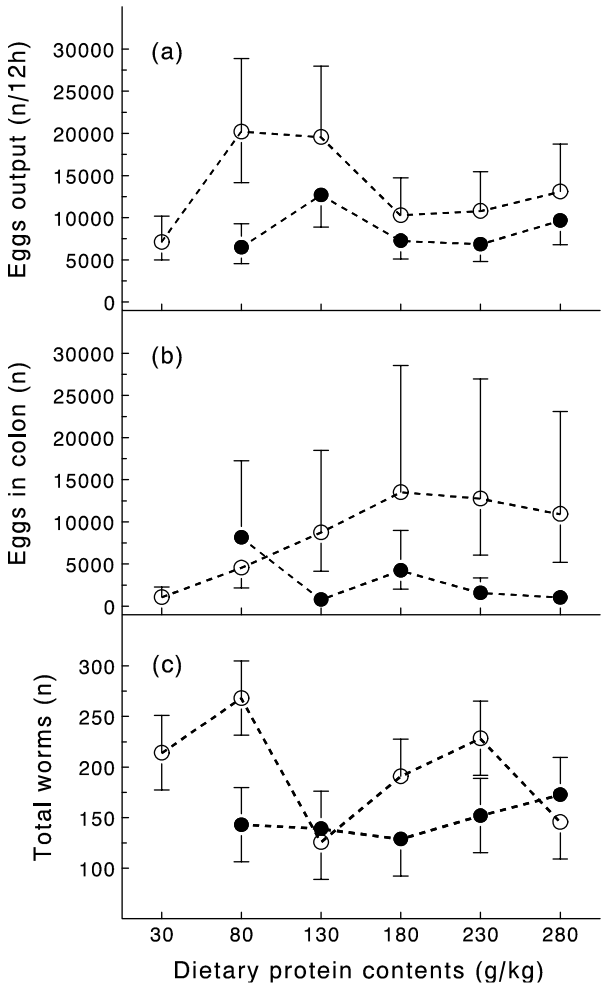

Fig. 4. (A) Back-transformed 12 h egg output averaged over days 17, 21 and 24 post-infection. (B) Back-transformed number of eggs in the colon on day 28 post-infection. (C) Total worm burden on day 28 post-infection for high (ROH – open circle) and low (ROL – closed circle) body weight mice, infected with *Heligmosomoides bakeri* and fed different levels of dietary crude protein (CP) for 28 days.

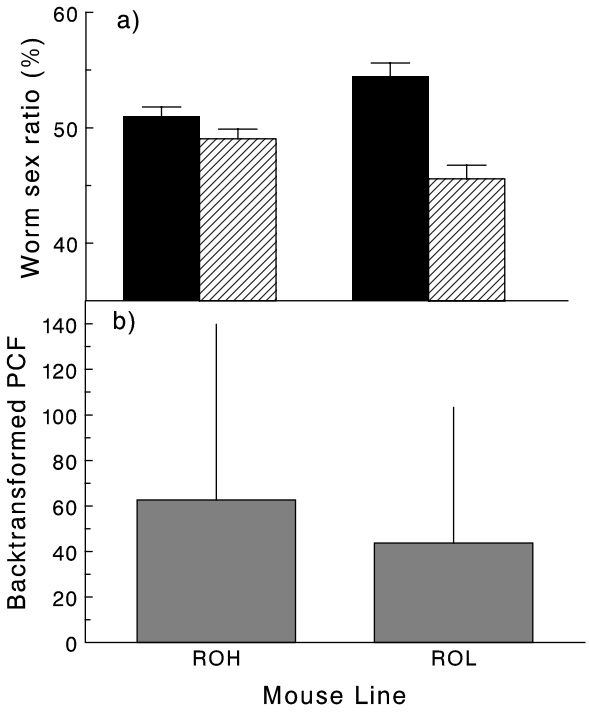

Fig. 5. (A) Sex ratio of worm burdens (% female worms-solid bar and % male worms-patterned bar) and (B) mean back-transformed *per capita* fecundity (PCF) for high (ROH) and low (ROL) body weight mice taken 28 days after infection with *Heligmosomoides bakeri*.

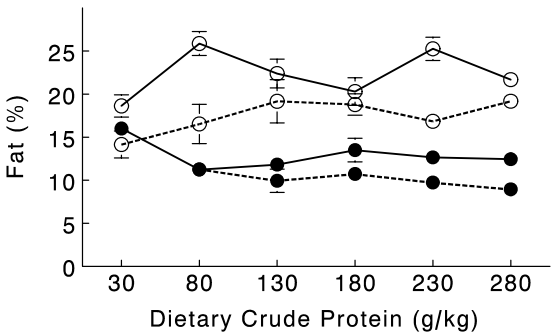

Fig. 6. Average body fat percentage of high (ROH – open circle) and low (ROL – closed circle) body weight mice either infected with *Heligmosomoides bakeri* (dashed line) or sham infected with water (solid line) at different levels of dietary CP, taken 28 days after infection.

achieved on the protein sufficient diet for the entire study (Tu *et al*. 2007). Taken together, these and our findings support the view that protein scarcity affects expression of immunity to a larger extent that acquisition of immunity (Coop and Kyriazakis, 1999).

The lower level of EO in ROH mice on diet 30 compared to diet 80 was an unexpected result but in agreement with similar findings in sheep, primary infected with the small intestinal nematode *Trichostrongylus colubriformis* (Athanasiadou *et al*. 2001). These observations may suggest that very low levels of nutrient supply can limit the parasite as well as the responses of the host (Houdijk and Athanasiadou, 2003). In the case of *H. bakeri*, these limitations may be incurred through villus atrophy that occurs under protein scarce environments (Tu *et al*. 2007). This in turn may reduce availability of epithelial cells as a food resource to *H. bakeri* (Bansemir and Sukhdeo, 1994, 1996).

The worm burden results were more subtly altered than egg count parameters in this current study with ROL mice showing a female worm bias. Such a bias is commonly reported in studies involving wild mice (Keymer and Dobson, 1987; Gregory *et al*. 1992). This difference in worm sex ratios between ROH and ROL may also be a product of potential genetic drift or inherent genetic correlation. Alternatively, differences in parasite sex ratios may be influenced by factors such as mating probabilities and disproportional survival between the sexes during larval stages in the host (Stien *et al*. 2005).

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Loss of ROL-30-I group

The loss of the ROL-30-I group made comparison of the treatment groups incomplete. Had this group been present, then the effect of protein on scarcity on resilience and resistance to *H. bakeri* infection may have been better understood. Clinical symptoms of infection were observed in this group from day 7 p.i. which corresponds to the time when larvae migrate from the intestinal mucosa to the lumen for their final moult to adult worms (Ehrenford, 1954*b*). This process is probably associated with mucosal damage, leading to loss of plasma proteins and epithelial cells, which are common features of intestinal parasitism (Coop and Holmes, 1996; van Houtert and Sykes, 1996). Because such losses would interfere with host maintenance, parasitized hosts would be expected to attempt to replenish them (Coop and Kyriazakis, 1999). Moreover, replenishment of these losses is the largest contributor to elevated protein requirements during gastrointestinal nematode infections (Houdijk *et al*. 2001). The clinical signs observed in the ROL-30-I mice may have arisen from their inability to respond to this temporary increased protein requirement. ROH-30-I mice did not show any clinical signs but displayed a small, temporal reduction in body weight on day 7 p.i. relative to day 4 and day 10 p.i. at similar levels of intake. This may suggest they were more able than their ROL counterparts to utilize body reserves to cope with the assumed temporal elevation of protein requirements at times of dietary protein scarcity. ROL mice infected with 250 L_3 *H. bakeri* and fed *ad libitum* a 50 g CP per kg food did not have any clinical signs of parasitism as observed in this study (Houdijk and Bünger, 2007). In addition, a single infection with 150 L_3 in 3 spare ROL-30-I mice also resulted in no clinical signs but a small temporal drop in body weight and an approximately 20% reduction in body weight gain over 28 days p.i. was observed, relative to ROL-30-C mice (data not shown). Taken together, these observations suggest that the inability of ROL-30-I mice to cope with the experimental treatment was likely a combination of the low dietary CP contents, the relatively high level of infection and their small body size.

The growth performance data obtained in the current experiment support the view that protein scarcity may only have been achieved in the ROH-30 mice; Fig. 2 suggests that growth performance did not differ between 80–280 g CP per kg in both lines whilst 30 g CP per kg causes a significant loss of performance in ROH mice when mice were not infected. This is in agreement with earlier findings, where BALB-c mice fed diets with 70 and 240 g CP per kg had similar growth performance, which was higher than that of mice fed diets with 30 g CP per kg (Boulay *et al*. 1998). The food intake data suggest ROL attempted to compensate for protein scarcity on the 30 g CP per kg diet through increasing their food intake. Such an increased intake would have been associated with a higher intake of energy, which in our experiment is reflected in the higher body fat percentage observed for ROL-30 mice compared to ROL mice on higher CP diets. In contrast, ROH mice were apparently unable to compensate for protein scarcity through an increased food intake; in fact, their intake on the 30 g CP per kg diet was significantly lower compared to ROH mice on higher CP diets. This result, is in accordance with the findings of Boulay *et al*. (1998) where food intake on the 30 g CP per kg diet was greatly reduced and the highest intake was observed on 70 g CP per kg diet. The significantly reduced intake of ROH-30 mice coincided with them having the lowest body fat percentage. It remains unclear why ROL and not ROH were able to overcome protein scarcity through displaying increased food intake but the data support the view that the protein requirement in ROL mice may be considerably lower than that in ROH mice. As such, the conditions for studying the effects of protein scarcity on genetic growth potential have not been met, which consequently confounds effects of protein scarcity with effects of genetic growth potential at the lowest levels of protein used in our study.

In conclusion, this study supports the view that narrow selection for a performance trait, body weight in this instance, may penalise immunocompetence and thus host ability to cope with a primary pathogen challenge and that, at least in terms of resilience, ensuring sufficient protein nutrition could minimize this penalty. Resistance *per se* may be due to genetic linkage or pleiotropy or alternatively through genetic drift during a primary infection, as this study found a lack of evidence supporting the allocation theory. Whether host protein nutrition affects the consequences of narrow selection for production traits on ability to cope with secondary infections remains to be addressed.

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