

Secondary infection of *Nippostrongylus brasiliensis* in lactating rats is sensitive to dietary protein content

Jos G. M. Houdijk^{1*}, Neil S. Jessop², David P. Knox³ and Ilias Kyriazakis¹

¹Animal Nutrition and Health Department, Scottish Agricultural College, West Mains Road, Edinburgh EH9 3JG, UK

²Institute of Atmospheric and Environmental Sciences, School of GeoSciences, University of Edinburgh, West Mains Road, Edinburgh EH9 3JG, UK

³Parasitology Division, Moredun Research Institute, Pentland Science Park, Bush Loan, Penicuik EH26 0PZ, UK

(Received 4 May 2004 – Revised 19 August 2004 – Accepted 3 December 2004)

Lactating mammals usually exhibit a breakdown of immunity to parasites, i.e. they have larger worm burdens than their non-lactating counterparts. Here, we tested the hypothesis that a secondary infection with *Nippostrongylus brasiliensis* in lactating rats is sensitive to dietary protein content. We also tested whether this infection affects host food intake. Rats either remained uninfected throughout the study or were given a single infection before mating (primary infection) and re-infected on day 2 of lactation (secondary infection) with 1600 infective larvae. Infected rats were fed foods during lactation formulated to supply 100 (low protein; LP), 200 (medium protein; MP) or 300 (high protein; HP) g crude protein per kg DM; non-infected rats were fed either the LP or HP food. Litter size was standardized to ten pups between parturition (day 0) and secondary infection (day 2). Ten days after secondary infection, MP and HP rats had excreted fewer nematode eggs, and had fewer adult nematodes in their small intestine and nematode eggs in their colon than the LP rats. Primary infection increased food intake in late pregnancy, and increased maternal body weight and litter size at parturition. Secondary infection did not affect mean food intake, maternal and litter weight, although food intake was reduced for 1 d following infection. These results support the view that a secondary infection with *N. brasiliensis* is sensitive to dietary protein content, and that the latter infection does not impair lactational performance. Future studies may focus on elucidating the nutritional sensitivity of immune responses underlying the reduced secondary *N. brasiliensis* infection.

Lactating rats: *Nippostrongylus brasiliensis*: Nutrition: Immunity: Protein

Animals acquire immunity to most parasites due to their continuous exposure towards them. However, the expression of acquired immunity to parasites can break down, particularly during the periparturient period. This phenomenon has long been recognized for gastrointestinal nematode parasites in sheep (Taylor, 1935), where it is associated with an increased gastrointestinal nematode burden and increased excretion of nematode eggs onto the pasture. This contributes to the epidemiology of infection, as it makes parasitized, periparturient sheep a major source of infection for their parasite-naïve offspring. However, periparturient breakdown of acquired immunity is not limited to gastrointestinal nematodes and sheep, but has been observed for a wide range of pathogens and hosts, including man (Houdijk *et al.* 2001a). Various hypotheses have been put forward to account for the phenomenon in sheep (Barger, 1993), but it remains poorly understood.

It has recently been suggested that periparturient breakdown of immunity to parasites may have a nutritional basis, as it could result from partially prioritized scarce protein allocation to reproductive functions (e.g. milk production) rather than to immune functions (Coop & Kyriazakis, 1999). Such a hypothesis would best be addressed in animals where relatively large differences in the degree of protein scarcity can be attained. The nutrient requirements of lactating rats can increase up to tenfold, relative

to nutrient requirements for maintenance, through manipulation of the number of suckling pups, and a wide range of protein supply can be achieved (Jessop, 1997). These manipulations suggest that the lactating rat is a suitable animal to achieve large differences in the degree of protein scarcity. In addition, rats acquire a strong immunity to the intestinal nematode *Nippostrongylus brasiliensis*; immune rats rapidly expel adult *N. brasiliensis* during a secondary infection (Jarrett *et al.* 1968), but a breakdown of immunity to *N. brasiliensis* occurs during re-infection in the lactating rat (Houdijk *et al.* 2003a). Moreover, immune expulsion of *N. brasiliensis* bears considerable similarities with those observed in small ruminants (Rothwell, 1989). Therefore, the *N. brasiliensis* re-infected lactating rat model would be an appropriate one for elucidating a possible nutritional basis of periparturient breakdown of immunity to parasites.

In a previous study (Houdijk *et al.* 2003a), feeding a high-protein (HP) food did not result in a reduced breakdown of immunity to *N. brasiliensis* in lactating rats. However, the observed food intake was considerably lower than that in parasite-free lactating rats, kept under the same nutritional protocol (Pine *et al.* 1994). Houdijk *et al.* (2003a) argued that the observed food intake may have resulted in a scarce protein supply for all rats, even for those on the HP food. Under those conditions, the increased

Abbreviations: CP, crude protein; HP, high protein; LP, low protein; MP, medium protein.

* **Corresponding author:** Dr Jos Houdijk, fax +44 131 535 3416, email jos.houdijk@sac.ac.uk

protein supply would not have been expected to improve expression of immunity to parasites (Coop & Kyriazakis, 1999; Houdijk *et al.* 2001a).

The present experiment re-addressed whether breakdown of immunity to *N. brasiliensis* in lactating rats is sensitive to dietary protein content, by using foods with higher protein contents and lower gross energy contents than used in Houdijk *et al.* (2003a). The lower gross energy contents were expected to result in increased food intake (Friggens *et al.* 1993). However, the lower than expected food intake in Houdijk *et al.* (2003a) could also have been due to the parasite infection *per se*. Therefore, in the present experiment, non-infected lactating rats were included to test whether infection with *N. brasiliensis* affects food intake during lactation. In the present experiment, changes in the degree of parasitism are considered as indirect indicators of changes in the degree of expression of immunity to *N. brasiliensis*. If it can be demonstrated that a secondary infection with *N. brasiliensis* in lactating rats is indeed sensitive to host (protein) nutrition, then future studies may focus on nutritional sensitivity of underlying immune responses.

Materials and methods

Animals and housing

Thirty-four second-parity, female Sprague–Dawley rats (Harlan Ltd, Loughborough, UK) were housed in a room regulated at 20–21°C with relative humidity between 45 and 65% and with a light period from 08.00 to 20.00 hours. The experiment started 45 d before the realized mean parturition date (day –45), with the parturition date being the morning when parturition was observed to have finished (day 0). Rats were individually housed in solid-bottom cages during most of the experiment, but were housed on wire-bottom cages for overnight faeces collection during the primary infection and during mating, as described previously (Houdijk *et al.* 2003a). Mating took place over a 9 d period and was confirmed through the presence of a vaginal plug.

Foods

All rats were given *ad libitum* access to standard rat chow (202 g digestible crude protein (CP) and 12.2 MJ digestible energy per kg DM; B&K Universal Ltd, Hull, UK) until mating was confirmed. Mated rats were given *ad libitum* access to a HP food (210 g CP/kg DM) from day –23 to day –13, followed by a low-protein (LP) food (60 g CP/kg DM) from day –13 until parturition. The diets were essentially the same as used previously (Houdijk *et al.* 2003a), with the exception of inclusion of cellulose (100 g/kg DM) to stimulate faecal formation. The two levels of CP nutrition during gestation were used to manipulate body protein content in order to minimize protein supply from such body protein reserves during lactation, since this can to some extent compensate for dietary protein scarcity during lactation (Pine *et al.* 1994).

Following parturition, lactating rats were given *ad libitum* access to one of three isoenergetic foods: LP (100 g CP/kg DM), medium protein (MP; 200 g CP/kg DM) or HP (300 g CP/kg DM). Three levels of CP were used in order to confirm that protein supply would not be limiting in this experiment. All diets were formulated to contain 18.0 MJ gross energy/kg DM. Table 1 presents the ingredients and the chemical analysis of the experimental foods offered during lactation.

Table 1. Composition and analysis of the experimental foods used during lactation

	Experimental group		
	LP	MP	HP
Ingredients (g/kg fresh)			
Casein	102	204	306
D,L-Methionine	1	2	3
Starch	304	255	206
Sucrose	152	127	103
Maize oil	198	169	139
Cellulose	94	94	94
Corn flour	46	46	46
Lecithin	2	2	2
Vitamins and minerals	101	101	101
Analysed chemical composition (g/kg DM)			
DM (g/kg fresh)	772	677	617
Crude protein	100	183	260
Diethyl ether extract	187	161	134
Acid-detergent fibre	63	47	56
Ash	23	25	23
Gross energy (MJ/kg DM)*	18.0	18.0	18.0

LP, low protein (100 g crude protein/kg DM); MP, medium protein (200 g crude protein/kg DM); HP, high protein (300 g crude protein/kg DM).

* Based on chemistry tables (Chemical Rubber Company, 2001).

Infection and experimental design

A subgroup of twenty-two rats received a primary infection on day –38, followed by a secondary infection on day 2 (infected), according to the previously established infection protocol (Houdijk *et al.* 2003a). Both infections consisted of 1600 third-stage infective *N. brasiliensis* larvae, which were suspended in 0.5 ml sterile PBS and were administered by subcutaneous injection in the hindlimb. The remaining rats were not infected but were injected with PBS at the same time (control).

The experiment consisted of five treatments, i.e. an incomplete 2 × 3 factorial combination of dietary protein and level of infection. The control rats were offered either the LP or HP food for 12 d during lactation, whilst infected rats were offered the LP, MP or HP food for the same period. The MP food was used to confirm that the protein supply for the HP food was not limiting, and was therefore not needed for both control and infected rats. The first two control rats whose litter size was standardized to ten pups were randomly allocated to either LP-C or HP-C. Likewise, the first three standardized infected rats were allocated to LP-I, MP-I or HP-I. These procedures were aimed to be repeated six times to obtain six replicates for each of the five treatments. However, one rat produced very few pups, two rats had dead litters and three rats were not pregnant. Therefore, achieved replications were six for LP-C, LP-I and MP-I, and five for HP-C and HP-I. Litter size standardization continued until day 2, using the pups from non-experimental rats. All infected rats were killed 10 d after secondary infection (day 12 of lactation) for the assessment of worm burdens and nematode egg counts in the colon contents (see later).

Sample collection and measurements

Body weight and food intake. All rats were weighed daily from arrival in the animal house onwards, to accustom them to the daily weighing during gestation and lactation. Food intake during pregnancy and lactation was measured daily by weighing used food jars and replacing them with filled food jars

of a known weight. Foods were sampled during their preparation for the analysis of DM, diethyl ether extract, acid-detergent fibre (cellulose), ash and CP ($6.25 \times \text{Kjeldahl-N}$). The latter was used to calculate achieved CP intake during lactation. From the morning of day 0 onwards, pups were counted, weighed and litter size was standardized. However, from day 2 onwards, litter sizes were no longer standardized.

Nematode egg counts and worm burden. Faeces were collected as described before (Houdijk *et al.* 2003a), from day -33 to day -26 (i.e. days 5 to 12 after primary infection), for monitoring the primary infection through the assessment of faecal egg counts. In addition, a faecal egg count was also carried out in the faeces produced over three 24 h periods during lactation. For this purpose, bedding of the solid-bottom cage was replaced with fresh bedding on the mornings of day 7, day 9 and day 11, and total faeces collection took place on the morning of day 8, day 10 and day 12. On day 12, rats were sedated through progressively increasing CO₂ inhalation, and then killed humanely by CO₂ asphyxiation. Large intestinal contents were collected, weighed and assessed for the number of nematode eggs, and adult nematodes were harvested from the small intestine, both as described before (Houdijk *et al.* 2003a). The nematodes obtained were stored in formaldehyde, pending the assessment of their number and sex.

Calculations and statistical analysis

Nematode egg excretion was calculated for each of the aforementioned three 24 h periods by multiplying the nematode egg count in the faeces with the amount of faeces produced. Likewise, the number of nematode eggs in the colon was calculated by multiplying the nematode egg count in the colon content with its amount. Because of their skewed nature, the data on nematode egg output and worm burden were transformed according to $\log(n+1)$ to normalize them before statistical analysis. These data are reported as back-transformed means with 95% CI, and were calculated as $10^a - 1$ with $a = \mu + 0.5\sigma^2$ (Johnson *et al.* 1988), where μ is the mean, lower or upper limit of the 95% CI of the transformed data and σ is the standard deviation of the transformed data. The arithmetic means and either SE or SED are reported for the non-transformed data.

The first hypothesis (secondary infection of *N. brasiliensis* in lactating rats is sensitive to dietary protein contents) was assessed through an ANOVA for the effect of feeding treatments LP-I, MP-I and HP-I on nematode egg output and worm burden. The second hypothesis (infection with *N. brasiliensis* affects host food intake) was assessed through a 2×2 factorial design on achieved DM intake during lactation, using dietary protein (LP and HP), infection status (control and infected) and their interaction as the independent factors. This model was also used to assess treatment effects on maternal and litter body weight. These analyses included pre-infection maternal body weight as a covariate. ANOVA for intake, maternal and litter body weight, and nematode egg excretion were used within a repeated-measures procedure (split-plot model). The main-plot factors (dietary protein, infection and their interaction) were tested against the main-plot error, which implicitly represented the random animal effect. Time and main factors \times time interactions were tested against the residual error in the split plot. The df from the split plot within the repeated-measures procedure were multiplied with the Greenhouse–Geisser ϵ factor, in order

to test effects measured over time more conservatively (Littel *et al.* 1998). An additional ANOVA was carried out using the factorial model on each time point during lactation when interactions with time were significant. The results on intake, maternal and litter body weight were considered significant at $P < 0.005$, i.e. these were adjusted for multivariate ANOVA. However, it was decided *a priori* to also analyse DM intake during lactation through an antedependence model for repeated measurements. Such a model for repeated measures was considered more appropriate than a split-plot model because it does not rely on the presence of interactions with time to identify whether infection would affect food intake. If such an effect was present, then it would be short-lived (Mercer *et al.* 2000), and it was anticipated that the split-plot model for repeated measurements ANOVA would not be sufficiently sensitive to detect such temporary changes. All statistical analyses were performed with Genstat 6 (Release 6.1.0.200; Lawes Agricultural Trust, Rothamsted, UK).

Results

Faecal egg counts during primary infection and performance until parturition

Ten of the twenty-two infected rats had started to excrete small numbers of nematode eggs in their faeces by day -33 , averaging 31 (95% CI 18, 52) eggs/g, and all rats excreted nematode eggs by day -32 . Mean faecal egg counts then increased rapidly to peak on day -30 at 9742 (95% CI 8648, 11 366) eggs/g. The faecal egg counts then decreased gradually, and all rats stopped excreting nematode eggs by day -26 , i.e. 12 d after primary infection.

Infection status did not affect body weight and food intake between day -23 and day -13 , when the rats were fed the HP gestation food (Fig. 1). The rats grew on average from 288 (SE 5) g to 328 (SE 5) g and their DM intake averaged 18.1 (SE 0.3) g/d during this period. However, infection status affected rat body weight and food intake from day -13 until parturition (Fig. 1). During this period, the control and infected rats grew to 363 and 405 g on day -1 , respectively (SED 8; df = 25; $P < 0.001$), and the DM intake of the LP gestation food averaged 13.7 and 17.4 g/d, respectively (SED 0.9; df = 25; $P < 0.001$).

Infection status affected the parturition weight of the rat and her litter. Body weight on day 0 averaged 286 and 313 g for the control and infected rats, respectively (SED 7; df = 25; $P < 0.001$) and the litter weight averaged 56.2 and 67.5 g, respectively (SED 4.7; df = 25; $P = 0.019$). Mean pup weight did not differ, averaging 5.5 ± 0.1 g, but natal litter size tended to be lower for the control rats than for the infected rats (10.5 v. 12.6 pups; SED 1.2; df = 28; $P = 0.104$).

Nematode egg excretion, eggs in colon and worm burden following secondary infection

Table 2 shows the back-transformed mean nematode egg excretion on days 8, 10 and 12 of lactation. Across time, HP-I rats excreted fewer eggs than LP-I and MP-I rats ($P = 0.023$). However, time and feeding treatment interacted for daily egg excretion ($P = 0.039$), which was reflected in different effects of feeding treatment at the different time points; HP-I rats excreted fewer eggs on day 8 and day 10 than LP-I and MP-I rats, while both MP-I and HP-I rats tended to excrete fewer nematode eggs than LP-I rats on day 12 (Table 2). Feeding treatment significantly affected the number of nematode eggs found in the colon and the

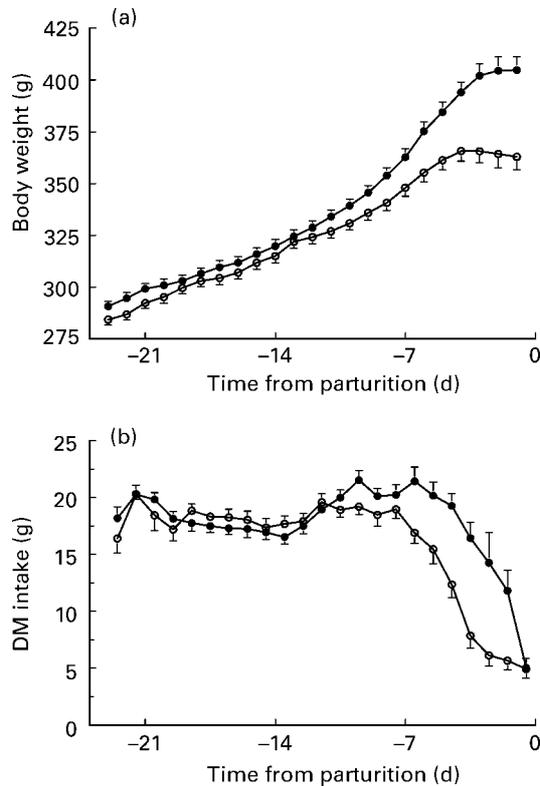


Fig. 1. Body weight during pregnancy (a) and DM intake (b) of rats either immunized through a single infection with *Nippostrongylus brasiliensis* on day -38 (●) or sham-infected (○). Rats were fed a high-protein food from day -23 until day -13 (210 g crude protein/kg DM), followed by a low-protein food until parturition (60 g crude protein/kg DM). Values are least square means with standard errors shown by vertical bars.

worm burden (Fig. 2), with LP rats having a larger number of nematode eggs in their colon ($P < 0.001$) and a larger number of worms in the small intestine than the MP and HP rats ($P = 0.005$). Feeding treatment did not significantly affect the percentage of male and female worms; the percentage of male worms averaged 31.6 ± 3.3 .

Performance during secondary infection

Feeding treatment and time significantly interacted for DM intake ($P < 0.001$); the LP rats had lower DM intake than the HP rats

from day 6 onwards, and this difference increased over time (Fig. 3). The associated mean achieved CP intake of the LP and HP rats was 2.0 and 7.9 g/d, respectively (SED 0.2 g/d; $df = 18$; $P < 0.001$). The split-plot model for repeated measures did not detect significant interactions between infection status and time ($P = 0.30$), and between feeding treatment, infection status and time ($P = 0.37$) for DM intake. However, the ANOVA through the antedependence model revealed that control rats had higher DM intake than infected rats on day 4 and day 5 ($P = 0.028$). DM intake of the control and infected rats averaged 23.7 and 17.8 g on day 4 (SED 1.6 g; $df = 18$; $P = 0.002$) and 25.0 and 22.0 g on day 5 (SED 1.5 g; $df = 18$; $P = 0.055$), respectively. Significant effects of interactions between feeding treatment and infection status on DM intake were not observed through the antedependence model.

None of the five experimental groups had a mean number of pups born that was significantly smaller than ten, i.e. the standardized litter size, and subsequent feeding treatment and infection status did not affect pup survival (mean litter size on day 12 was 9.9 ± 0.1 across treatment groups). Feeding treatment and time significantly interacted for maternal and litter body weight ($P < 0.001$); the LP rats had lower maternal and litter body weights than the HP rats from day 6 and day 2 onwards, respectively, and this difference increased over time (Fig. 3). Infection status affected maternal but not litter body weight; pooled over feeding treatment and time during lactation, the control rats weighed less than the infected rats (281 v. 295 g; SED 6 g; $df = 18$; $P = 0.048$). There were no significant infection status \times time or feeding treatment \times infection status \times time interactions on maternal and litter body weight, although there was a tendency for maternal body weight to converge, especially for the HP rats (Fig. 3). Mean DM intake, achieved CP intake, maternal and litter body weight of the MP-I rats during lactation was 30.6 (SE 1.3) g/d, 5.6 (SE 0.2) g/d, 334 (SE 10) g and 133 (SE 11) g, respectively. These were similar to those from the HP-I rats.

Discussion

The hypothesis tested was that a secondary infection with *N. brasiliensis* in lactating rats is sensitive to dietary protein content. This hypothesis could not be tested in Houdijk *et al.* (2003a) because the food intake achieved was lower than expected. In the present experiment, DM intake of the MP-I rats was approximately 20% higher than that in the aforementioned study, whilst gross energy intake was similar. Since the lactational performance of the HP and MP rats was similar, it can be concluded that protein

Table 2. Daily nematode egg excretion in lactating rats secondarily infected with *Nippostrongylus brasiliensis* and offered foods calculated to supply 100 (LP), 200 (MP) or 300 (HP) g crude protein/kg DM

(Values are expressed as eggs/d with 95% CI in parentheses)

	Feeding treatment			P value*
	LP	MP	HP	
Days in lactation				
Day 8	3405 (963, 12 035)	1669 (1258, 2214)	341 (58, 1993)	0.041
Day 10	1855 (1444, 2383)	2608 (1905, 3571)	958 (189, 4838)	0.013
Day 12	3603 (3160, 4108)	1936 (586, 6390)	722 (566, 921)	0.097

LP, low protein; MP, medium protein; HP, high protein.

* Effect of feeding treatment (main plot): $P = 0.020$; effect of time and time \times feeding treatment (split plot): $P = 0.118$ and $P = 0.039$, respectively.

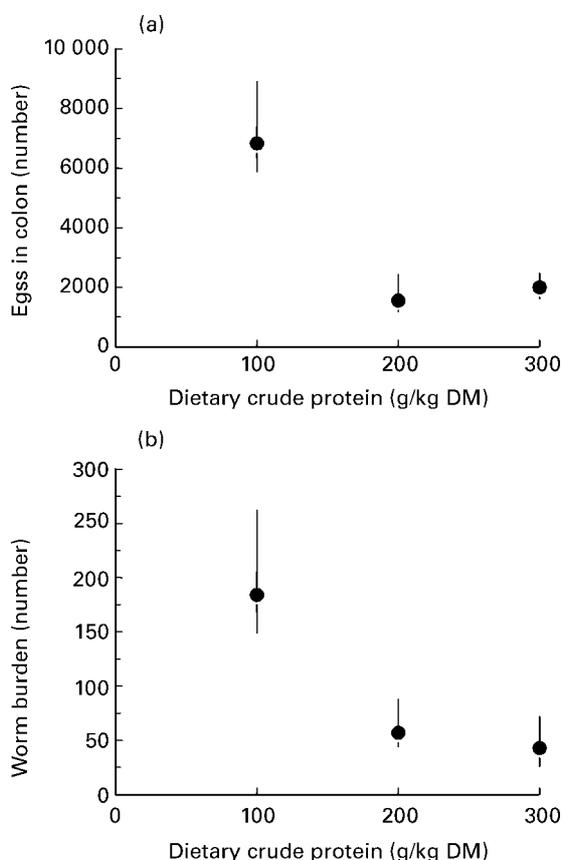


Fig. 2. Number of parasite eggs in the large intestine (eggs in colon, (a)) and adult parasites in the small intestine (worm burden, (b)) of lactating rats offered foods calculated to supply 100, 200 or 300 g crude protein/kg DM. Rats were secondarily infected with *Nippostrongylus brasiliensis* on day 2 of lactation and sampled on day 12 of lactation. Values are back-transformed means with 95% CI shown by vertical bars.

supply from the MP and HP foods was not scarce. Hence, the hypothesis can be addressed by comparing nematode egg excretion and worm burdens of the LP-I rats with those of the MP-I and HP-I rats.

Relative to the LP rats, faecal nematode egg excretion, number of nematode eggs in the colon and worm burden of the MP and HP rats were on average reduced by 53, 74 and 63%, respectively. These results may have been the outcome of at least one of three mechanisms. First, these results may be the outcome of improved expression of immunity due to an increased protein intake. Expression of immunity to *N. brasiliensis* is sensitive to host protein supply (Bolin *et al.* 1977; Cummins *et al.* 1987), and studies in lactating farm animals would support this view (Chartier *et al.* 2000; Donaldson *et al.* 2001; Houdijk *et al.* 2003c; Kahn *et al.* 2003).

Second, the observed effects may be the outcome of improved expression of immunity due to an increased intake of any nutrient and/or energy, as the increased protein intake from the MP and HP foods coincided with an increased intake *per se*. Most studies in ruminants have shown that expression of immunity to gastrointestinal parasites is not sensitive to moderate changes in energy supply (Bown *et al.* 1991; Donaldson *et al.* 1998; Houdijk *et al.* 2000). Whilst there is no evidence to suggest that intake of carbohydrates *per se* affects immunity, fat intake may affect host immunity (Yaqoob, 2004). The latter is, however, more likely related to increased intake of *n*-3 rather than *n*-6 fatty acids,

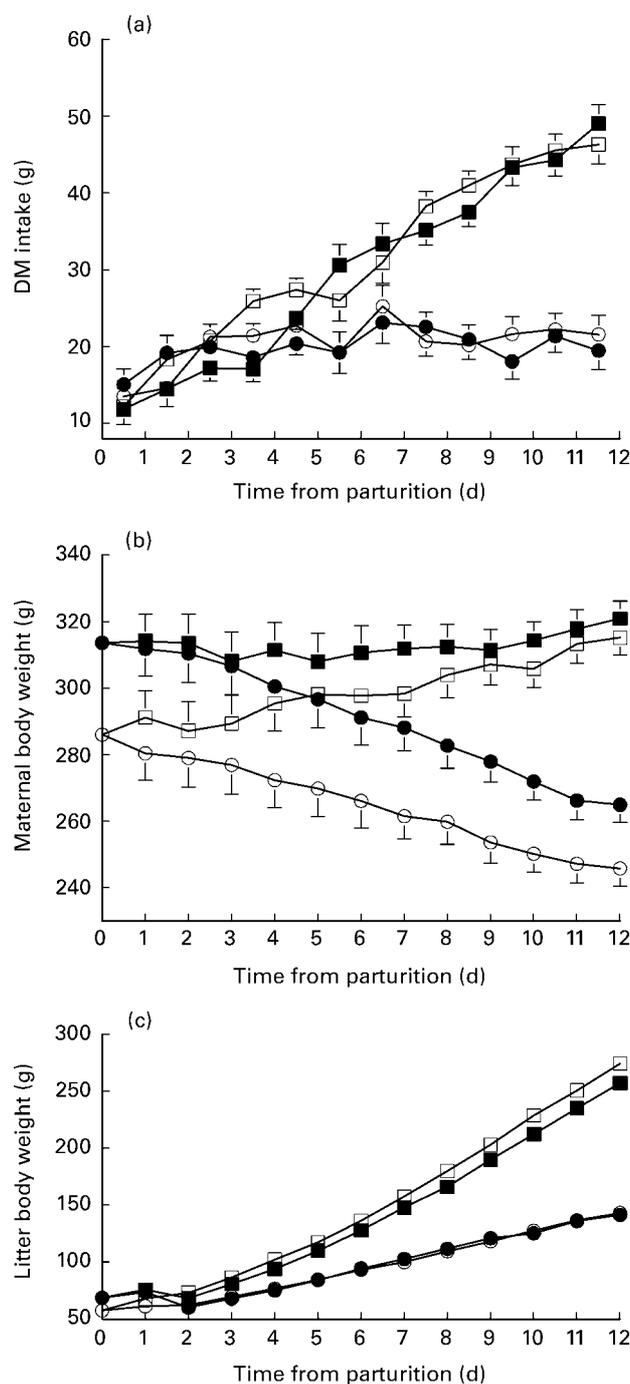


Fig. 3. DM intake (a), maternal body weight (b) and litter body weight (c) of rats either secondarily infected with *Nippostrongylus brasiliensis* (●, ■) or not (○, □), and offered foods calculated to supply 100 (●, ○) or 300 (■, □) g crude protein/kg DM. Values are least square means with standard errors shown by vertical bars. The pooled SE for litter body weight was too small to be visible, but increased gradually from 3.5 g on day 0 to 7.6 g at day 12.

derived from the maize oil used in the present experiment. Vitamin and mineral intake has also been associated with immunity (Koski & Scott, 2001). However, the foods used were formulated to provide more than sufficient minerals and vitamins (National Research Council, 1995) for any level of lactational performance, suggesting that the latter were unlikely to be scarce.

Third, the increased food intake could have changed the gut environment into a less favourable one for parasite survival, mediated through effects of rate of passage or intake of specific food components. There is evidence that fibre nutrition can affect gastrointestinal parasitism (Herbert & Nickson, 1969; Pearce, 1999; Petkevicius *et al.* 1999). Achieved mean fibre (cellulose) intake for the LP, MP and HP rats was 1.1, 1.7 and 1.6 g/d (SED 0.1; df = 15; $P < 0.001$). However, it is unlikely that the increased intake of cellulose affected the degree of parasitism, as the latter would be mediated through intake of highly fermentable fibre (Petkevicius *et al.* 2003). Furthermore, it has been shown that reducing nutrient demand in lactating sheep resulted in a reduced degree of parasitism without affecting food intake *per se* (Houdijk *et al.* 2003b). The latter supports the view that changing gut environment through changes in food intake *per se* is not necessarily required to affect parasitism.

This discussion supports the view that the observed reduced level of parasitism in the present experiment likely resulted from effects of protein supply on expression of immunity to *N. brasiliensis*. However, future studies may confirm this through assessing underlying immune responses. In addition, this *N. brasiliensis* re-infected lactating rat model may also be used to: (i) re-examine the discrepancy between effects of protein and energy supply on parasitism, as its monogastric nature allows more rigorous testing than possible in the ruminant host; (ii) confirm the absence of effects of changes in gut environment on gastrointestinal parasitism.

Food intake of the infected rats during lactation was temporarily reduced relative to the non-infected controls on day 4 and to a lesser extent on day 5, i.e. days 2 and 3 after infection. Although this pattern is consistent with anorexia observed during the first days following a single infection with *N. brasiliensis* in parasite-naïve rats (Mercer *et al.* 2000), more observations of the effects of infection on intake in (partially) immune animals are required to establish its significance, as the scarce information available suggests that anorexia is not likely to occur during a secondary infection (Kyriazakis *et al.* 1998). The infection with *N. brasiliensis* did not affect averaged DM intake, maternal and litter body weight during lactation, although there was a tendency that the non-infected, lactating HP rats grew faster than their infected counterparts over the total lactation period. Therefore, it remains unclear what caused the lower than expected food intake in Houdijk *et al.* (2003a), but it was unlikely a consequence of the rodent–parasite model used. The absence of an effect of infection on litter weight in the LP rats during lactation supports the view that scarce nutrient allocation to milk production takes priority over expression of immunity (Coop & Kyriazakis, 1999). This is because in the converse situation, i.e. had immunity been given a higher priority than milk production for the allocation of scarce nutrients, infection would be expected to have penalized litter growth.

The previously infected rats had higher DM intake and growth during late pregnancy than their non-infected counterparts, which was reflected in larger natal litter sizes and maternal body weights. These unexpected effects agree with observations from wild-type house mice, infected with the gastrointestinal nematode parasite *Heligmosomoides polygyrus*, which produced more pups than their non-infected counterparts (Kristan, 2004). However, in the present study, these results were obtained at times when the rats were not actually being challenged. This suggests that the host immune status, rather than infection or immune responses

per se, may be involved in modulating (scarce) resource allocation to reproductive effort. This hypothesis may provide some explanation for why the non-infected rats did not increase their intake to achieve a similar reproductive output.

DM intake of the LP rat was considerably lower than DM intake of the MP and HP rats (Fig. 3). A low intake on low-protein foods during lactation has been observed across species, e.g. in rats (Pine *et al.* 1994), pigs (Mahan & Mangan, 1975), sheep (Houdijk *et al.* 2001b) and dairy cows (Tolkamp *et al.* 1998). It is unclear why lactating animals do not increase food intake to achieve higher protein intake from LP foods. It has been suggested that lactating animals cannot metabolize the associated excess energy, at times of body fat mobilization (Pine *et al.* 1994). However, Tolkamp *et al.* (1998) suggested that such excess energy could be put into non-protein milk solids and body fat. Intake of LP foods may also be constrained by an inability to lose heat to the environment (Emmans & Kyriazakis, 2000). However, this cannot have played a major role, as heat production will undoubtedly have been much larger in the MP and HP rats.

In conclusion, the results from the present study support the view that a secondary infection with *N. brasiliensis* in lactating rats is sensitive to dietary protein content, and that the latter infection does not impair food intake. As feeding HP foods was associated with increased food intake *per se*, additional studies would be required to exclude the possibility that these effects are the result of (i) an increased intake of non-nitrogenous food ingredients or (ii) changes in gut environment into one less favourable for parasite survival. However, having demonstrated the nutritional sensitivity of a secondary *N. brasiliensis* infection in the lactating rat, it is now timely to elucidate the nutritional sensitivity of underlying immune responses in future studies.

Acknowledgements

We wish to thank Graeme Allan, Ellie McGiver, John Verth, John Tweedy, Vicky Brown and Sheena Boath for their assistance in taking care of the animals, sampling, and analytical and parasitological support. The Scottish Agricultural College and the Moredun Research Institute receive support from the Scottish Executive, Environment and Rural Affairs Department.

References

- Barger IA (1993) Influence of sex and reproductive status on susceptibility of ruminants to nematode parasitism. *Int J Parasitol* **23**, 463–469.
- Bolin TD, Davis AE, Cummins AG, Duncombe VM & Kelly JD (1977) Effect of iron and protein deficiency on the expulsion of *Nippostrongylus brasiliensis* from the small intestine of the rat. *Gut* **18**, 182–186.
- Bown MD, Poppi DP & Sykes AR (1991) The effect of post-ruminal infusion of protein or energy on the pathophysiology of *Trichostrongylus colubriformis* infection and body composition in lambs. *Austr J Agric Res* **42**, 253–267.
- Chartier C, Etter E, Hoste H, Pors I, Mallereau M-P, Broqua C, Mallet S, Koch C & Massé A (2000) Effects of the initial level of milk production and of the dietary protein intake on the course of natural nematode infection in dairy goats. *Vet Parasitol* **92**, 1–13.
- Chemical Rubber Company (2001) *Handbook of Chemistry and Physics*, 82nd ed. London: CRC Press.
- Coop RL & Kyriazakis I (1999) Nutrition–parasite interaction. *Vet Parasitol* **84**, 187–204.

- Cummins AG, Duncombe VM, Bolin TD, Davis AE & Yong J (1987) The response of the small intestine of the protein-deficient rat to infection with *Nippostrongylus brasiliensis*. *Int J Parasitol* **17**, 1445–1450.
- Donaldson J, van Houtert MFJ & Sykes AR (1998) The effect of nutrition on the periparturient parasite status of mature ewes. *Anim Sci* **67**, 523–533.
- Donaldson J, van Houtert MFJ & Sykes AR (2001) The effect of dietary fish-meal supplementation on parasite burdens of periparturient sheep. *Anim Sci* **72**, 149–158.
- Emmans GC & Kyriazakis I (2000) Issues arising from genetic selection for growth and body composition characteristics in poultry and pigs. In *The Challenge of Genetic Change in Animal Production*, pp. 36–53 [WG Hill, SC Bishop, B McGuirk, JC McKay, G Simm and AJ Webb, editors]. Edinburgh: British Society of Animal Science.
- Friggens NC, Hay DEF & Oldham JD (1993) Interactions between major nutrients in the diet and the lactational performance of rats. *Br J Nutr* **69**, 59–71.
- Herbert IV & Nickson EW (1969) Dietary factors and the production of *Oesophagostomum* sp. ova in breeding pigs. *Vet Rec* **84**, 569–570.
- Houdijk JGM, Kyriazakis I, Jackson F & Coop RL (2000) Effects of protein and energy supply on the resistance to nematodes in pregnant ewes. *Proc Br Soc Anim Sci* **116**, 33.
- Houdijk JGM, Jessop NS & Kyriazakis I (2001a) Nutrient partitioning between reproductive and immune functions in animals. *Proc Nutr Soc* **60**, 515–525.
- Houdijk JGM, Kyriazakis I, Coop RL & Jackson F (2001b) The relationship between protein nutrition, reproductive effort and breakdown in immunity to *Teladorsagia circumcincta* in periparturient ewes. *Anim Sci* **72**, 595–606.
- Houdijk JGM, Jessop NS, Knox DP & Kyriazakis I (2003a) Breakdown of immunity to *Nippostrongylus brasiliensis* in lactating rats. *Br J Nutr* **90**, 809–814.
- Houdijk JGM, Kyriazakis I, Jackson F & Coop RL (2003b) Reducing the degree of protein scarcity rapidly increases immunity to nematodes in ewes. *Proc Br Soc Anim Sci* **119**, 29.
- Houdijk JGM, Kyriazakis I, Jackson F, Huntley JF & Coop RL (2003c) Is the allocation of metabolisable protein prioritised to milk production rather than to immune functions in *Teladorsagia circumcincta*-infected lactating ewes? *Int J Parasitol* **33**, 327–338.
- Jarrett EEE, Jarrett WFH & Urquhart GM (1968) Quantitative studies on the kinetics of establishment and expulsion of intestinal nematode populations in susceptible and immune hosts. *Nippostrongylus brasiliensis* in the rat. *Parasitology* **58**, 625–639.
- Jessop NS (1997) Protein metabolism during lactation. *Proc Nutr Soc* **56**, 169–175.
- Johnson NL, Kotz S & Balakrishnan N (1988) *Continuous Univariate Distributions*, 2nd ed., vol. 1. Boston, MA: PWS-KENT.
- Kahn LP, Knox MR, Gray GD, Lea JM & Walkden-Brown SW (2003) Enhancing immunity to nematode parasites in single-bearing Merino ewes through nutrition and genetic selection. *Vet Parasitol* **112**, 211–225.
- Koski KG & Scott ME (2001) Gastrointestinal nematodes, nutrition and immunity: breaking the negative spiral. *Annu Rev Nutr* **21**, 297–321.
- Kristan DM (2004) Intestinal nematode infection affects host life history and offspring susceptibility to parasitism. *J Anim Ecol* **73**, 227–238.
- Kyriazakis I, Tolkamp BJ & Hutchings MR (1998) Towards a functional explanation of the occurrence of anorexia during parasitic infections. *Anim Behav* **56**, 265–274.
- Littel RC, Henry PR & Ammerman CB (1998) Statistical analysis of repeated measures data using SAS procedures. *J Anim Sci* **76**, 1216–1231.
- Mahan DC & Mangan LT (1975) Evaluation of various protein sequences on the nutritional carry over from gestation to lactation with first litter sows. *J Nutr* **105**, 1291–1298.
- Mercer JG, Mitchell PI, Moar KM, Bisset A, Geissler S, Bruce K & Chappell LH (2000) Anorexia in rats infected with the nematode *Nippostrongylus brasiliensis*: experimental manipulations. *Parasitology* **120**, 641–647.
- National Research Council (1995) *Nutrient Requirements of Laboratory Animals*, 4th ed. Washington, DC: National Academy Press.
- Pearce GP (1999) Interactions between dietary fibre, endo-parasites and *Lawsonia intracellularis* bacteria in grower-finisher pigs. *Vet Parasitol* **87**, 51–61.
- Petkevicius S, Nansen P, Bach KKE & Skjoth F (1999) The effect of increasing levels of insoluble dietary fibre on the establishment and persistence of *Oesophagostomum dentatum* in pigs. *Parasite* **6**, 17–26.
- Petkevicius S, Knudsen KEB, Murrell KD & Wachmann H (2003) The effect of inulin and sugar beet fibre on *Oesophagostomum dentatum* infection in pigs. *Parasitology* **127**, 61–68.
- Pine AP, Jessop NS & Oldham JD (1994) Maternal protein reserves and their influence on lactational performance in rats. *Br J Nutr* **71**, 13–27.
- Rothwell TLW (1989) Immune expulsion of parasitic nematodes from the alimentary tract. *Int J Parasitol* **19**, 139–168.
- Taylor EL (1935) Seasonal fluctuation in the number of eggs of trichostrongylid worms in the faeces of ewes. *J Parasitol* **21**, 175–179.
- Tolkamp BJ, Dewhurst RJ, Friggens NC, Kyriazakis I, Veerkamp RF & Oldham JD (1998) Diet choice by dairy cows. 1. Selection of feed protein content during the first half of lactation. *J Dairy Sci* **81**, 2657–2669.
- Yaqoob P (2004) Fatty acids and the immune system: from basic science to clinical applications. *Proc Nutr Soc* **63**, 89–104.