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The effects of changes in nutritional demand on gastrointestinal parasitism in lactating rats

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Lactating rats experience a breakdown of immunity to parasites, i.e. they carry larger worm burdens after re-infection compared to their non-lactating counterparts. Feeding high-protein foods to lactating rats results in reduced worm burdens. This could be attributed to changes in gastrointestinal environment or to overcoming effects of nutrient scarcity on host immunity. The latter hypothesis was addressed through a manipulation of nutrient demand by manipulating litter size. Twenty-three rats were immunized prior to mating and re-infected on day 2 of lactation with 1600 infective *Nippostrongylus brasiliensis* larvae. From parturition onwards, rats received *ad libitum* a low-protein food (100 g crude protein/kg). Litter sizes were standardised to nine (LS9), six (LS6) or three (LS3) pups, by day 2 of lactation. After a further 10 d, LS9 and LS6 rats carried more worms than LS3 rats. However, feeding treatments did not affect concentrations of mucosal inflammatory cells. Achieved feed intake did not differ consistently between the treatment groups. However, LS9 and LS6 rats lost weight, whilst LS3 rats gained weight during lactation. The results support the view that resistance to *N. brasiliensis* is sensitive to changes in nutrient demand, and the improved resistance to *N. brasiliensis* is likely due to effects of overcoming nutrient scarcity on host immunity.

Lactating rats: *Nippostrongylus brasiliensis*: Nutrition: Immunity: Litter size

There has been renewed interest into why previously immune periparturient hosts are predisposed to a relaxation in immunity towards pathogens, including gastrointestinal parasites (Coop & Kyriazakis, 2001). The nutritional changes associated with pregnancy and lactation have been considered to be an important factor influencing this host–parasite relationship. It has been proposed that a reproductive animal prioritises the degree to which it allocates scarce nutrient resource(s) to various body functions (Coop & Kyriazakis, 1999). This nutrient-partitioning framework suggests that the allocation of scarce nutrients to the functions associated with parasite control have a low partial priority relative to reproductive effort. As a consequence, at times of nutrient scarcity, the periparturient animal would experience a breakdown of immunity to parasites, evidenced by an increased gastrointestinal nematode burden and faecal egg count.

The proposed nutritional basis of periparturient breakdown of immunity to parasites implies that the degree of parasitic burden would be affected by changes in the degree of nutrient scarcity. This hypothesis has been addressed in a rodent model. Earlier studies using previously naïve rats have shown that immune expulsion of a primary infection of the gastrointestinal nematode *Nippostrongylus brasiliensis* is impaired in lactating rats relative to their nulliparous controls (Dineen & Kelly, 1972). This model has now been extended to previously immune rats, demonstrating that lactating rats exhibit a breakdown of immunity to *N. brasiliensis* (Houdijk *et al.* 2003*a*). A recent study has shown that this breakdown has indeed a nutritional basis, as feeding a high-protein diet resulted in a reduced level of parasitism relative to a low-protein diet (Houdijk *et al.* 2005*a*). However, it could not be excluded that this increased resistance was due to dietary components directly changing the gastrointestinal environment into a less favourable one for parasite survival, as offering the high-protein diet also resulted in increased food intake *per se* (Houdijk *et al.* 2005*a*). There is evidence, for example, that fibre nutrition can affect gastrointestinal parasitism through manipulation of the gut environment (Petkevicius *et al.* 1999).

In addition to increasing nutrient supply, nutrient scarcity can also be reduced and eventually overcome through a reduction in nutrient demand, e.g. through manipulating (reducing) litter size. In recent studies on sheep, such manipulation has not resulted in differences in food intake (Houdijk *et al.* 2001, 2006). Hence, this methodology may be used to study

Abbreviations: CP, crude protein; LS3, litter size of three pups; LS6, litter size of six pups; LS9, litter size of nine pups.

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effects of reducing or overcoming nutrient scarcity on gastrointestinal nematode parasitism, whilst direct effects of diet on gut environment can be avoided. It was hypothesised that the degree of *N. brasiliensis* infection occurring in the lactating rat offered a low-protein food would be sensitive to changes in nutrient demand. A reduction in nutrient demand, achieved through reducing litter size, would be expected to result in a reduced worm burden.

Materials and methods

Animals and housing

Twenty-three second parity female Sprague-Dawley rats (Harlan Ltd, Oxfordshire, UK) were housed in a room where ambient temperature was maintained at 21°C, relative humidity ranged from 45 to 65 %, and artificial lighting was provided between 08.00 and 18.00 hours. Rats were housed in solid-bottomed cages with fresh sawdust being provided weekly and a handful of shredded plastic bubble wrapping for nesting material from 3 d before expected parturition until the end of the experiment. Wire-bottomed cages were used during mating and for faeces collection during the primary infection as described previously (Houdijk *et al.* 2003*a*). For mating, female rats were placed with a proven male breeder and mating was confirmed through the presence of a vaginal plug.

Foods

All rats were given *ad libitum* access to standard rat chow (222 g digestible crude protein (CP) and 12·2 MJ digestible energy per kg DM), until mating was confirmed. Mated rats were given *ad libitum* access to a high-protein food (210 g CP/kg DM) for 10 d followed by a low-protein food (60 g CP/kg DM) until parturition. This feeding protocol was used to reduce body protein reserves during the second half of gestation in order to maximize the degree of protein scarcity during lactation when rats are on low-protein foods (Pine *et al.* 1994; Houdijk *et al.* 2005*a*). Parturition was considered as day 0 and from then until day 12 of lactation rats were given *ad libitum* access to a low-protein food, formulated to supply 100 g CP/kg DM. Ingredients and chemical analysis of the experimental food offered during lactation is shown in Table 1.

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

	Experimental diet
Ingredients (g/kg fresh matter)	
Casein (plus 1 % methionine)	103
Starch	304
Sucrose	152
Maize oil	197
Vitamins	47
Minerals	47
Cornflour	46
Choline	7
Lecithin	2
Alphacel	94
Analysed chemical composition	
DM (g/kg fresh matter)	783
Gross energy (MJ/kg DM)*	19·7
Crude protein (g/kg DM)	124
Diethyl ether extract (g/kg DM)	189
Ash (g/kg DM)	42

* Calculated from feed tables.

Infection protocol and experimental design

All rats were infected through a subcutaneous injection with *N. brasiliensis* according to a previously established protocol (Houdijk *et al.* 2003*a*). Rats received on day −37 (37 d before the realised mean parturition date) a primary infection of 1600 third-stage infective larvae of *N. brasiliensis*, which were suspended in 0·5 ml sterile PBS. A secondary infection of 1600 third-stage infective larvae of *N. brasiliensis* was administered on day 2 of lactation.

The experiment consisted of three treatments, with litter size being standardised on day 2 of lactation at three (LS3), six (LS6) or nine (LS9) pups. The different litter sizes were chosen to result in different degrees of nutrient scarcity. Using previous data collected by Houdijk *et al.* (2005*a*), it was hypothesised that a nursing dam fed the low-protein food could support normal growth of three pups, resulting in nutrient abundance. However, when nursing six or nine pups, achieved intake of the low-protein food would limit her lactational performance, thus resulting in nutrient scarcity.

The aim was to obtain seven replicates for each of the three treatments. However, five rats did not conceive, one was killed for unrelated reasons and data from one LS6 rat was omitted because litter size was not maintained at six pups. Therefore, the achieved number of replicates were six for LS9 and LS3, and four for LS6.

All rats were killed on day 12 (i.e. 10 d post secondary infection) for the assessment of worm burdens, number of nematode eggs in the colon contents, and concentration of inflammatory cells in the small intestinal mucosa (see later).

Sample measurements and collection

Body weight and food intake. Rats were weighed daily throughout the experiment. Feed intake was measured daily during gestation and lactation. The pups were counted and weighed daily from day 0. Foods offered during gestation and lactation were sampled during their preparation for the analysis of DM, CP (Kjeldahl-N × 6·25), ether extract and ash.

Nematode egg counts and worm burdens. From 5 d post primary infection (day −32), faeces were collected daily for 7 d as described previously (Houdijk *et al.* 2003*a*) for the assessment of faecal egg counts (eggs/g faeces). This was done to provide evidence that a primary infection had established.

During the secondary infection, faeces were collected for three 24 h periods, starting in the morning of days 7, 9 and 11. On the first morning of faeces collection, the solid-bottomed cages were cleaned and a small amount of fresh sawdust was added at 08.00 hours. Fresh faeces were then collected every 2–3 h until 18.00 hours. Half of the collected fresh faeces were kept refrigerated in a sealed plastic bag, pending the assessment of faecal egg counts. The other half was used to determine its air DM content, which was estimated through drying at room temperature until the next

morning. The rest of the faeces produced overnight were collected in the morning of days 8, 10 and 12, respectively. This enabled the total wet faeces production and nematode egg excretion over three 24 h periods to be calculated (see later). We needed to estimate total faeces production to account for potential effects of faeces volume on faecal egg counts. The latter is a concentration measure, and the litter size treatments could have resulted in different volumes of faeces produced.

Colon contents and worm burden. Rats were sedated by gradually increasing ambient concentration of CO_2 and humanely killed by CO_2 asphyxiation on day 12 and dissected to collect the small and large intestine. Large intestinal contents were weighed and assessed for the concentration of nematode eggs, and nematodes were harvested from the small intestine, both as described previously (Houdijk *et al.* 2003*a*). A small 2 cm section of small intestine, 15 cm down from the stomach, was placed in 4 % paraformaldehyde for 6 h and then transferred to 70 % ethanol. The small intestine sections were embedded in paraffin wax and mounted on slides for histochemical quantification of inflammatory cells. Sections of tissue were stained with toludine blue at pH 0·5 for the assessment of mucosal mast cell counts, whilst globule leucocyte and eosinophil counts were assessed after staining with carbol chromatrope and differentiation on morphological criteria (Huntley *et al.* 1995).

Calculations and statistical analysis

Due to their skewed nature, nematode egg excretion, number of eggs in the colon, nematode numbers and inflammatory cell counts were transformed according to log $(n + 1)$. This normalised the data before statistical analysis was carried out. These results are expressed as back-transformed means with 95 % CI. The non-transformed data are reported using the arithmetic mean values with their standard errors.

Nematode egg excretion during lactation (eggs/d) was calculated by multiplying the faecal egg count in the fresh faeces collected during the day (eggs/g) with the amount of fresh faeces produced over 24 h (g/d). The latter was calculated as the sum of the fresh faeces collected during the day and overnight: the latter was calculated from the dry faeces collected overnight and the estimated air DM content.

A one-way ANOVA was used to assess the hypothesis that the secondary infection of *N. brasiliensis* was sensitive to nutrient demand in lactating rats. The three levels of litter size were used to analyse the effects on nematode egg excretion, nematode eggs in colon, worm burden and immune responses (mucosal mast cells, globule leucocytes and eosinophils). The expectation was that parasitism would reduce and immune responses would increase with reduced litter size.

Effects of litter size on dam body weight, dam feed intake and litter body weight were assessed through repeated measure one-way ANOVA, taking into consideration the effect of time and interactions between litter size and time. Body weight at parturition was used as a covariate when analysing the effects of litter size on dam performance during lactation. All statistical analyses were performed using Genstat 6 for Windows release 6.1, 2002 (Lawes Agricultural Trust, Rothamsted, UK) and Minitab 12 release 12.1, 1998 (Minitab, Coventry, UK).

Results

Faecal egg counts during the primary infection and performance until parturition

All rats showed signs of a primary infection, and had started excreting nematode eggs by day −32. This then peaked at 52,479 (95 % CI 46,880, 58,748) eggs/g faeces by day −29. The faecal egg counts then gradually decreased and by day −26 all rats stopped excreting nematode eggs.

During the first 10 d of gestation, rats grew from 279 (SE 3·9) g to 315 (SE 5·1) g, with an average DM intake of 26·6 (SE 0·6) g/d. From then onwards and until parturition, the pregnant rats continued to grow to a mean weight of 349 (SE 9·1) g with an average DM intake of 18·3 (SE 0·1) g/d, which dropped to an average of 9·41 (SE 0·03) g/d just before parturition.

Food intake, dam and litter body weight during lactation

Figure 1(a) shows the DM intake for the dams during lactation. DM intake increased rapidly over time until day 3 of lactation ($P<0·001$; F 13·02; df 11,143), whilst litter size did not affect mean feed intake during the 12 d lactation period ($P=0·37$; F 1·06; df 2,12). However, litter size and time interacted for achieved feed intake ($P=0·029$; F 2·39; df 22,143) due to small differences in achieved food intake on two occasions. On days 10 and 12 only, DM intake of LS6 rats was lower than that of LS3 rats, whilst during the same days the intake of LS9 rats was intermediate to these groups (Fig. 1(a)).

Dam performance

Figure 1(b) shows dam body weight during lactation. At parturition, mean dam body weight was 270 (SE 14·2) g. Litter size and time significantly interacted for dam body weight during lactation ($P<0·001$, F 11·13, df 22,143). LS9 and LS6 rats had smaller body weight than LS3 rats from day 5 onwards. Over the 12 d lactation period, LS9 and LS6 rats lost 24·0 and 24·6 g, respectively, whilst LS3 rats gained 35·2 g (SED 14·7 g; $P<0·01$; F 11·00; df 2,13).

Litter performance

Mean litter weight from day 0 during lactation is shown in Fig. 1(c). At parturition, mean litter body weight was 53·1 (SE 2·8) g. Litter size and time significantly interacted for litter body weight during lactation ($P<0·001$, F 11·03, df 22,143). Litter size had a significant effect on mean litter weight from day 2 onwards, and this effect increased over time. Final litter weight did not differ between LS9 and LS6 rats. As expected, final pup weight increased with reduced litter size, and averaged 13·2, 18·5 and 20·3 g for LS9, LS6 and LS3 rats, respectively (SED 1·78 g; $P<0·01$; F 8·25; df 2,13).

Nematode egg excretion during lactation

Time and litter size each affected faeces production during lactation, but there was no interaction between them ($P=0·46$; F 0·86; df 4,26). Mean faeces production averaged

5·1, 3·3 and 3·5 g for day 7–8, 9–10 and 11–12 of lactation, respectively (SED 0·58 g; $P=0·026$; F 5·56; df 2,26), whilst it averaged 3·7, 3·4 and 4·7 g for LS9, LS6 and LS3, respectively (SED 0·43 g; $P=0·026$; F 4·91; df 2,13). Effects of time and litter size on nematode egg excretion are therefore expressed as daily egg output to account for observed effects on faeces volume. Mean nematode egg excretion significantly increased over time from 1802 (range 729–4451), to 3791 (2257–6368) and 5826 (3909–8683) eggs/d for days 7–8, 9–10 and day 11–12, respectively ($P=0·017$; F 5·50; df 2,26). For the different litter sizes, nematode egg excretion averaged 3310 (range 2652–4132), 8438 (4021–17 704) and 1731 (1278–2345) eggs/d for LS9, LS6 and LS3, respectively, but these differences failed to reach formal statistical significance ($P=0·11$; F 2·60; df 2,13).

Colon egg count and worm burden

The effects of litter size on the mean number of nematode eggs found in the colon, shown in Fig. 2(a), were in a similar direction to the effect on worm burden (see later), but these effects were not significant ($P=0·36$, F 1·12, df 2,13). However, litter size had a significant effect on worm burden ($P<0·01$, F 7·08, df 2,13), as shown in Fig. 2(b). Worm burden of LS9 and LS6 rats were similar but were both significantly larger than that of LS3 rats. Litter size did not affect the percentage of male and female worms ($P=0·81$; F 0·21; df 2,13); the percentage of male worms averaged 41·6 (SE 4·2).

Inflammatory cells

Feeding treatments had no significant effect ($P=0·80$, F 0·22, df 2,13) on the number of mucosal mast cells and eosinophils ($P=0·13$, F 2·36, df 2,13) in the small intestine. Overall, the mean concentration of mucosal mast cells was 51 (range

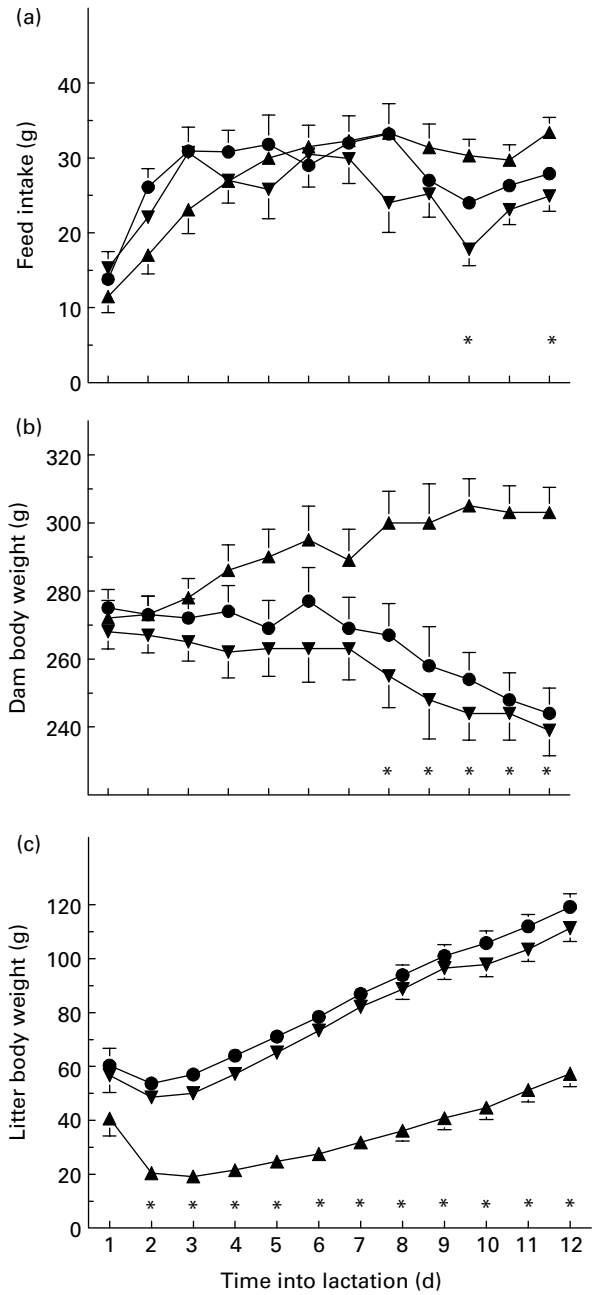

Fig. 1. Dam food intake (a), dam body weight (b) and litter body weight (c) of lactating rats offered a low-protein food (100 g crude protein/kg DM) and nursing nine (●), six (▼) or three (▲) pups. For details of procedures, see pp. 105–106. Values are least square means with their standard errors depicted by vertical bars. *Effects of litter size at $P<0·05$ at specific time-points.

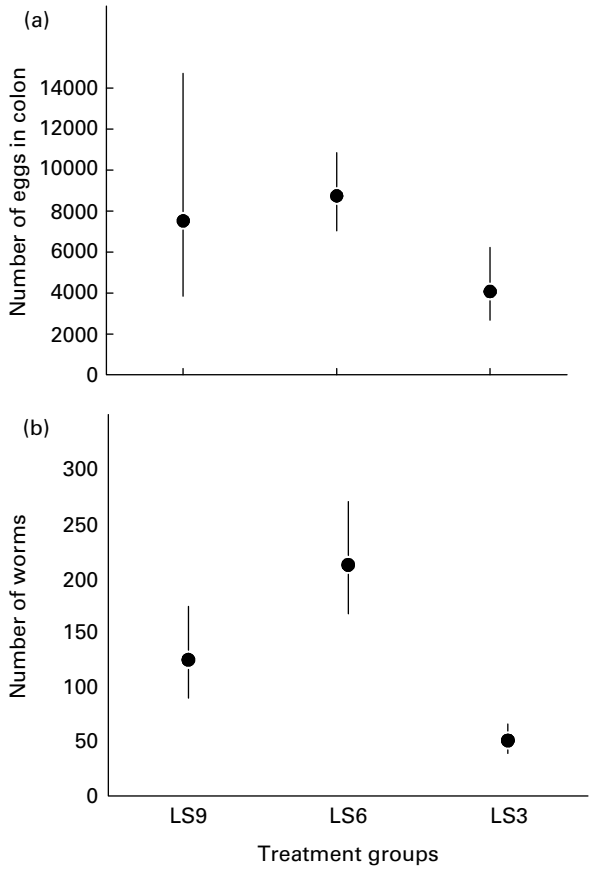

Fig. 2. Number of eggs in colon contents (a) and worm burden (b) of lactating rats offered a low-protein food (100 g crude protein/kg DM) and nursing nine (LS9), six (LS6) or three (LS3) pups. For details of procedures, see pp. 105–106. Values are mean back-transformed with 95% CI depicted by vertical bars.

39–69) per mm^2, and that of eosinophils was 172 (147–205) per mm^2. Globule leucocytes were absent in all tested sections.

Discussion

At times of nutrient scarcity, the periparturient animal may experience a breakdown of immunity to parasites, evidenced by an increased gastrointestinal nematode burden and a faecal egg count. Houdijk *et al.* (2005*a*) used a rodent model to assess whether a reduction in nutrient scarcity during lactation resulted in a reduced degree of parasitism. Feeding high-protein foods resulted in a reduced worm burden, but this was confounded by the concomitant increase in food intake. Therefore, effects observed on parasitism may not necessarily have been associated with an increased nutrient supply, but could have been related to changes in the gut environment, as a consequence of increased food intake. The current experiment aimed to account for this uncertainty by testing the hypothesis that parasitism during lactation is sensitive to nutrient demand in the absence of changes in food intake. This would then further support the view that nutritional control of gastrointestinal parasitism may be associated with immunological changes in the gut environment.

Firstly, it is important to establish whether the current experiment had achieved the correct conditions under which the hypotheses could be tested. The hypothesis stated that worm burden during the periparturient period would be sensitive to changes in nutrient demand. This was to be evidenced by comparing the LS9, LS6 and LS3 feeding treatments. The aim was to create differences in nutrient demand, so that LS9 and LS6 rats would be in a nutrient-scarce state while LS3 rats would achieve nutrient abundance. It is evident from the data obtained that these litter size treatments affected dam performance, i.e. body weight change and litter gain (proxy for milk production). At similar levels of achieved food intake over the 12 d lactation period, LS6 and LS9 rats lost a significant amount of weight, whilst LS3 rats gained weight, and pup weight progressively increased from LS9 to LS3. Hence, the data support the view that LS6 and LS9 rats were indeed in a more nutrient-scarce state than LS3 rats. Although it cannot be exclusively concluded from the current data, it might be argued that since LS3 rats were lactating and increasing in body size, they had indeed achieved nutrient adequacy. Hence, the hypothesis that the degree of *N. brasiliensis* infection is sensitive to differences in nutrient demand could be properly addressed in this experiment. The discussion will hereafter focus on the worm burden, as changes in the level of worm burden act as a reflection of how the host has coped with the parasite challenge. We focus on worm burdens rather than on nematode egg excretion and/or nematode eggs in colon because the worm burdens are much less sensitive to between-animal variation in achieved food intake/faeces production and/or colon content (e.g. Athanasiadou *et al.* 2001; Houdijk *et al.* 2003*b*). These sources of variation have likely contributed, to a large extent, to the absence of significant feeding treatment effects on nematode egg excretion and the number of nematode eggs in the colon contents (Fig. 2). However, these effects were in the same direction and, on average, of the same magnitude as those on worm burdens.

The present results show that at similar food intake over the 12 d lactation period used, a clear reduction in worm burden with reduced litter size was observed. Intuitively, one might have expected that the LS9 rats, and to a lesser extent the LS6 rats, would consume more of the low-protein food than the LS3 rats, in order to compensate for the protein shortage relative to the extra demand placed on them. However, the low protein to energy ratio of food used may have prevented an increased food intake to satisfy protein requirements, because of the metabolic consequences that would arise from the associated excess energy intake (Friggens *et al.* 1993). The observed reduction in worm burden at similar achieved food intake indicates an improvement of host resistance in the absence of direct changes in gut environment. Therefore, this supports the view that the nutritionally achieved reduction in worm burden in the present experiment, as well as in Houdijk *et al.* (2005*a*), is likely achieved through effects of nutrient availability on expression of immunity, and not through changes in the gastrointestinal environment.

The observation that LS3 rats had significantly smaller worm burdens than LS6 and LS9 rats is consistent with results obtained from farm animal studies. Parasitised single-rearing ewes have usually lower faecal egg counts and smaller worm burdens than their twin-rearing counterparts (Donaldson *et al.* 1998; Houdijk *et al.* 2001; Kahn, 2003), whilst similar effects have been seen in low-yielding dairy goats compared to high-producing goats (Chartier *et al.* 2000). In addition, the effects of nutrient supply on worm burdens in the *N. brasiliensis*-infected lactating rat (Houdijk *et al.* 2005*a*) are also consistent with a large number of farm animal studies (e.g. Donaldson *et al.* 1998, 2001; Houdijk *et al.* 2003*b*; Kahn, 2003). Hence, the evidence obtained to date shows that our *N. brasiliensis* lactating rat model can reproduce similar nutritional sensitivities of host resistance to gastrointestinal nematode parasites during the periparturient period as observed in other lactating hosts. This supports the view that this model may be used to assess nutritional sensitivity of periparturient immune responses that may be associated with nutritionally improved resistance to parasites.

In the current experiment, we investigated some of the possible underlying immune responses that may be associated with nutritionally improved resistance to *N. brasiliensis* during lactation, through the measurement of specific inflammatory cells. Mucosal mast cells have long been thought to be important effector cells in expulsion of nematode parasites in general, and this role has been well documented in infections with *N. brasiliensis* (Befus & Bienenstock, 1979). However, more recent studies have suggested that the final phase of immune expulsion of *N. brasiliensis* in primary infections is mast cell independent (Nawa *et al.* 1994), although a role for mucosal mast cells in secondary infections may not be excluded (Katona *et al.* 1988). The current view is that mast cells may play a role in leading to the outcome of the immune response, rather than serve simply as late-stage effector cells involved with the actual immune expulsion of the parasite (Maizels & Holland, 1998; Maizels *et al.* 2004). Previous work indicated that dietary protein deficiency in growing rats resulted in delaying the expulsion of *N. brasiliensis* from the gastrointestinal tract and that this was associated with a reduction in the number of mucosal mast cells compared to protein-replete controls (Cummins *et al.* 1987). This points towards at least some degree of nutritional sensitivity of the mast cell response to *N. brasiliensis* infection.

Here, we compared the number of mucosal mast cells and eosinophils in the different treatment groups, on the basis of the activity of the latter being influenced by the former (Capron *et al.* 1978). The present analysis showed that reducing nutrient demand had no effect on either parameter. Future work will expand upon the immunological measurements to include isotype-specific humoral responses and the release of cell-specific factors such as mast cell proteases, to provide additional measures of cellular activity. It may also be possible to expand the immune response analysis by accounting for goblet cells. It is known that these cells undergo dramatic expansion in the parasitised gut epithelium. Evidence has accumulated to implicate goblet cells and their mucus production as key elements in the expulsion of *N. brasiliensis* during a primary infection. High levels of mucus production may trap parasites in the lumen and minimise their ability to attach in the gut (Nawa *et al.* 1994). However, whether goblet cells are also involved in immune expulsion of secondary *N. brasiliensis* infections, as in our lactating rat model, remains to be investigated.

Globule leucocytes were absent in mucosal tissues collected in the present experiment. A possible explanation as to why no globule leucocytes were found could be related to the timing of sampling used. Connan (1973) found globule leucocytes from samples obtained after more than 10 d of a single secondary infection with *N. brasiliensis*. Another reason could be due to the mode and short period of infection. In nature, both the primary and secondary infection would be a gradual, trickle one, acquired over a period of time. The presence of an effect of protein supplementation on globule leucocytes has been consistently observed in other parasitised hosts, where a gradual, trickle infection has been used (Houdijk *et al.* 2005*b*).

In conclusion, the results of the present experiment support the view that the extent of *N. brasiliensis* infection during the periparturient period is sensitive to changes in nutrient demand, and that these effects are independent of changes in the gastrointestinal environment. This supports the view that nutritional control of resistance to parasites during lactation is mediated through host immunity. This rodent model can now be used to understand fully the underlying mechanisms of the nutritional basis of relaxation in immunity during the periparturient period.

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References

Athanasiadou S, Kyriazakis I, Jackson F & Coop RL (2001) The effects of condensed tannins supplementation of foods with different protein content on parasitism, food intake and performance of sheep infected with *Trichostrongylus colubriformis*. *Br J Nutr* **86**, 697–706.

Befus AD & Bienenstock J (1979) Immunologically mediated intestinal mastocytosis in *Nippostrongylus brasiliensis* infected rats. *Immunology* **38**, 95–101.

Capron M, Capron A, Torpier G, Bazin H, Bout D & Joseph M (1978) Eosinophil-dependent cytoxicity in rat schistosomiasis. Involvement of IgG_{2a} antibody and role of mast cells. *Eur J Immunol* **8**, 127–133.

Chartier C, Etter E, Hoste H, Pors I, Mallereau M-P, Broqua C, Mallet S, Koch C & Masse 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.

Connan RM (1973) The immune response of the lactating rat to *Nippostrongylus brasiliensis*. *Immunology* **25**, 261–267.

Coop RL & Kyriazakis I (1999) Nutrition-parasite interaction. *Vet Parasitol* **84**, 187–204.

Coop RL & Kyriazakis I (2001) Influence of host nutrition on the development and consequences of nematode parasitism in ruminants. *Trends Parasitol* **17**, 325–330.

Cummins AG, Duncombe VM, Bolin TD, Davis AE & Young J (1987) The response of the small intestine of the protein-deficient rat to infection with Nippostrongylus brasiliensis. *Int J Parasitol* **17**, 1445–1450.

Dineen JK & Kelly JD (1972) The suppression of rejection of *Nippostrongylus brasiliensis* in lactating rats: the nature of the immunological defect. *Immunology* **22**, 1–12.

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.

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.

Houdijk JGM, Jackson F, Coop RL & Kyriazakis I (2006) Rapid improvement of immunity to *Teladorsagia circumcincta* is achieved through a reduction in the demand for protein in lactating ewes. *Int J Parasitol* **36**, 219–227.

Houdijk JGM, Jessop NS, Knox DP & Kyriazakis I (2003*a*) Breakdown of immunity to *Nippostrongylus brasiliensis* in lactating rats. *Br J Nutr* **90**, 809–814.

Houdijk JGM, Jessop NS, Knox DP & Kyriazakis I (2005*a*) Secondary infection of Nippostrongylus brasiliensis in lactating rats is sensitive to dietary protein content. *Br J Nutr* **93**, 493–499.

Houdijk JGM, Kyriazakis I, Jackson F & Coop RL (2001) The relationship between protein nutrition, reproductive effort and breakdown in immunity to *Teladorsagia circumcincta* in periparturient ewes. *Anim Sci* **72**, 595–606.

Houdijk JGM, Kyriazakis I, Jackson F, Huntley JF & Coop RL (2003*b*) 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.

Houdijk JGM, Kyriazakis I, Jackson F, Huntley JF & Coop RL (2005*b*) Effects of protein supply and reproductive status on local and systemic immune responses to *Teladorsagia circumcincta* in sheep. *Vet Parasitol* **129**, 105–117.

Huntley JF, Patterson, Mackellar A, Jackson F, Stevenson LM & Coop RL (1995) A comparison of the mast cell and eosinophil responses of sheep and goats to gastrointestinal nematode infections. *Res Vet Sci* **58**, 5–10.

Kahn LP (2003) Regulation of the resistance and resilience of periparturient ewes to infection with gastrointestinal nematode parasites by dietary supplementation. *Aus J Exp Agric* **43**, 1477–1485.

Katona IM, Urban JF & Finkelman FD (1988) The role of L3T4 + and Lyt-2 + T cells in the IgE response and immunity to *Nippostrongylus brasiliensis*. *Parasite Immunol* **140**, 3206–3211.

Maizels RM, Balic C, Gomez-Escobar N, Nair M, Taylor MD & Allen JE (2004) Helminth parasites – masters of regulation. *Immunol Rev* **201**, 89–116.

Maizels RM & Holland MJ (1998) Parasite immunity: pathways for expelling intestinal helminths. *Curr Biol* **8**, R711–R714.

Nawa Y, Ishikawa N, Tsuchiya K, Horii Y, Abe T, Khan AL, Bing-shi Itoh H, Ide H & Uchiyama F (1994) Selective effector mechanisms for the expulsion of intestinal helminths. *Parasite Immunol* **16**, 333–338.

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.

Pine AP, Jessop NS & Oldham JD (1994) Maternal protein reserves and their influence on lactational performance in rats. *Br J Nutr* **71**, 13–27.