Parasite-induced anorexia and its association with the immune response and plasma leptin concentrations in lambs of two genotypes

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Parasite-induced anorexia and its association with the immune response and plasma leptin concentrations in lambs of two genotypes

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Abstract. The degree of anorexia (i.e. reduction in voluntary food intake) in lambs following nematode infection may be sensitive to host production potential. An increase in plasma leptin concentrations (PLC) following infection may be related to the occurrence of anorexia. Our study tested the hypotheses that (i) the degree of anorexia will be greater in parasitized lambs selected more intensively for growth, and (ii) nematode infection will increase PLC in lambs. In Experiment I, 48 Suffolk x Greyface (S) and 48 Scottish Blackface (B) were used. Half lambs of each genotype were infected with the nematode *Teladorsagia circumcincta* and fed *ad libitum*. The remaining were not infected and either fed *ad libitum* or pair-fed to infected lambs. In Experiment II, previously infected lambs were either not re-infected or re-infected 4- or 8-weeks after the end of the primary infection. S lambs were more susceptible to infection than B lambs, as shown by faecal egg counts and IgA results in Experiment I. A mild anorexia was observed in both S and B lambs during the primary infection only. Neither primary nor secondary infections lead to an increase in PLC. Our data suggest that differences in intrinsic capacity for growth in lambs are associated with differences in the ability to cope with infection. Leptin seems unlikely to be responsible for *T. circumcincta*-induced anorexia.

Keywords. Anorexia – Infection – Genotype – Sheep – *Teladorsagia circumcincta.*

L'anorexie induite par les parasites et son implication dans la réponse immunitaire et les concentrations de la leptine dans le plasma de deux races d'agneaux

Résumé. *Le degré d'anorexie (réduction de l'ingestion volontaire des aliments) chez les agneaux après une infestation par les nématodes pourrait être sensible au potentiel de production de l'hôte. Une augmentation des concentrations de leptine dans le plasma (PLC) après infestation pourrait être reliée à la présence de l'anorexie. Notre étude a permis de tester deux hypothèses : (i) le degré d'anorexie serait plus élevé chez les agneaux parasités sélectionnés pour la croissance, et (ii) l'infection par les nématodes augmenterait le PLC chez les agneaux. Dans l'expérience I, 48 agneaux de race Suffolk x Greyface (S) et 48 autres agneaux de race Scottish Blackface (B) ont été utilisés. La moitié des agneaux de chaque race a été infestée avec le nématode* Teladorsagia circumcincta *et a été nourrie à volonté. Le reste des animaux n'a pas été infesté et a été nourri à volonté ou alimentés de façon similaire que les animaux infestés. Dans l'expérience II, les agneaux ayant été infestés auparavant n'ont pas été réinfestés ou réinfestés 4 ou 8 semaines après la fin de la première infestation. Les agneaux du groupe S ont été plus sensibles à l'infestation que ceux du groupe B, comme le montre la concentration des œufs fécaux et les résultats de l'IgA issus de l'expérience I. Une anorexie atténuée a été observée au niveau des groupes S et B uniquement pendant la première infestation. Les première et deuxième infestations ont entrainé une augmentation du PLC. Nos données suggèrent que les différences de la capacité intrinsèque de la croissance des agneaux sont associées aux différences de la capacité de faire face à l'infection. La leptine ne semble pas responsable du* T. circumcincta *induit par l'anorexie.*

Mots-clés. *Anorexie – Infection – Génotype – Brebis – Teladorsagia circumcincta.*

I – Introduction

It has been well established that anorexia (i.e. reduction in voluntary food intake) is a major cause of the impaired productivity of the nematode infected lambs (Coop and Kyriazakis, 1999). Breeds of sheep that have been selected more intensively for production efficiency (e.g. growth) are generally more susceptible to gastrointestinal infections than breeds that have been selected less intensively (Baker, 1998; Bisset *et al.*, 2001). Recently, it has been suggested that also the degree of anorexia in growing lambs is likely to be affected by genotype as lambs with higher growth capacity showed a greater degree of anorexia (Zaralis *et al.*, 2008). However, in the latter study, observed genotype differences in the degree of anorexia could have been affected by the immune status of the lambs prior to infection. The same study showed that genotypes selected more intensively for growth are likely to show renewed anorexia following a re-exposure to nematode parasites after 2 weeks of absence of infection; however, the effect was not very strong (Zaralis *et al.*, 2008). Therefore, there is some uncertainty as to whether re-infection of previously infected animals results in renewed anorexia or not and whether the degree of anorexia is strongly affected by the intrinsic capacity for growth in lambs. In the current study it was hypothesized that (i) the degree of anorexia following nematode (re)infection will be greater in lambs that have been selected more intensively for growth and (ii) the magnitude of anorexia in re-infected lambs will depend on the length of the parasite-free period between the end of the primary and the start of the secondary infection.

Moreover, in an attempt to understand better the mechanism responsible for the parasite induced anorexia in sheep, recent studies have investigated the implication of the hormone leptin in the immune response of nematode infected sheep (Valderrábano *et al.*, 2006; Fox *et al.*, 2006). However, these studies do not allow a comparison in plasma leptin concentrations (PLC) between infected and non-infected sheep. A recent study with non-infected restrictedly fed lambs showed that PLC is elevated in infected lambs, when data were corrected for the level of food intake (Zaralis *et al.*, 2008). However, the level of food restriction in the study of Zaralis *et al.* (2008) resulted in intakes that were lower than those of the infected animals showing anorexia. In the present study, correction for the level of food intake in order to study the effect of infection on PLC was achieved by the involvement of non-infected lambs that were pair-fed to infected lambs. Therefore, it was hypothesised that (iii) nematode infected lambs will have increased PLC compared to non-infected controls with similar food intakes.

II – Materials and methods

1. Experiment I

Forty-eight Suffolk × Greyface (S) and 48 Scottish Blackface (B) lambs were used in this experiment. All lambs were born and weaned indoors and prior to lambing all ewes were orally drenched with anthelminthics to ensure that lambs would not become infected from contact with ewe-derived parasites. After weaning, lambs were transferred to the experimental shed (week -3) and acclimatized for a period of 3 weeks, during which all lambs were fed *ad libitum*. On week -2 lambs were allocated randomly to individual pens, in which they stayed until the end of the experiment (week 10). All pens contained a food trough that allowed measurement of individual food intake.

On week 0, half of the lambs (24 of each breed) were trickle infected with 7,000 L3 larvae of the nematode *Teladorsagia circumcincta*, 3 days a week for a period of 10 weeks and fed ad libitum (treatment INF). Half (12 of each breed) of the non-infected controls, were fed *ad libitum* (treatment AL) to allow estimation of anorexia in INF lambs. To measure effects of food intake on PLC, the remaining 12 control lambs in each breed were pair-fed (treatment PF) with 12 lambs of the same breed and

gender of the INF treatment. The PF lambs received the same amount of food per kg BW as consumed by their *ad libitum* fed and infected counterpart during the previous three to four days.

Lambs were assigned randomly to the above treatments after blocking for breed, sex and BW in week -1, which guaranteed an equal number of males and females per breed of similar BW per treatment. The mean BW on week -1 was 31.8 ± 0.43 kg and 21.3 ± 0.43 for S and B lambs, respectively. All lambs during the experiment were fed the same type of food (grass pellets) with an average composition per kg as-fed of 948 g DM, 166 g CP and 479 g NDF.

2. Experiment II

The 48 previously infected lambs in Experiment I (24 S and 24 B) were used in Experiment II. Lambs were allocated randomly into three treatments of 8 lambs per breed (i.e. treatments CON, R8 and R4) after blocking for breed, sex and BW, and housed as treatment groups. Trickle infection for lambs in the CON and R8 treatments was ceased immediately after the end of experiment I and at the same time these lambs were drenched with antihelminthics, to remove worm burdens. Lambs in the R4 treatment continued to receive the trickle infection (as described for experiment I) for another 4 weeks, after which, they were drenched. Four weeks after the end of infection in the lambs of the R4 treatment, a secondary infection with *T. circumcincta* was applied to the lambs of both the R8 and R4 treatment and lasted for 12 weeks. This design resulted in lambs being re-infected either 4 weeks (R4) or 8 weeks (R8) after the primary infection was terminated. The lambs were moved to individual pens one week prior to the start of the secondary infection and food intake was started to be recorded. Throughout the experiment all lambs were fed *ad libitum* grass/straw pellets with an average composition per kg as-fed of 917 g DM, 103 g CP and 532 g NDF. The experimental facilities and infection details were identical to those used in experiment I.

3. Sample collection and measurements

In both experiments, measurements and procedures were carried out over similar time-scales using identical protocols. Amounts of distributed food were recorded daily and any residues were weighed twice weekly to allow calculation of intakes. Fresh faecal samples were taken twice weekly, directly from the rectum from week -1 onwards. The samples were analysed for faecal egg counts (FEC) according to a modified flotation method (Christie and Jackson, 1982) and were expressed as the number of eggs per gram (epg) of fresh faeces. Blood samples were taken weekly and blood plasma was analysed for leptin (as described by Zaralis *et al.*, 2008) and IgA (as described by Sinski *et al.*,1995) concentrations.

4. Statistical analysis

All data were analyzed by ANOVA using the MIXED procedure of SAS (SAS 9.1.3; SAS Institute Inc., Cary, NC, USA). The statistical model for the ADFI, BW, IgA, FEC and PLC data contained the fixed effects of breed, treatment, time, and their interactions and the random effect was animal nested within breed (model 1). A heterogeneous first-order auto-regressive structure was selected in the model to account for non-constant residual variation throughout the weeks, were appropriate. In addition, PLC data were analyzed by a similar model (model 2) that contained the relative ADFI to BW (RADFI) of the lambs as a co-variable, in addition to the main effects of used in model 1. The relationships between PLC and RADFI were tested by comparing the respective co-variable coefficient with its associated standard error. All models for leptin included an assay effect to take into account the between-assay variation. Data are reported as least square means and their standard error (SE) and their differences were tested by a t-test. Prior to statistical analysis FEC data were log-transformed according to log10(x+1), in order to normalise residuals. FEC data are reported as back-transformed means (according to 10^{α}, with $\alpha = \mu + 0.5 \times \sigma 2$) with 95% confidence intervals (CI; lower and upper limit).

II – Results and discussion

1. Faecal egg counts (FEC) and IgA levels

The FEC data showed that all lambs were parasite naïve prior to the start of the primary trickle infection. During experiment I, FEC in INF lambs reached a maximum value during the third week of infection with mean epg of 154 (95% CI was 100 to 184) and 59 (95% CI was 41 to 106) for S and B lambs, respectively. From the fourth week of infection, FEC declined which suggests that development of immunity had started to occur. There was a significant breed by time interaction (P=0.001), attributable to the fact that FEC were raised to higher values in S than in B lambs especially during the third week of infection. These results suggest that S lambs are more susceptible to infection than B lambs, which is a finding that concurs with the results of earlier studies that compared breeds of sheep of different production levels (Amarante *et al.*, 2004; Good *et al.*, 2006; Zaralis *et al.*, 2008). During experiment II FEC remained very low in both breeds (at all time points FEC was below 5 eggs per g faeces), which suggests that a loss of the acquired immunity does not occur in growing lambs within an 8-week parasite free period.

Primary infection resulted in a significant increase in IgA and the effect was more pronounced in B that in S lambs, during experiment I as shown by the interaction between infection, breed and time (P=0.013). In experiment II a rapid increase in IgA was also observed in B lambs but only in the R8 treatment. IgA response to infection is regarded to be the most important manifestation of immunity in growing lambs as it affects worm growth and causes a reduction in worm fecundity (Stear *et al.*, 1995; Strain and Stear, 2001). Therefore, the differences in immune response to infection between S and B lambs as shown both by IgA and FEC results, could well be mediated by differences in the host's prioritization of nutrients towards its various physiological functions, as suggested by Colditz (2008).

2. Food Intake and plasma leptin

The average daily food intake (ADFI) for S and B lambs in INF and AL treatments are shown in Fig. 1 (panel a). There was an expected, significant genotype effect (P<0.001) as S lambs had higher ADFI than B lambs. Nematode infection caused a significant reduction in ADFI from the third week of infection and onwards (P=0.02). In both breeds the reduction in ADFI was around 6%, which is relatively small compared to depressions of appetite of around 12% or more that earlier studies report in infected lambs with the same parasite (Coop *et al.*, 1977; Sykes and Coop, 1977; Symons *et al.*, 1981). There is no a clear explanation about this finding. However, this is interesting in view of the results of the study of Zaralis *et al.* (2008), where the same dose of *T. circumcincta* resulted in a well established anorexia (of around 12%), at least in infected S lambs. In the study of Zaralis *et al.* (2008) lambs of the same genotypes were fed a similar type of food (grass pellets), which had a protein content of 186 g/kg per fresh food, while the lambs in the current experiment were fed on grass pellets with a slightly less protein content (i.e.166 g per kg fresh food). It is therefore unlikely that the dietary protein content of the food could have contributed in the relatively small degree of anorexia observed in the current study. The results of the current study do not support the hypothesis that differences in the degree of anorexia exist between these genotypes.

As expected, the ADFI differed between the two breeds as S lambs had significantly higher intakes than B lambs throughout the experiment II (P<0.001; Fig. 2a). Re-infection did not cause any reduction in ADFI in both R4 and R8 treatments and the interaction between breed, treatment and time was not significant (P=0.1; Fig. 2a). These results suggest that secondary infection is unlikely to result in renewed anorexia; however, this finding should be interpreted with caution as the reduction in ADFI during the primary infection was relatively small.

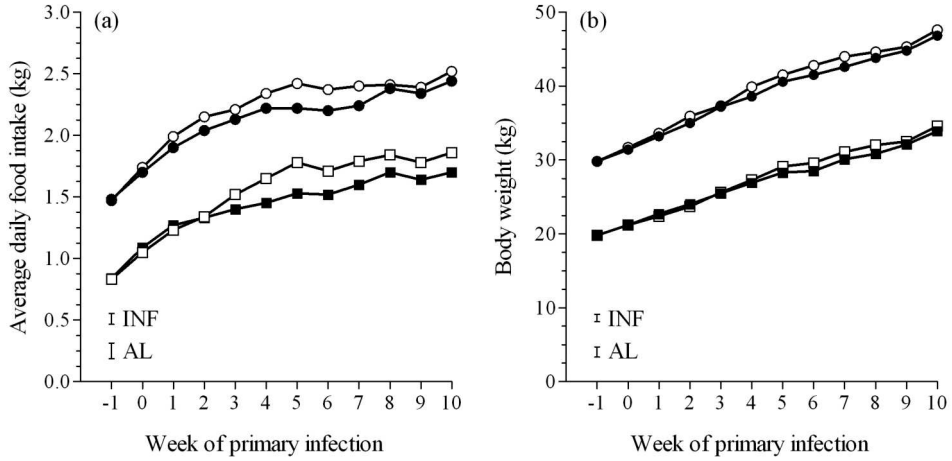

Fig. 1. Average daily food intake (panel a) and body weight (panel b) of non-infected (AL; ❍, ❑) and infected (INF; ●, ■) fed *ad libitum* Suffolk x Greyface (❍, ●) and Scottish Blackface (❑, ■) lambs during the experiment I (primary infection). Standard errors of the means are shown by vertical bars and are based on error mean squares pooled over treatments.

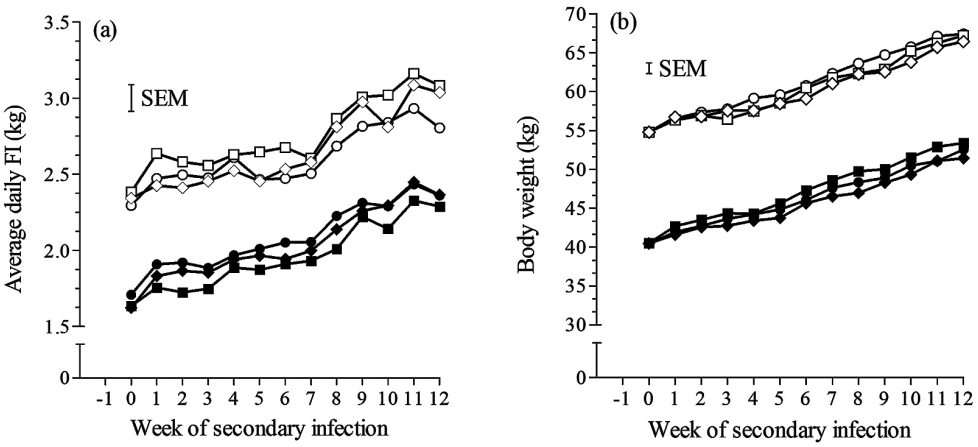

Fig. 2. Average daily food intake (panel a) and body weight (panel b) of non-infected (CON; ❍, ●), re-infected after 8 weeks of the end of the primary infection (R8; ✧, ✦), and re-infected after 4 weeks of the end of the primary infection (R4; ❑, ■) fed *ad libitum* Suffolk x Greyface (❍, ✧, ❑) and Scottish Blackface (●, ✦, ■) lambs during the experiment II (secondary infection).

During both experiments the BW of the lambs did not differ between infected and control lambs (Figs 1b and 2b) and the interaction between breed and infection was not significant (P>0.2). This outcome is probably attributable to the fact the reduction in ADFI was not large enough to affect the body weight gain of the lambs.

Analysis of the PLC data with models 1 and 2 showed that there were no significant effects of breed (P>0.17) and infection (P>0.58) on PLC or their interaction (P>0.41) during both primary and secondary infections. All models showed that PLC was affected by time (P<0.001) as PLC

increased gradually in all lambs towards the end of each experiment. A statistically significant positive relationship between PLC and RADFI was observed only during experiment II, as evidenced by the co-variable coefficient (P=0.012). The estimated co-variable coefficient showed that a difference in RADFI of 10 g was associated with a difference in PLC of 0.72 ng/ml (SE 0.205; P=0.018).

These results concur with earlier findings in parasitised sheep where infection did not result in an acute increase in PLC (Liu *et al.*, 2007; Zaralis *et al.*, 2008). However, a recent study suggested that PLC was significantly affected by infection, when leptin data were corrected for the level of food intake (Zaralis *et al.*, 2008). In the current study it was suggested that this was not the case when PLC data were corrected for the level of food intake (model 2). Because infection resulted in a relatively small anorexia in experiment I in both breeds, the ADFI of the pair-fed lambs was not significantly reduced compared to the intake of their infected counterparts. It is therefore unlikely that the food intake data of the pair-fed lambs have provided significant correction for the effect of the level of food intake on PLC. The PLC results of the pair-fed lambs suggest that a reduction in ADFI of around 6% is not strong enough to result in significant and systematic lower PLC in lambs. Therefore, whilst the hypothesis that gastrointestinal nematode infection results in an increase of PLC in lambs may not be rejected, the evidence from this study do not seem to provide support for it. The absence of any clear association between RADFI and changes in PLC in experiment I during which a reduction in intake was observed, suggests that leptin may not be essential for the induction of anorexia during parasitism in sheep.

III – Conclusion

The data show that differences in intrinsic capacity for growth are associated with susceptibility to infection in these genotypes, as S lambs had higher FEC and weaker IgA response than B lambs. The results showed that a primary infection is associated with anorexia in young lambs; however, the degree of anorexia was not affected by genotype differences in growth capacity. Re-infection of previously infected lambs did not result in renewed anorexia when lambs were re-infected after 4 or 8 weeks of the end of the primary infection. These data suggest that anorexia might be associated with the development of immunity in parasitized sheep but not with the expression of immunity. Finally the results suggest that nematode (re)infection did not result in an acute increase of PLC and leptin itself is unlikely to be responsible for the anorexia of nematode infection in lambs.

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References

Amarante A.F., Bricarello P.A., Rocha R.A. and Gennari S.M., 2004. Resistance of Santa Inés, Suffolk and Ile de France sheep to naturally acquired gastrointestinal nematode infections. In: *Veterinary Parasitology*, 120, p. 91-106.

Baker R.L., 1998. A review of genetic resistance to gastrointestinal nematode parasites in sheep and goats in the tropics and evidence for resistance in some sheep and goat breeds in sub-humid coastal Kenya. In: *Animal Genetics Resources Information Bulletin*, 24, p. 13-30.

Bisset S.A., Morris C.A., McEwan J.C. and Vlassoff A., 2001. Breeding sheep in New Zealand that are less reliant on anthelmintics to maintain health and productivity. In: *New Zealand Veterinary Journal*, 49, p. 236-246.

Colditz I.G., 2008. Six costs of immunity to gastrointestinal nematode infections. In: *Parasite Immunology*, 30, p. 63-70.

Coop R.L. and Kyriazakis I., 1999. Nutrition-parasite interaction. In: *Veterinary Parasitology*, 84, p. 187-204.

Coop R.L., Sykes A.R. and Angus K.W., 1977. Effect of a daily intake of *Ostertagia- Circumcincta* larvae on body-weight, food intake and concentration of serum constituents in sheep. In: *Research in Veterinary Science*, 23, p. 76-83.

Christie M. and Jackson F., 1982. Specific identification of strongyle eggs in small samples of sheep Feces. In: *Research in Veterinary Science*, 32, p. 113-117.

Fox M.T., Reynolds G.W., Scott I., Simcock D.C. and Simpson H.V., 2006. Vagal and splanchnic afferent nerves are not essential for anorexia associated with abomasal parasitism in sheep. In: *Veterinary Parasitology*, 135, p. 287-295.

Good B., Hanrahan J.P., Crowley B.A. and Mulcahy G., 2006. Texel sheep are more resistant to natural nematode challenge than Suffolk sheep based on faecal egg count and nematode burden. In: *Veterinary Parasitology*, 136, p. 317-327.

Liu S.M., Adams N.R., Briegel J.B., Smith T.L. and Martin G.B., 2007. Effect of genetic resistance to gastrointestinal nematodes on plasma concentrations of insulin-like growth factor- 1 and leptin in Merino sheep. In: *Australian Journal of Experimental Agriculture*, 47, p. 905-911.

Valderrábano J., Gómez-Rincón C. and Uriarte J., 2006. Effect of nutritional status and fat reserves on the periparturient immune response to *Haemonchus contortus* infection in sheep. In: *Veterinary Parasitology*, 141, p. 122-131.

Sinski E., Bairden K., Duncan J.L., Eisler M.C., Holmes P.H., McKellar Q.A. and Stear M.J., 1995. Local and plasma antibody responses to the parasitic larval stages of the abomasal nematode *Ostertagia circumcincta*. In: *Veterinary Parasitology*, 59, p. 107-118.

Stear M.J., Bishop S.C., Duncan J.L., Mckellar Q.A. and Murray M., 1995. The repeatability of fecal egg counts, peripheral eosinophil counts, and plasma pepsinogen concentrations during deliberate infections with *Ostertagia-Circumcincta*. In: *International Journal for Parasitology*, 25, p. 375-380.

Strain S.A.J. and Stear M.J., 2001. The influence of protein supplementation on the immune response to *Haemonchus contortus*. In: *Parasite Immunology*, 23, p. 527-531.

Sykes A.R. and Coop R.L., 1977. Intake and utilization of food by growing sheep with abomasal damage caused by daily dosing with *Ostertagia-Circumcincta* larvae. In: *Journal of Agricultural Science*, 88, p. 671-677.

Symons L.E.A., Steel J.W. and Jones W.O., 1981. Effects of level of larval intake on the productivity and physiological and metabolic responses of lambs infected with *Ostertagia-Circumcincta*. In: *Australian Journal of Agricultural Research*, 32, p. 139-148.

Zaralis K., Tolkamp B.J., Houdijk J.G.M., Wylie A.R.G. and Kyriazakis I., 2008. Changes in food intake and circulating leptin due to gastrointestinal parasitism in lambs of two breeds. In: *Journal of Animal Sciences*, 86, p. 1891-1903.