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Consequences of protein supplementation on anorexia and expression of immunity in two parasitized sheep breeds

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Abstract. The periparturient breakdown of immunity to parasites has a nutritional basis and it is likely to be associated with host production potential. However, not much is known whether periparturient hosts experience a reduction in food intake (anorexia). Here we investigate: (i) whether parasite infection during the periparturient period results in anorexia and protein supplementation can affect the degree of anorexia in periparturient ewes; and (ii) whether breeds that differ in production potential differ also in the extent of the breakdown of immunity and the degree of anorexia. A 2 × 2 × 2 factorial design was used with two breeds of twin-bearing ewes [Greyface cross, G (n = 32) and Scottish Blackface, B (n = 32)]. Half of the ewes in each breed were trickle infected with 30,000 larvae per week of the abomasal parasite *Teladorsagia circumcincta*, while the other half was not infected. In addition, half of the ewes in each infection treatment in each breed were fed either a high or a low protein diet. Infection with *T. circumcincta* resulted in breakdown of immunity and in a reduction in food intake in both breeds. The study has shown that breeds can differ in the extent of the breakdown of immunity, but these breed differences were not associated with a significant difference in the degree of anorexia. The results showed that protein supplementation can assist periparturient ewes in reducing the breakdown of immunity, but protein supplementation did not significantly affect the degree of anorexia.

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

Conséquences de la supplémentation protéique sur l'anorexie et l'expression immunitaire chez deux races ovines parasitées

Résumé. *Le déficit immunitaire dû aux parasites de la brebis en fin de gestation et début de lactation est d'origine alimentaire et est probablement associé au potentiel productif de l'hôte. Cependant, la diminution d'ingestion (anorexie) chez la brebis en fin de gestation et début de lactation n'est pas bien connue. Ici, nous étudions : (i) si l'infestation parasitaire des brebis durant la période périnatale provoque une anorexie et si une supplémentation protéique peut modifier le degré d'anorexie ; et (ii) si des races à potentiel productif différent présentent différents degrés de baisse immunitaire et d'anorexie. Deux races de brebis [croisement Greyface (n = 32) et Scottish Blackface (n = 32)], portant des jumeaux, ont été utilisées dans un plan factoriel 2 × 2 × 2. La moitié des brebis de chaque race a été infectée expérimentalement par le parasite de la caillette,* Teladorsagia circumcincta*, à raison de 30 000 larves par semaine et l'autre moitié servait de témoin. De plus, la moitié des brebis infectées recevaient soit un régime hyperprotéiné ou hypoprotéiné. L'infection par* Teladorsagia circumcincta *provoqua une baisse d'immunité et une diminution de l'ingestion dans les deux races. L'étude a montré que le degré de déficit immunitaire variait entre les races, mais que le degré d'anorexie n'était pas significativement différent. Les résultats ont montré que la supplémentation protéique pouvait réduire le déficit immunitaire de la brebis en fin de gestation et début de lactation, mais n'affectait pas significativement le degré d'anorexie.*

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

I – Introduction

Anorexia, which is the reduction in voluntary food intake, is a prominent feature of infectious

diseases, including gastrointestinal parasitism of sheep. This has been mainly studied in nematode infections with parasite naïve lambs (Coop *et al.*, 1982) and the results show that anorexia is likely to recover when animals acquire full immunity to parasites (Kyriazakis *et al.*, 1996). Reproductive ewes however, experience a breakdown of their acquired immunity to parasites during the periparturient period (late pregnancy and early lactation), generally referred to as the periparturient relaxation of immunity (PPRI). Although PPRI has been well documented (Barger, 1993), little is known whether periparturient ewes experience a reduction in food intake, and whether there are breed differences in the degree of anorexia, during the periparturient period. Coop and Kyriazakis (1999), in a nutrient partitioning framework have suggested that PPRI has a nutritional basis. Later studies have shown that the extent of the PPRI can be reduced by an increased intake of metabolizable protein (MP) (Houdijk *et al.*, 2000, 2001). Whether enhanced protein nutrition can affect also the degree of anorexia in infected periparturient ewes is not known.

In addition, it has been suggested that the production potential of the host is likely to associate with its resistance to infection in growing lambs (Bisset *et al.*, 2001) and that resistant breeds display a reduced PPRI when compared with susceptible or unselected breeds of sheep (Barger, 1993). Whether breed differences in production potential or in resistance associate also with differences in the degree of anorexia during infection, is not known. The aim of the present study was to investigate: (i) whether parasite infection during the periparturient period results in anorexia and protein supplementation can affect the degree of anorexia in periparturient ewes; and (ii) whether breeds that differ in production potential differ also in the extent of PPRI and the degree of anorexia.

II – Materials and methods

Sixty four pregnant, twin bearing, multiparous ewes, 32 Greyface cross (G) and 32 Scottish Blackface (B) were used. The ewes were brought indoors 57 days (day-57) before the realized mean parturition day (day-0) and drenched with Ivermectin (Oramec, Merial, UK) and Levamisole (Nilverm Gold, Schering-Plough, Welwyn Garden City, UK) according to manufacture's instructions, to remove residual worm burdens from previous natural exposure to parasites. The mean body weight (BW) and condition score (CS) at day-57 were 73.1 (SE 1.13) kg and 3.1 (SE 0.04) for the G ewes and 53.2 (SE 1.00) kg and 2.5 (SE 0.04) for B ewes, respectively. The ewes were housed in a naturally illuminated and ventilated shed in individual pens until 4 weeks into lactation (day-28). Each ewe had access to *ad libitum* water and a feeding bin for pelleted feed (see below). Since the lambs stayed with the ewes, feeding bins were raised above floor level to minimize access for, and hence feed consumption by the lambs. From housing until day-28 (28 days before realized mean parturition) all ewes were offered ad libitum hay. Following this day, ewes were subjected to feeding treatments.

Ewes of the both breeds were assigned randomly to treatments on the basis of their BW and CS measured on day-57. Half of the ewes in each breed were trickle infected (treatment: +) with the nematode *Teladorsagia circumcincta* at a rate of 10,000 infective third-stage larvae three times per week (Monday/Wednesday/Friday). The trickle infection started at day-47. Similar rates of infection have been shown to lead to the establishment of *T. circumcincta* worm burden in periparturient ewes (Leyva *et al.*, 1982; Houdijk *et al.*, 2003). Non-infected ewes (treatment: –) were given a similar volume of water ("sham" infection) at the same time, thus undergoing the same amount of handling stress as the infected ewes. Half of the ewes in each infection treatment in each breed, were fed ad libitum a low protein pelleted diet (treatment: LP). This diet provided sufficient energy, minerals and vitamins but less than the estimated MP requirements. The diet was formulated to provide 7 g MP/MJ metabolisable energy (ME), while the requirements for twin bearing/lactating ewes are estimated to be 9 g MP/MJ ME (AFRC, 1993; Houdijk *et al.*, 2001). The other half of the ewes, in each infection treatment in each breed, were fed the same pelleted diet plus a protein supplement (Soypass) which topped up the protein intake to around 11 g MP/MJ ME (treatment: HP). During late pregnancy and early parturition (3 weeks before and 1 week after parturition; Period 1) mean intake in periparturient ewes reduces but it increases considerably shortly after

parturition (1 to 4 weeks after parturition; Period 2) (Houdijk *et al.*, 2001). In order to maintain the ratio of MP/MJ ME approximately constant for those two periods the amount of Soypass added to the diet was formulated according to the estimated food intake of the ewes during these periods.

Amounts of distributed food were recorded daily and any residues were weighed twice weekly (Monday and Thursday) to allow calculation of intakes. Fresh faecal samples were taken twice weekly, directly from the rectum from day–57 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 from the ewes were taken weekly from day-57 and blood plasma was analysed for pepsinogen concentrations. Plasma pepsinogen concentration indicates the degree of abomasal leakage during *T. circumcincta* infections and was determined by the modified method of Paynter (1992).

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 food intake (FI) data contained the fixed effects of breed, treatment, time, and their interactions and the random effect was animal nested within breed. A heterogeneous first-order auto-regressive structure was selected in the model to account for non-constant residual variation throughout the weeks. 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 of FEC and pepsinogen data were log-transformed according to log10 (x + 1), in order to normalise residuals. Log-transformed data were analysed by repeated measures ANOVA. FEC and pepsinogen data are reported as back-transformed means (according to 10α, with $\alpha = \mu + 0.5 \times \sigma^2$) with 95% confidence intervals (CI; lower and upper limit).

III – Results and discussion

1. Faecal egg counts and pepsinogen levels

Mean back-transformed FEC for the infected ewes are shown in Fig. 1a. Most ewes had started to excrete nematode eggs by day-28 and FEC gradually increased to an average FEC of 136 (95% CI: 76, 243) epg on day7. Non infected animals (sham infected) had zero FEC throughout the experiment. G ewes had higher FEC that B ewes as indicated both by the significant effect of breed ($P = 0.007$) and the breed by time interaction ($P = 0.005$). Protein supplementation resulted in significant lower FEC in both breeds ($P = 0.025$) but the interaction between feeding treatment and time was not significant ($P = 0.15$). Plasma pepsinogen, as an indirect indicator of inflammatory reactions (Coop *et al.*, 1995), was higher in the infected ewes than in the non infected ewes in both breeds ($P < 0.0001$). Protein supplementation resulted in lower pepsinogen levels in G ewes and the effect was significant from day-0 onwards ($P = 0.015$). No differences in pepsinogen were observed between high and low protein fed B ewes (Fig. 1b).

A nutrient partitioning framework developed by Coop and Kyriazakis (1999) suggests that the periparturient relaxation of immunity has a nutritional basis in terms of allocation of scarce nutrients. The framework proposes that periparturient animals prioritise scarce nutrient allocation to reproductive functions (e.g. milk production) rather than to immune functions and thus immunity to parasites is compromised during the periparturient period (Coop and Kyriazakis, 1999). Our results support the position of this framework, since protein supplementation reduced the breakdown of immunity in both breeds. A possible explanation of the breed differences in the extent of the periparturient relaxation of immunity are likely to relate with breed differences in the reproductive effort (Houdijk *et al.*, 2001, 2006) since ewes of the G breed has been selected more intensively for higher production potential than ewes of the B breed.

2. Food intake

The mean daily FI for the G and B ewes are shown in Fig. 2. During the periparturient period, *T. circumcincta* infection resulted in a significant reduction in FI in both breeds within each feeding treatments ($P < 0.0001$). In most of the treatments, significant differences in FI between infected

and non infected ewes established mainly after parturition (day-0), which coincide with the rise in FEC. These results concur with an earlier study in periparturient ewes (Leyva *et al.*, 1982). This suggests therefore that anorexia in periparturient ewes is more severe during early lactation rather than during late pregnancy.

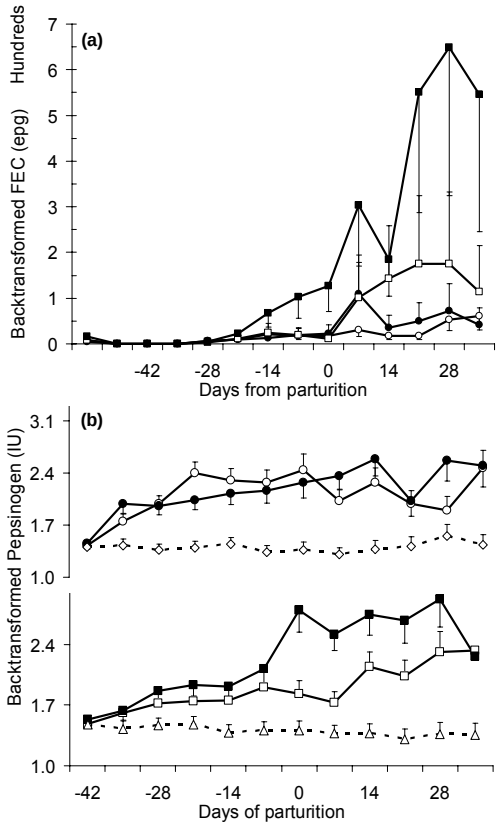

Fig. 1. Mean Backtransformed log_{10} FEC (a) and Pepsinogen (b) levels of infected (G-LP: ■; G-HP: □; B-LP: ●; B-HP: ○) and non infected (G: ─△─, B ─◇─) ewes during the periparturient period.

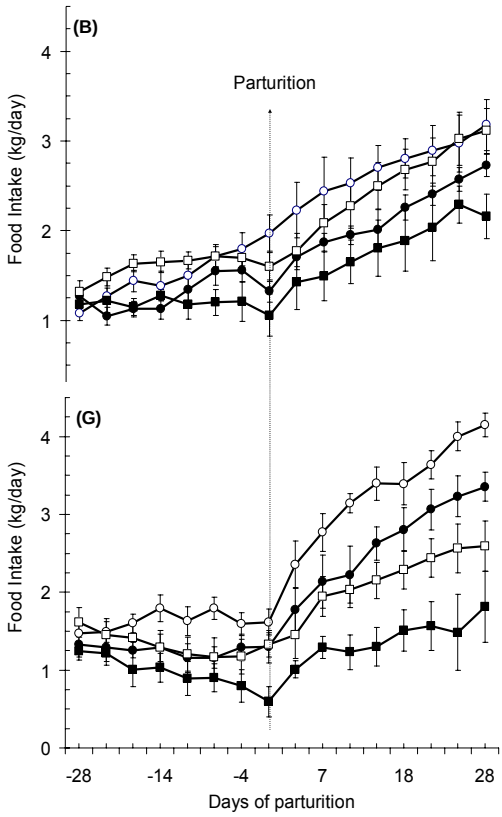

Fig. 2. Mean FI of non-infected fed *ad libitum* Blackface (B) and Greyface (G) ewes during the periparturient period (HP–: ○; HP+: ●; LP–: □; LP+: ■).

Our study is the first to investigate whether there are breed differences in the magnitude or duration of anorexia and whether enhanced protein nutrition can affect the degree of anorexia in periparturient ewes. The results showed that although the reduction in FI in the B ewes appeared to be of less magnitude when compared with that of G ewes within each protein treatment, this breed difference was not statistically significant (P = 0.19). Within each infection treatment, protein supplementation resulted in significant higher intakes in both breeds (P = 0.002). The average reduction in FI in the infected B ewes was 15% and 20% for the HP and LP protein treatments. Similarly, the average reduction in FI in the infected G ewes was 26% and 32% for the HP and LP protein treatments. However, the effect of protein supplementation on the reduction of food intake was not statistically significant in either of the breeds (P = 0.7). These data are not consistent with the view that protein supplementation has a significant effect on the degree of anorexia in periparturient ewes.

IV – Conclusions

This study has shown that nematode infection of ewes results not only in breakdown of immunity as evidenced by the rise in FEC but also in anorexia. The study has also shown that a breed selected more intensively for higher productivity showed a greater breakdown of immunity during the periparturient period than an unselected breed. However, the breed differences in the breakdown of immunity were not associated with a significant difference in the degree of anorexia. The study adds to the growing body of evidence that protein supplementation can assist periparturient ewes in reducing the breakdown of immunity. This effect of protein however, was not associated with a significant effect on the degree of anorexia.

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