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Further studies on the role of suckling in the parasite status of very young lambs infected with *Teladorsagia circumcincta*.

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ABSTRACT

This study investigated the role of suckling on the resistance and resilience of lambs during the period - six to 20 weeks after lambing. Eighty, 42-day old twin-born lambs were allocated to four treatment groups in which lambs continued to suckle (S-) or were weaned (W-), while concurrently infected with either 0 (-N) or 1000 (-I) L3 *Teladorsagia circumcincta* larvae d⁻¹. Faecal egg counts peaked at 680, 1600, 260, and 225 eggs g⁻¹ of fresh faeces in SI, WI, SN and WN lambs, respectively. Live weight gain was reduced from day 98 of age as a consequence of weaning (p<0.001) and infection (p=0.008). Serial slaughter on days 84, 112, and 140 indicated that suckling reduced the number of adult worms in SI compared with WI lambs though significantly so only on day 112 (p<0.05). *In vitro* larval establishment decreased (p<0.001) with slaughter age but was not influenced by suckling or infection (p>0.05). These results suggest that suckling may inhibit nematode development at a later stage than the immediate entry of incoming L3 larvae. In addition, suckling does not appear to confer greater resilience during *T. circumcincta* infection in young lambs.

Keywords: *Teladorsagia circumcincta*; suckled lambs; resistance; resilience; *in vitro* larval rejection.

INTRODUCTION

The role of a milk diet in resistance and resilience of lambs to abomasal nematode parasitism has received considerable interest in recent years; however, the exact benefit of extended suckling remains unclear. Resistance has been defined as the ability of a host to prevent or limit the establishment or development of infection, usually measured by faecal egg counts, worm counts, worm length, eggs *in utero* etc.; while resilience was defined to be the ability of the host to maintain a reasonable level of production under parasitic challenge, usually measured by weight gain, milk and wool yield etc. (van Houtert; Sykes, 1996).

Bovine milk fed to *Teladorsagia circumcincta*-infected lambs from six weeks after lambing has been observed to reduce worm burdens in lambs except when fed in combination with solid feed (Zeng, *et al.*, 2001). *In vitro* studies showed that bovine milk proteins, or components associated with the proteins, reduced the motility of *T. circumcincta* larvae (Zeng, *et al.*, 2003), which may account for the reduced worm burdens in the milk-fed animals. In our previous study with six-week old infected lambs that were weaned or suckled and necropsied at week 12 after lambing, weaned lambs were able to show comparable resilience to their suckled contemporaries in terms of weight gain, despite harbouring almost twice the

worm burden of the suckled lambs (Iposu, *et al.*, 2005). It was argued that the lambs in that study may have been too young to exhibit an immune response (Kambara, *et al.*, 1993; Colditz, *et al.*, 1996) sufficiently strong enough to affect performance, either through reduced intake (Greer, *et al.*, 2005) or diversion of nutrients to immune functions (Coop; Kyriazakis, 1999), thereby reducing the chance of seeing the beneficial effect of milk on resistance or resilience in lambs of that age.

This study was therefore designed to investigate the possible role of suckling in the resistance and resilience of lambs to *T. circumcincta* infection during the period - six to 20 weeks after lambing when the immune response was anticipated to be strengthening.

MATERIALS AND METHODS

Eighty, twin-born Coopworth lambs were allocated, along with their dams, to one of two infection (larval dose) groups viz. 0 (-N) or 1000 (-I) L3 *T. circumcincta* larvae d⁻¹ from 42 days old. One member of each set of twins was weaned (W-) while its twin was allowed to continue suckling (S-) until the end of trial on day 140. This created four treatment groups that were balanced for live weight viz. weaned and not infected (WN; n = 16), suckled and not infected (SN; n = 16), weaned and infected (WI; n = 24) and suckled and infected (SI; n = 24).

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Suckled lambs and their dams were maintained on a ryegrass-white clover sward, while weaned lambs were reared in a separate paddock of the same sward. A three-paddock rotational grazing of the two flocks was adopted to enable the nursing ewes, which had earlier been dosed with a controlled-release worm capsule (Extender™ 100; 3.85 g/capsule of Albendazole @ a minimum dose of 0.5 mg/kg LW per day; Captec New Zealand Ltd, Auckland, New Zealand), to help reduce pasture larval contamination and also maintain an equivalent pasture contamination for weaned and suckled groups.

Lamb live weight was recorded weekly and faecal samples collected weekly for the determination of nematode eggs in the faeces (FEC), which were measured by using a modification of the McMaster method (M.A.F.F., 1979) and expressed as eggs g⁻¹ (epg) of fresh faeces. Lambs were allocated hierarchically by lambing date to slaughter groups. Necropsy was carried out on slaughter groups of five and six lambs from each of the uninfected and infected treatments, respectively, on days 84 (week 12), 112 (week 16), and six lambs each from all groups on day 140 (week 20) after lambing. In addition, six 'parasite-naïve' lambs were reared indoors, two of which were necropsied at each slaughter date. Slaughter and worm recovery were carried out as described by Donaldson, *et al.* (2001).

The ability of abomasal mucosa to prevent larval establishment was measured using an *in vitro* direct challenge (IVDC) technique, as described by Jackson *et al.* (2004). Briefly, six abomasal tissue explants were taken from each lamb at slaughter and placed into a Corning six-well plate and surrounded, but not submerged, by warm Hank's medium. Varying doses of exsheathed larvae were added into three of the six isolation cylinders, which were formed with the barrel of 5ml syringes and contained the larvae within a given area on the abomasal mucosal surface. The remaining three tissue explants did not receive any larvae, effectively acting as controls. The plates were then flushed with oxygen and incubated for 3 h at 37°C. Tissues and isolation cylinders were then 'washed' by repeated plunging in warm physiological saline to recover any larvae that were loosely associated with tissues while the larvae that had penetrated the tissues were recovered after digestion of the tissue with 1% HCL pepsin. Larvae found in the saline wash and pepsin digest for each well were counted

separately. Larval rejection was calculated as the number of larvae recovered in the wash divided by the total number recovered (washings and digest) for each well and expressed as a percentage.

The experiment was carried out under the authority of the Lincoln University Animal Ethics Committee (Approval No. 58).

Statistical Analyses

All data were analysed using the Genstat suite of statistical packages (GenStat Release 8.2 Copyright 2005, Lawes Agricultural Trust, Rothamsted Experimental Station). Abomasal worm burdens and FEC were log-transformed [$\text{LOG}_{10}(\text{count}+1)$] before analysis. Weekly FEC and cumulative live weight gains underwent sequential comparison of antedependence structure for repeated measures before being analysed using Restricted Maximum Likelihood, with time, nutrition (weaned or suckled) and infection as factors. Abomasal worm burdens, female worm length, and percentage *in vitro* larval rejection were analysed as an unbalanced design using analysis of variance (ANOVA) with slaughter age, nutrition, and infection as factors. Percentage *in vitro* larval rejection for the naïve lambs was analysed using a one-way ANOVA with slaughter group as a factor.

RESULTS

There were no clinical signs of parasitism in any of the lambs. Log transformed and geometric back-transformed FEC means are shown in Figures 1a and 1b, respectively. Overall, FEC was reduced by suckling ($p < 0.001$) in addition to a time X infection interaction ($p < 0.001$) that was reflected in greater FEC in infected than in uninfected lambs from days 63 to 84 and again on day 119.

Abomasal worm burdens, female worm length and percent *in vitro* larval rejection are given in Table 1. Overall, total abomasal worm burdens were influenced by slaughter age ($p < 0.001$) reflected in significantly lower burdens on slaughter day 84 compared with days 112 and 140, which were similar ($p > 0.05$). Also, total worm burdens were lower in suckled than in weaned animals ($p = 0.003$), while also showing a slaughter group X infection interaction ($p < 0.001$) as a result of greater burdens in the infected groups on day 84 but similar burdens in both dose

TABLE 1: Worm counts (geometric means in parenthesis), female worm length (mm) and *in vitro* larval rejection (%) in lambs either weaned (W-) at 42 days of age or suckled (S-) till day 140 and concurrently infected with either 0 (-N) or 1000 (-I) L3 *T. circumcincta* larvae d⁻¹ from day 42 onwards, and necropsied on days 84, 112, and 140 after lambing, respectively.

Age at necropsy (Days)	Lamb groups (n)	Worm Counts				Female Worm Length (mm)	% <i>In vitro</i> larval rejection
		Worm developmental stages			Total worm		
		L3	L4	Adults			
84	WN (5)	0.21 ^c (1)	0.77 ^d (5)	2.91 ^c (806)	2.91 ^b (818)	11.9 ^a	53 ^c
	SN (5)	0.26 ^c (1)	0.72 ^d (4)	2.82 ^c (655)	2.83 ^b (669)	11.2 ^a	57 ^c
	WI (6)	0.25 ^c (1)	3.30 ^{bc} (2003)	4.02 ^{ab} (10567)	4.10 ^a (12722)	11.0 ^a	75 ^{abc}
	SI (6)	0.00 ^c (0)	2.72 ^c (521)	3.94 ^{ab} (8769)	3.99 ^a (9850)	10.9 ^{ab}	61 ^{bc}
112	WN (5)	2.29 ^{ab} (196)	4.29 ^a (19542)	4.21 ^a (16254)	4.58 ^a (37669)	8.5 ^{de}	65 ^{bc}
	SN (5)	2.58 ^{ab} (377)	3.30 ^{bc} (1985)	2.37 ^c (236)	3.61 ^b (4035)	9.8 ^b	55 ^c
	WI (6)	2.41 ^{ab} (255)	3.83 ^{ab} (6713)	3.84 ^{ab} (6981)	4.17 ^{ab} (14927)	8.9 ^d	78 ^{abc}
	SI (6)	2.50 ^{ab} (314)	3.69 ^{ab} (4886)	2.46 ^c (289)	3.78 ^b (5969)	7.4 ^e	85 ^{ab}
140	WN (6)	2.41 ^{ab} (254)	3.87 ^{ab} (7412)	4.11 ^{ab} (12822)	4.37 ^a (23495)	9.1 ^c	94 ^a
	SN (6)	2.13 ^b (132)	4.10 ^a (12588)	3.59 ^{ab} (3889)	4.25 ^a (17864)	7.2 ^e	93 ^a
	WI (6)	2.72 ^a (529)	3.95 ^{ab} (8809)	4.07 ^{ab} (11802)	4.34 ^a (21826)	8.4 ^{dc}	86 ^{ab}
	SI (6)	2.27 ^{ab} (186)	3.34 ^{bc} (2177)	3.14 ^{bc} (1386)	3.77 ^a (5860)	8.4 ^{dc}	95 ^a
	SEM	0.15	0.16	0.12	0.1	0.2	3

abcde - Column means with similar superscript are not significantly different ($p > 0.05$).

SEM - Standard error of means.

groups on slaughter days 112 or 140. For adult worm burdens, there was a slaughter group X nutrition interaction ($p=0.044$) that was reflected in no difference at day 84, but greater burdens in weaned than in suckled lambs on slaughter days 112 and 140. In addition, there was a slaughter X infection interaction ($p = 0.015$) due to greater adult worm burdens of infected animals at day 84 but not on days 112 or 140. Overall, L4 worm burdens were lower in suckled than in weaned animals ($p=0.023$), while also displaying a slaughter group X infection interaction ($p < 0.001$) as a result of greater burdens in the infected groups on day 84 but not on slaughter days 112 or 140. Overall, L3 worms were influenced by slaughter date only ($p < 0.001$), being lower on day 84 than on days 112 and 140. Overall, a small intestinal worm population was made up of an average of 2400 *Trichostrongylus colubriformis* (46%), 1800 *Nematodirus species* (33%), 990 *Ostertagia species* (19%), and 90 *Cooperia species* worms.

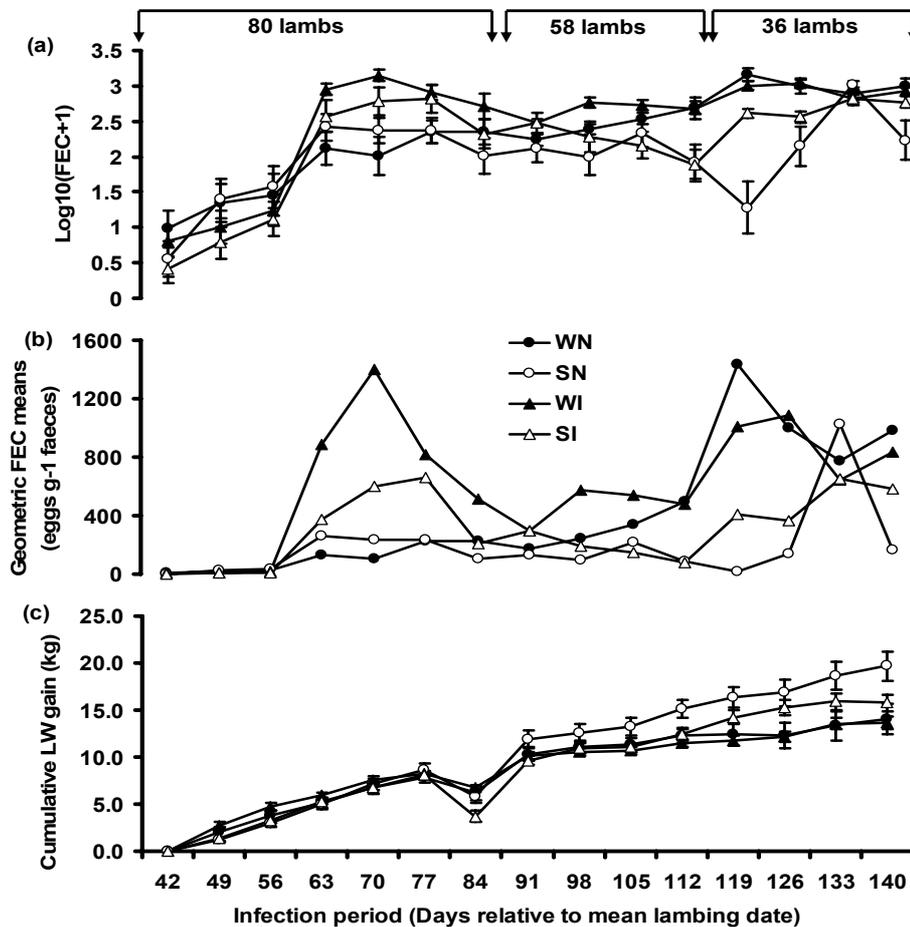
For female worm length, there was a slaughter group X nutrition X infection interaction ($p < 0.001$) reflected in no difference between

treatments on slaughter day 84; however, infection caused a reduction in length of worm in suckled animals on day 112 ($p < 0.05$) and in weaned animals on day 140 ($p < 0.05$) compared with their uninfected contemporaries.

Percentage *in vitro* larval rejection tended to be greater in infected animals ($p=0.056$) and was influenced by slaughter date ($p < 0.001$) as there was higher rejection on day 140 compared with days 112 and 84. No L3s were recovered from either the wash or digest of the control tissue explants that did not receive any larvae. Percentage *in vitro* larval rejection in naïve animals was influenced by slaughter date ($p=0.028$), rejection being lower on day 112 (43%) than on days 84 (67%) and 140 (61%) (SEM=5.63).

Cumulative live weight gain (CLWG) is shown in Figure 1c. The sudden reduction in weight of all lamb groups on day 84 was probably due to extremely cold and wet weather conditions during that period. There was a nutrition X infection ($p < 0.001$) interaction as infection reduced CLWG in the suckled lambs but not in weaned lambs. In addition, there were time X

FIGURE 1: Showing (a) Transformed FEC ($\text{Log}_{10}\text{FEC}+1$), (b) Geometric back-transformed FEC (eggs g^{-1}), and (c) Cumulative LW gain (kg) - of lambs either weaned (W-) at 42 days of age, or continually suckled (S-) till day 140 and concurrently infected with either 0 (-N) or 1000 (-I) L3 *T. circumcincta* larvae d^{-1} from day 42 onwards, with necropsies of selected lambs on days 84 (n=22), 112 (n=22), and 140 (n=24), respectively.



nutrition ($p < 0.001$), and time X infection ($p = 0.008$) interactions; these reflected a general increase in lamb weight with time but a faster rate of gain in both groups of suckled lambs and in the suckled but uninfected lambs, respectively, from day 112.

DISCUSSION

This study supports the hypothesis that suckling could help in reducing worm burdens and FEC, but may not necessarily be beneficial for resilience of lambs, in terms of weight gain, against *T. circumcincta* infection during the acquisition of immunity. While suckled lambs consistently showed lower FEC than their weaned counterparts, the evidence for the reduced worm burden due to suckling was more equivocal. There was little evidence for an effect of suckling on worm burden at day 84, which is in agreement with our earlier findings in 12-week old lambs (Iposu, *et al.*, 2005) and those of Zeng *et al.* (2001) in which worm burden was similar in lambs fed milk in

combination with solid feed and those weaned to solid feed alone. Rather, the effect of suckling appeared during the second phase (day 84 – 112), as indicated by lower FEC and worm burdens in suckled than their weaned counterparts. Moreover, the increase in FEC and greater worm burden at day 112 in uninfected groups suggest these lambs experienced considerable (not measured) natural infection from pasture. The consequence of this appears to have been more severe in the weaned lambs as indicated by the larger worm burdens of the WN lambs. This either suggests greater herbage and larval intake in weaned lambs, or a possibly greater development of resistance in the suckled groups. Given that the average growth rate of suckled lambs was in excess of 200g d^{-1} , it can be calculated that the milk production of their dams by day 100 would provide less than 20% of total energy requirement. Therefore, the difference in worm burdens between WN and SN would seem less likely to be due to difference in herbage (and consequently L3) intake than a more rapid

development of immunity in suckled lambs. Short periods of enhanced protein intake in young lambs have previously been shown to provide delayed and long term effects on FEC (Datta, *et al.*, 1999). Moreover, Bown *et al.* (1991) and van Houtert *et al.* (1995) observed that the beneficial effect of protein supplements occurred at least six weeks after the commencement of infection, possibly by speeding up the acquisition of immunity rather than enhancing innate immunity. Indeed, the pattern of worm burdens, particularly in the suckled infected lambs and progressive shortening of worm length that appeared to be independent of adult worm populations (Stear, *et al.*, 1995) tended to confirm that the lambs had entered the immunity acquisition phase. While the adult worm population at each stage was reflected in a future FEC pattern of the lambs (Figure 1b), the greater proportion of the worm population at the L4 stages in suckling lambs on days 112 and 140 suggests regulation of worm development, which may also signal the onset of an immune response.

The increase in percent larval rejection by abomasal explants as the lambs aged in the IVDC study further confirms a possible immune development at the tissue level. However, suckling did not appear to provide any benefit here regardless of slaughter age. Moreover, the similar larval rejection by naïve and experimental lambs at day 84 are in agreement with the apparent absence of immune competence in the lambs in our previous study, which were slaughtered at that age (Iposu, *et al.*, 2005). However, the relatively low larval rejection by the naïve lambs compared with the infected lambs at second slaughter (day 112) and by all lamb groups at the last slaughter (day 140) could mean these lambs had acquired some immune competence by those slaughter ages. The percentage rejection in the third phase of this study (95%) is similar to that reported in abomasal tissues of animals with a fully established immunity by Jackson *et al.* (2004). The potential of the IVDC as a means of examining the critical first phase of larval establishment (Jackson, *et al.*, 2004), however, needs to be further investigated as the present study showed some inconsistencies between the larval rejection and worm burdens of the lambs. For example, the large adult worm burdens of the weaned lambs in the second and last slaughters were not consistent with the high larval rejection in these lambs during IVDC in the last slaughter. On the other hand, this may be interpreted as indicating that the effect of suckling (milk) on immunological development, with respect to reduced worm burdens, may occur at a later stage than the immediate entry of incoming L3 larvae.

From the performance of the lambs as shown by cumulative LW gain in Figure 1c, it is clear, contrary to our expectation, that suckling may not have improved the resilience of lambs to infection as infection reduced CLWG in the suckled lambs but not in weaned lambs. Similarly, Iposu *et al.* (2005) found a smaller difference in performance than anticipated between control and infected-weaned than between control and infected-suckled lambs. One interpretation is that suckling, and the presumed improved protein nutrition, speeds up the development of immunity, for which there is evidence in the present work and in earlier works (Bown, *et al.*, 1991; Coop, *et al.*, 1995; van Houtert, *et al.*, 1995), but that the consequences of such acquisition of immunity are metabolic costs (Greer, *et al.*, 2005). The fact that the weaned lambs appeared to tolerate infection without metabolic cost may well indicate that they may still not have entered the phase of acquisition of immunity with its associated costs (Greer, *et al.*, 2005). We would, however, have been more confident in this hypothesis had we had lower residual infection on pasture.

We conclude, therefore, that suckling could have a delayed beneficial effect on worm establishment and development while there was no evidence for a beneficial effect on resilience.

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