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The effect of weaning age on animal performance in lambs exposed to naturally acquired nematode infections

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ABSTRACT

The effects of mixed gastrointestinal nematode (GIN) infections on animal growth and post-weaning activity patterns were investigated in grazing intact ram lambs when naturally exposed to two different infection levels and weaned at different ages. Ewes and their twin-born lambs were turned-out to graze in two permanent pasture enclosures naturally contaminated with GIN the previous year. Ewes and lambs in the low parasite exposure group (LP) were drenched before turn-out and at weaning, respectively, with 0.2 mg ivermectin per kg body weight, whereas those in the high parasite exposure group (HP) were left untreated. Two weaning ages were applied, early weaning (EW) (10 weeks) and late weaning (LW) (14 weeks), respectively. The lambs were then allocated to one out of four groups based on parasite exposure level and weaning age (EW-HP, n = 12; LW-HP, n = 11; EW-LP, n = 13; LW-LP, n = 13). Body weight gain (BWG) and faecal egg counts (FEC) were monitored, in all groups, from the day of early weaning and every four weeks, for 10 weeks. In addition, nematode composition was determined using droplet digital PCR. Activity patterns measured as Motion Index (MI; the absolute value of the 3D acceleration) and lying time were monitored continuously from the day of weaning until four weeks post-weaning using IceQube® sensors. Statistical analyses were performed in RStudio, using mixed models with repeated measures. BWG was 11% lower in EW-HP compared with EW-LP ($P = 0.0079$) and 12% lower compared with LW-HP ($P = 0.018$), respectively. In contrast, no difference in BWG was observed between LW-HP and LW-LP ($P = 0.97$). The average EPG was higher in EW-HP compared with EW-LP ($P < 0.001$), as well as in EW-HP compared with LW-HP ($P = 0.021$), and LW-HP compared with LW-LP ($P = 0.0022$). The molecular investigation showed that animals in LW-HP had a higher proportion of *Haemonchus contortus* compared with EW-HP. MI was 19% lower in EW-HP compared with EW-LP ($P = 0.0004$). Daily lying time was 15% shorter in EW-HP compared with EW-LP ($P = 0.0070$). In contrast, no difference in MI ($P = 0.13$) and lying time ($P = 0.99$) between LW-HP and LW-LP was observed. The results suggest that a delayed weaning age may reduce the adverse effects of GIN infection on BWG. Contrarily, an earlier weaning age may reduce the risk of *H. contortus* infection in lambs. Moreover, the results demonstrates a potential use of automated behaviour recordings as a diagnostic tool for the detection of nematode infections in sheep.

1. Introduction

Infection with gastrointestinal nematode (GIN) parasites is well known globally as a major problem in pasture-based sheep production. They are often associated with reduced animal health and welfare, thereby affecting farm productivity and profitability (Charlier et al., 2020). Current control practices of GIN infections depend largely on the use of anthelmintic drugs, often in conjunction with grazing management strategies (Sutherland and Scott, 2010). However, misuse of these drugs has led to widespread development of drug resistance, which now

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threatens the sustainability of traditional worm control strategies (Rose Vineer et al., 2020). In many sheep management situations, lambs are weaned by the ewe at an age between 14 and 26 weeks, with most being weaned around week 21 (Arnold et al., 1979). Under such conditions, milk provision is the main factor involved in the maintenance of the ewe-lamb relationship, but along with a gradual decrease in milk supply, the lamb increases its grazing activity (Penning and Gibb, 1979). Natural weaning rarely occurs in commercial settings. Instead, weaning often occurs around 12 weeks of age and results in an abrupt dissolution of the ewe-lamb bond, which can be advantageous from a production perspective (Napolitano et al., 2008; Orgeur et al., 1998). However, it may also have adverse effects linked to stress responses, presented by increased levels of bleating and locomotion (Alexander, 1977; Mears and Brown, 1997).

In Sweden, lambs in pasture-based production systems are commonly separated from ewes at an age of 14–17 weeks (Arnesson et al., 2015). The relocation, at the time of weaning, from an infected pasture to a relatively uncontaminated, so called "clean", pasture with abundant high-quality herbage can prevent adverse effects on health and production, including GIN exposure (Sutherland and Scott, 2010). For example, in Denmark, it has been shown that this strategy may work regardless of whether the move is accompanied by anthelmintic treatment or not (Githigia et al., 2001). Still, how weaning age affects the infection level of grazing lambs after the move to a new pasture is limited and somewhat conflicting. For example, Watson and Gill (1991) found that lambs weaned at eight weeks had a higher faecal egg count (FEC) compared with control lambs which remained with their dam for 16 weeks. In line with these results, Campbell et al. (2017) observed that delayed weaning improved overall performance on pasture and reduced the overall parasite load. Contrarily, in a recent study, delayed weaning at an age of 17 weeks did not affect the parasite resilience in grazing lambs, but proved beneficial for lamb growth (Campbell et al., 2021). It is therefore of importance that this is further investigated.

Despite all of this, the use of anthelmintics is still an integral part of most grazing-based livestock production systems. On many farms, the "dose-and-move" strategy is still applied. In Sweden, lambs are most often dewormed but usually based on the results from faecal examination, before they are moved to a parasite-safe pasture (Halvarsson et al., 2022). Although this deworming strategy usually is effective in suppressing pasture contamination, it has been suggested that it may select for anthelmintic resistance (AR), especially in absence of *refugia* (Van Wyk, 2001; Waghorn et al., 2008). Dose and move is therefore discouraged in the United Kingdom, for example, where nematode control methods that maintain an anthelmintic susceptible population are advocated (Bartley et al., 2020). Targeted selective treatment (TST), where only individual animals within a group are treated, aims to control the negative impact of GIN infections and maintain individual benefits to animal health and at the same time decrease the risk for the development of AR. However, such an approach requires correct identification of the animals in need of treatment (as reviewed by Charlier et al., 2014). Several TST indicators have been proposed for sheep, i.e. faecal egg count (FEC), body weight gains (BWG) and other production traits, as well as pathophysiological indicators such as the FAMACHA© system (Chylinski et al., 2015; McBean et al., 2016; Westers et al., 2016; Keegan et al., 2018). Still, the implementation of TST approaches is today limited on commercial farms and future integration is dependent on user-friendly, affordable and robust indicators, that ideally can demonstrate the presence of subclinical parasitism and the potential need for anthelmintic intervention.

Behavioural changes associated with infectious disease have been described in a variety of species (typically referred to as sickness behaviours) (Hart, 1987; Shakhar and Shakhar, 2015). The monitoring of such behaviours has been suggested as an applicable indicator for disease monitoring. Furthermore, deviating feeding behaviour and general activity can provide specific information about an animal's health and welfare (Weary et al., 2009). The advancement of Precision Livestock Farming (PLF) enables real-time monitoring of such behaviours (Berckmans, 2017) and could potentially be utilized to monitor the level of GIN challenge. Studies in first-season grazing (FSG) steers infected with GIN have shown that infected animals have a lower activity level compared with dewormed animals as well as a higher number of conducted lying bouts 74 – 86 days after turn-out (Högberg et al., 2019). In addition, FSG steers inoculated with *Ostertagia* and *Cooperia* at turn-out showed a longer daily lying time during the 40 first days on pasture as well as a higher number of steps days 62 – 69, compared with dewormed animals (Högberg et al., 2021a). Until recently, the knowledge of responses in host activity in response to GIN infections in small ruminants was limited (Vercruysse et al., 2018). However, several recent studies have assessed activity patterns with a sensor approach as an indicator of GIN infections in sheep on pastures. Burgunder et al. (2018) observed with 3D-accelerometers that GIN-infected sheep exhibited a smaller behavioural complexity compared with dewormed animals, suggesting that organizational patterns of their behaviour change with GIN infections. Ikurior et al. (2020) showed that sheep naturally exposed to strongyles had a lower activity level compared with dewormed animals after 42 – 46 days on pasture. Furthermore, Högberg et al. (2021b) showed that lambs naturally challenged with GIN had a lower activity level, in addition to having a shorter daily lying time, in connection to weaning, compared to dewormed animals. Still, the possible interaction effect between GIN infection and weaning age on activity patterns remains to be explored.

This study aimed to: 1) investigate if standard diagnostic indicators (BWG and FEC) of GIN parasite infection in naïve grazing lambs are influenced by weaning age under natural grazing conditions. 2) Evaluate if activity patterns (lying time, number of lying bouts and total activity) are affected when exposed to contrasting levels of GIN under natural grazing conditions, at different weaning ages.

We predicted that lambs that were untreated at weaning would have a lower activity level as well as weight gain during the first month after weaning compared to anthelmintic-treated lambs. We also predicted that lambs weaned at 10 weeks would have a lower weight gain compared with lambs weaned at 14 weeks.

2. Material and methods

The study took place at Götala Beef and Lamb Research Centre, Sweden (58° 42′N, 13° 21′E) from May 4th until August 17th 2021.

2.1. Experimental design

The trial consisted of two periods, i) a pre-experimental period in which two groups of ewes were released with their lambs into one of two similar enclosures on a permanent semi-natural pasture with contrasting parasite exposure levels (high, HP, and low, LP, exposure) (Fig. 1). These levels originated from larval contamination generated by grazing of sheep the previous years. Contrast in exposure levels were acquired by deworming ewes in group LP before turn-out (see Animals). The pre-experimental period was followed by ii) an experimental period when data were collected from the lambs following weaning, when moved to one of four 1.0 ha ley enclosures at two weaning ages: at 10 weeks (early = EW) and 14 weeks (late = LW).

2.2. Animals

A total of 41 multiparous ewes with two lambs from the same commercial herd were initially included. The ewes consisted of 34 pure Dorset breed and 7 Dorset and Swedish Finewool crossbreeds. All lambs had a purebred Dorset father and were reared in litters of two lambs. At turn-out on May 4th, ewes with their twin-born lambs were allocated to either the enclosure with high (HP) or low (LP) pasture larval contamination where they were grazed for 5 or 9 weeks, respectively, depending on the time of weaning (8th of June and 6th of July). Ewes

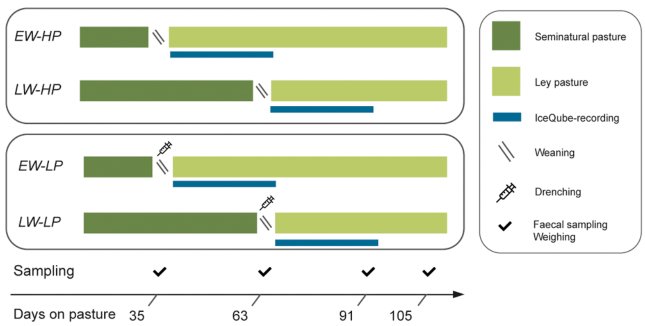

Fig. 1. Timeline illustrating the experimental design. The study consisted of two periods, i) a pre-experimental period in which two groups of ewes were released with their lambs into one of two similar enclosures on a permanent semi-natural pasture, followed by, ii) an experimental period when data were collected from the lambs following weaning to one of four ley enclosures at two weaning ages: at 10 weeks (early = EW) and weeks 14 (late = LW). LP groups (EW-LP and LW-LP) were dewormed with Noromectin® (0.2 mg kg^{-1}) at weaning. Body weight gain and faecal egg counts were monitored, in all groups, from the day of early weaning and every four weeks. Activity patterns were monitored from the day of weaning of each group, EW and LW respectively, and for the following four weeks using IceQube® sensors.

were first sorted according to age and time of weaning and then randomly assorted to one of the two treatment groups. At weaning, intact ram lambs were allocated to one out of four groups based on experimental group and weaning age (EW-HP, n = 12; LW-HP, n = 11; EW-LP, n = 13; LW-LP, n = 13). Each group was balanced for days after lambing (EW-HP: 36.6 ± 2.5; EW-LP: 35.1 ± 3.9; LW-HP: 31.1 ± 2.2; LW-LP: 29.9 ± 1.8). The mean weaning age expressed in days for the four groups were; EW-HP: 72 ± 2; EW-LP: 70 ± 3; LW-HP: 95 ± 2; LW-LP: 93 ± 2. Ewes in group EW-LP and LW-LP were treated with 0.2 mg ivermectin (Noromectin vet., oral suspension, Norbrook Laboratories Ltd., Northern Ireland) per kg body weight before turn-out, whereas their lambs were dewormed at the time of weaning, before being moved (see below). Ewes and lambs in groups EW-HP and LW-HP did not receive any anthelmintic. At weaning, ewes and ewe lambs were relocated to a different farm. The stocking rate in the pre-experimental period was determined according to previous experience of grazing pressure in the semi-natural pasture. Only ram lambs were used in the experimental period due to management reasons. One ewe from EW-HP, LW-HP and EW-LP, respectively, were treated for mastitis before weaning and were excluded from the study. One lamb in EW-LP was treated with Metacam® (20 mg/mL) for lameness three days post-weaning. In addition, one lamb in EW-HP was treated for myiasis and was excluded from the study six weeks after weaning. Altogether, 49 lambs were used in the experiment.

2.3. Pasture

The pasture used pre-weaning consisted of permanent semi-natural pastures with minor areas of former arable land. For the present experiment, the pasture was divided into two enclosures with comparable stocking rates. The grass-dominated pasture was mainly open including areas of deciduous trees. Due to a cold and late spring, the ewes and lambs were from turn-out to weaning supplementary fed 30 kg of concentrate and 40 kg dry matter (DM) grass/clover silage daily equally distributed between the two groups of sheep, HP and LP. The ley grazed by the lambs post-weaning consisted of a mixed sward of grasses (timothy, meadow fescue, perennial ryegrass) and legumes (red clover, white clover). It had not been grazed the previous 22 months. The leys were stocked 19 days for EW-HP and EW-LP and 11 days for LW-HP and LW-LP after a first harvest cut, harvest times set to reach similar grass swards in all four enclosures. To ensure similar conditions, sward height and chemical composition of the herbage was measured at days 36 (the weaned groups EW-HP and EW-LP only), 63, 91 and 107 days after turnout in the four enclosures. In each enclosure, sward height measurements were made according to Frame (1993), with 30–39 recordings performed with a rising plate meter. The chemical composition was estimated by taking 5–6 herbage mass samples in circles (3 m in diameter) along the route. The composed herbage samples from each enclosure were analysed for concentration of crude protein (CP), neutral detergent fibre (NDF) and in vitro organic matter digestibility. The CP was determined according to Dumas (1831) and NDF was determined according to Chai and Udén (1998). Metabolisable energy (ME) concentration was calculated in vitro disappearance of rumen organic matter according to Lindgren (1979). The animals had ad libitum access to fresh water in a large plastic container, a salt stone, and vitaminized minerals in a bucket. Along the fences grass was not cut, thus offering the lambs a place to hide and eat from. The deciduous forest offered both bushes and trees of different species that the lambs could feed from.

2.4. Host performance, nematode egg counts and larval specification

The body weights of lambs were registered on a digital scale from day 36 at pasture and thereafter every fourth week until the end of the study. Rectal faecal samples were collected at the same intervals. FEC was determined using a modified McMaster method based on 3 g faeces dispersed in 42 mL saturated NaCl, where each egg counted represented 50 strongyle eggs per gram (EPG) faeces. In addition, pooled group samples, from each experimental group, were collected and analysed at the same intervals as individual samples. This was achieved by adding approximately 3 g of faeces per animal to a common plastic container with Vermiculite® and thereafter keeping it under humid conditions at 20 °C for 10 days to culture the eggs to the L3 stage. The larvae were harvested using the Petri dish method (Elmahalawy et al., 2018), concentrated and stored in separate 1.5 mL Eppendorf tubes. Total DNA was extracted using the NucleoSpin XS Tissue kit (assessed by: Högberg et al., 2022), following the guidelines issued by the manufacturer (Macherey Nagel, Germany). The proportions of *Haemonchus* spp., *Teladorsagia* spp. and *Trichostrongylus* spp. DNA copies situated in the internal transcribed spacer region 2 (ITS2) of the ribosomal RNA gene array were then determined in relation to the universal strongyle egg DNA copies in duplex reactions using a droplet digital (dd)PCR assay (BioRad), as described earlier by Elmahalawy et al. (2018). During handling for sample collections, mentioned above, an overall clinical examination was conducted. This included signs of malaise, diarrhoea and anaemia.

2.5. Activity measurements

On the day of weaning, lambs were fitted with IceQube® 3D-accelerometers (IceRobotics Ltd, Edinburgh, UK; Validated for use in lambs by Högberg et al. (2020)), on the left hind leg above the fetlock. The sensor operates with a sample rate of 4 Hz, a time resolution of 15 min and a 9-day internal memory. Dimensions were 55 × 55 × 27 mm and 75 g. The IceQube® continuously recorded standing time, lying time, the number of conducted Lying bouts and Motion Index (the measured net acceleration, indicates total activity). Recordings from IceQubes expressed as minutes per 24 h and lying bouts per 24 h, were downloaded at weekly intervals from the time of weaning and then for four consecutive weeks, using the download station IceReader. Recordings from the first and last day were not included, so each analysed day contained 24 h of data.

2.6. Statistical analysis

The statistical analyses were performed using RStudio (2021.09.1). Assumptions of variance homogeneity and normal distribution of residuals of the data were checked by inspection of residual plots. For EPG data, log transformation was used to meet assumptions of normal distribution. Activity data were analysed using mixed models, with repeated measures using the LME function in the NLME package (Pinheiro et al., 2021). For activity data (Lying time, Lying bouts and Motion Index), experimental group (EW-HP, LW-HP, EW-LP; LW-LP), and day from weaning were treated as fixed factors with the individual as nested random effects. Start weights were treated as a covariate in the model. To account for autocorrelation, a continuous autoregressive structure for a continuous time covariate (CorAr1) was fitted. The final model selection was based on Akaike's information criterion. Data from one lamb in group EW-LP, treated for lameness, was excluded from the day of weaning and for the two consecutive weeks. In addition, data from one lamb in group EW-HP, treated for myiasis was removed from the group 43 days after weaning. Pairwise differences were compared with ANOVA in the NLME package. Differences between experimental groups during the different periods were compared using Tukey's pairwise comparisons with the emmeans package (Lenth et al., 2018). Activity recordings of time spent standing were not analysed, as it is a direct mirroring of time spent lying. BWG and EPG were analysed in a repeated measure mixed model with the experimental group (EW-HP, LW-HP, EW-LP; LW-LP) and day as fixed factors with the individual as nested random effects. A continuous time covariate (corAr1) was also fitted to account for autocorrelation. Results are reported as the arithmetic mean values and standard deviation (SD). Significance was set at $P < 0.05$. All illustrations were made using the ggplot2 package (Wickham, 2016).

3. Results

3.1. Pasture

All weaned lamb groups had similar nutritional conditions with similar amounts and nutritional quality of pasture herbage. Across the post-weaning period, the average sward height was 4.4–5.7 (± 0.9–1.4) cm in the four enclosures. The ranges of chemical composition across the enclosures were 152–170 g CP, 449–471 g NDF, and 10.2–11.0 MJ ME per kg of DM herbage.

3.2. Host performance, nematode egg counts and larval specification

The mean body weight throughout the study is presented in Fig. 2.

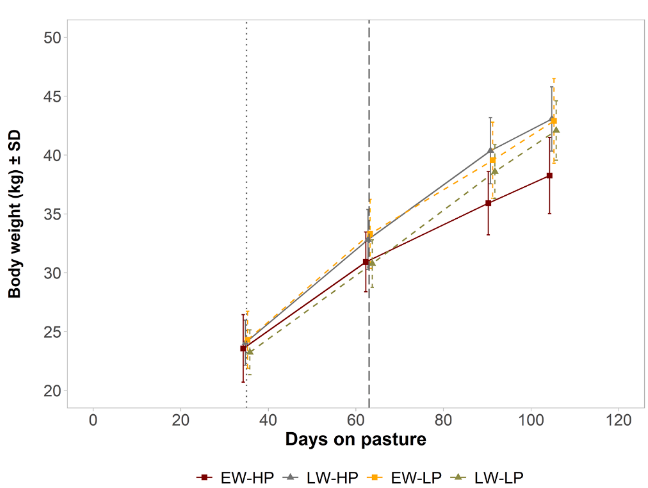

Fig. 2. Mean body weight ± SD from four experimental groups, based on infection level group (LP = low; HP = high) and weaning age (EW = early; LW = late), in grazing lambs exposed to overwintering strongyle larvae at pasture. LP groups (EW-LP and LW-LP) were dewormed with Noromectin® (0.2 mg kg^{-1}) at weaning (EW-LP, n = 13; LW-LP, n = 13), exposing them to a lower parasite challenge compared with HP groups (EW-HP, n = 12; LW-HP, n = 11). The dotted and dashed vertical line indicates the day of early and late weaning, respectively.

There was an interaction effect of experimental group and day on the BWG ($P = 0.0004$) from day 36 on pasture until day 105 (end of experiment). The average daily BWG was 11% higher ($P = 0.0079$) in EW-LP (229 ± 18 g) than in EW-HP (207 ± 20 g). In contrast, there was no observed difference ($P = 0.97$) in BWG between LW-LP (265 ± 6 g) and LW-HP (231 ± 17 g) during the same period. There was no effect of weaning age on BWG between the dewormed groups ($P = 0.99$). However, LW-HP had a 12% higher BWG ($P = 0.0175$) compared with EW-HP.

Fluctuations in FEC and cumulative FEC are shown in Figs. 3a and b, respectively. Strongyle eggs were apparent in all experimental groups at 36 days on pasture. There was an interaction effect of experimental group and day ($P = 0.0004$) on EPG throughout the study. The pairwise comparisons between the groups are presented in Table 1. The average EPG was higher ($P < 0.021$) in EW-HP 36 days after turnout compared with LW-HP, EW-LP and LW-LP. Moreover, the average EPG was higher in both EW-HP ($P < 0.0005$) and LW-HP ($P < 0.0034$) compared with EW-LP and LW-LP, respectively, 105 days after turnout. There was an interaction effect of experimental group and day ($P < 0.0001$) on the cumulative EPG throughout the study. The pairwise comparisons between the groups are presented in Table 1. The cumulative EPG was

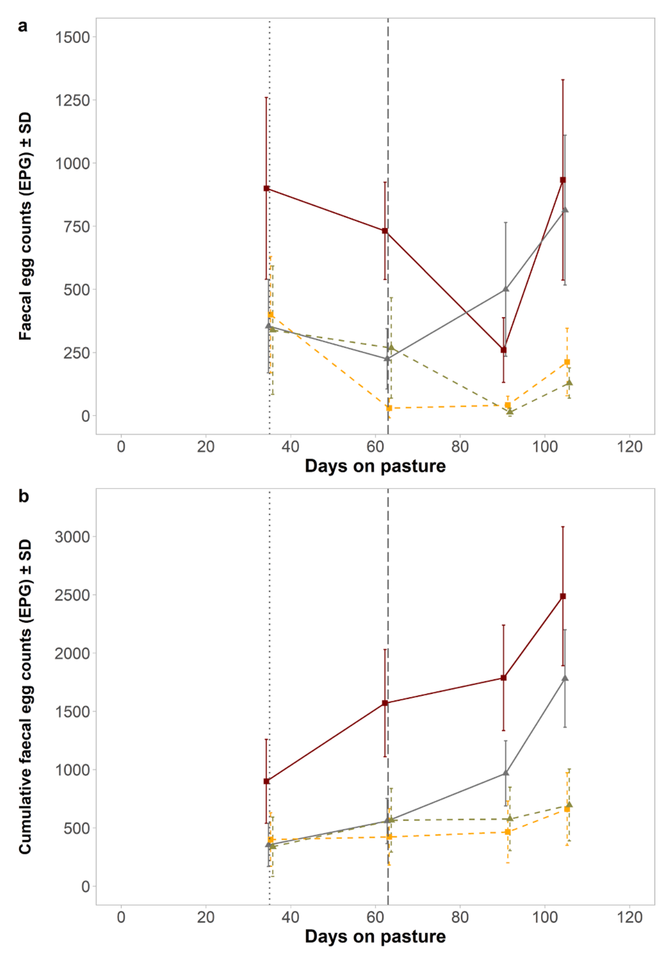

Fig. 3. a) Mean gastrointestinal nematode faecal egg counts (EPG) ± SD, and b) mean cumulative gastrointestinal nematode faecal egg counts (EPG) ± SD, from four experimental groups, based on infection level group (LP = low; HP = high) and weaning age (EW = early; LW = late), in grazing lambs exposed to overwintering strongyle larvae at pasture. LP groups (EW-LP and LW-LP) were dewormed with Noromectin® (0.2 mg kg^{-1}) at weaning (EW-LP, n = 13; LW-LP, n = 13), exposing them to a lower parasite challenge compared with HP groups (EW-HP, n = 12; LW-HP, n = 11). Dotted and dashed vertical lines indicate the day of early and late weaning, respectively.

Table 1
Significance levels of differences in faecal egg counts and cumulative faecal egg counts, respectively, in four experimental groups, in grazing lambs exposed to overwintering strongyle larvae at pasture. Faecal egg counts were monitored, in all groups, from the day of early weaning and every four weeks, for 10 weeks. LP groups (EW-LP and LW-LP) were dewormed with Noromectin® (0.2 mg kg−1) at weaning (EW-LP, n = 13; LW-LP, n = 13), exposing them to a lower parasite challenge compared with HP groups (EW-HP, n = 12; LW-HP, n = 11).

Faecal egg count	*LW-LP*	*EW-HP*	*LW-HP*
EW-LP	$P = 0.99$	$P < 0.0001$	$P = 0.0014$
LW-LP	-	$P < 0.0001$	$P = 0.0022$
EW-HP	-	-	$P = 0.021$
Cumulative Faecal egg count	*LW-LP*	*EW-HP*	*LW-HP*
EW-LP	$P = 0.99$	$P < 0.0001$	$P = 0.11$
LW-LP	-	$P < 0.0001$	$P = 0.20$
EW-HP	-	-	$P = 0.0013$

higher in EW-HP compared to the other groups ($P < 0.0013$). However, no difference in cumulative FEC was observed between the other groups. The proportion of *Haemonchus* spp., *Teladorsagia* spp. and *Trichostrongylus* spp. eggs in each group are shown in Fig. 4. No clinical signs of parasitism, such as diarrhoea or anaemia, were observed.

3.3. Activity measurements

Motion Index (Fig. 5a) had an interaction between the experimental group and the day from weaning ($P = 0.0019$). The average Motion Index was 19% lower ($P = 0.0004$) in EW-HP (11,024 ± 391) compared with EW-LP (13,613 ± 377). In contrast, no difference was observed ($P = 0.13$) between the mean Motion Index in LW-HP (11,109 ± 410) and LW-LP (12,350 ± 379). Average daily lying time (Fig. 5b) was associated with an effect of experimental group ($P = 0.0006$). The average daily lying time was 15% shorter ($P = 0.0070$) in EW-HP (608 ± 23 min) compared with EW-LP (718 ± 22 min). In contrast, no difference was observed ($P = 0.99$) in average daily lying time between LW-HP (599 ± 24 min) and LW-LP (597 ± 23 min). The number of recorded lying bouts (Fig. 5c) had an interaction between the experimental group and the day from weaning ($P = 0.0026$). However, the pairwise comparisons did not show any differences.

4. Discussion

The results of this study showed that overall activity and lying time decreased by the higher infection levels in the early weaned non-treated experimental group. The animal growth was only significantly reduced in the early weaned lambs exposed to the high infection level. Contrarily, late weaning seemed to increase the risk for *Haemonchus* infection, which may have dramatic effects beyond the studied period.

According to the ddPCR investigation *Teladorsagia* and *Trichostrongylus* were identified in all groups, whereas *Haemonchus* was only observed in groups that were not dewormed (HP) and in a larger proportion in the later weaning group (LW-HP). According to a recent study of the nemabiome composition in Swedish sheep (Halvarsson and Höglund, 2021), *H. contortus*, *Teladorsagia circumcincta*, *Trichostrongylus vitrinus*, *Oesophagostomum venulosum* and *Chabertia ovina* are the dominating species in Swedish sheep flocks. Thus, the genera identified most likely conform with these species whereas the unidentified ratio is a mixture mainly composed of *O. venulosum* and *C. ovina*, which are considered apathogenic (Sutherland and Scott, 2010). Similar species composition was observed in an earlier study conducted with animals recruited from the same farm and on the same pasture as in the present study (Högberg et al., 2021b). *Trichostrongylus* spp. was more abundant in EW-HP, appearing from day 35, thereby contributing to a high proportion of eggs shed. *H. contortus* appeared first on day 63 in this group and had a low proportion. Similarly, *H. contortus* appeared in group LW-HP also at day 63, but at a larger proportion. The pattern of *H. contortus* eggs appearing long after turn-out suggests that the lambs were exposed to larvae that originated from eggs shed by the ewes rather than from overwintering pasture contamination, in agreement with the findings of Troell et al. (2005). This is further underlined by the fact that no *H. contortus* eggs were observed in the dewormed groups. Noticeably, the later weaned group (LW-HP) exhibited a higher proportion of *H. contortus* than the earlier weaned group (EW-HP). These findings suggest that earlier weaning may reduce the risk of exposure to *H. contortus*. This could be linked to a shorter pre-weaning grazing period of group EW-HP, in combination with a poor overwintering pasture survival of *H. contortus* larvae. In line with the differences in FEC, EW-HP had a lower BWG compared with both LW-HP and EW-LP. In contrast, there was no difference between LW-HP and LW-LP. This suggests that the higher observed FEC in LW-HP, compared with LW-LP, was not large enough to affect the BWG during the experimental period. As no clinical signs of parasitism, such as diarrhoea and anaemia, were observed, this implies a subclinical course of disease also in the HP groups.

Our results indicate that a low to moderate GIN infection level at a later weaning age may have less adverse effects of the infection on BWG compared with earlier weaning ages. This agrees with Campbell et al. (2021) that observed that animals with delayed weaning, at 116 days of age, had a higher daily weight gain than control animals that grazed the same pasture, but were weaned in connection to turn out at 60 days of age. In contrast, they observed no difference in FEC between the groups, suggesting that the differences in BWG are linked to nutritional benefits associated with a later weaning age, rather than parasite exposure. The

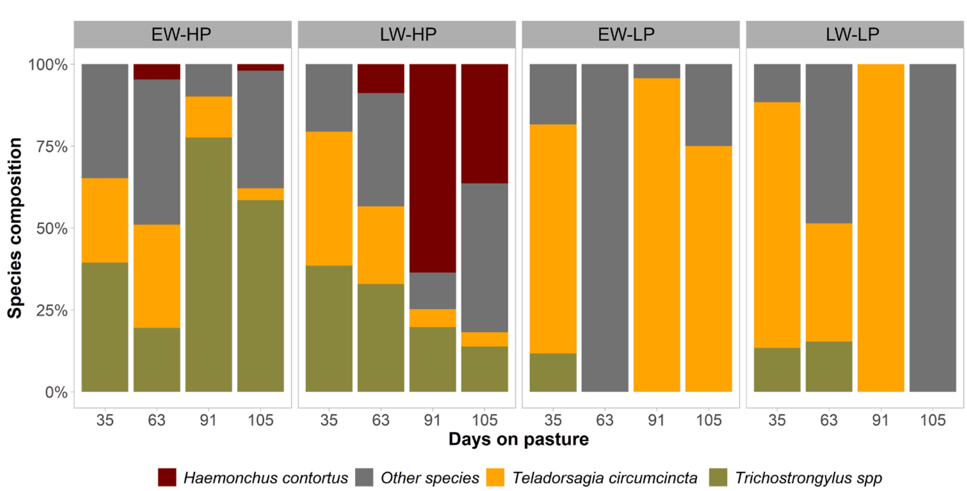

Fig. 4. Species composition (%) of *Haemonchus* spp., *Teladorsagia* spp., *Trichostrongylus* spp. and other species in faecal samples from four experimental groups, based on infection level group (LP = low; HP = high) and weaning age (EW = early; LW = late), in grazing lambs exposed to overwintering strongyle larvae at pasture. LP groups (EW-LP and LW-LP) were dewormed with Noromectin® (0.2 mg kg^{-1}) at weaning (EW-LP, n = 13; LW-LP, n = 13), exposing them to a lower parasite challenge compared with HP groups (EW-HP, n = 12; LW-HP, n = 11).

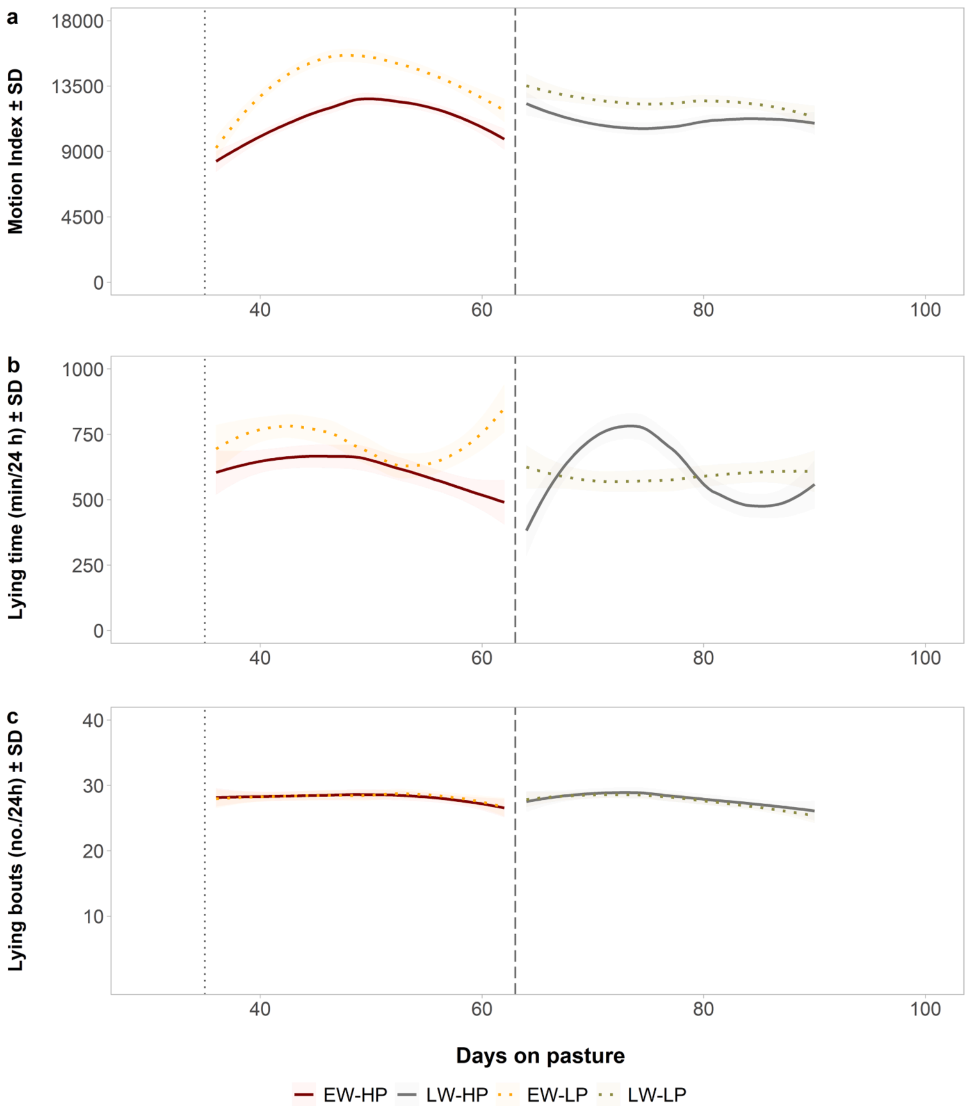

Fig. 5. a) Mean Motion Index (/24 h), b) duration of mean lying time ± SD (min/24 h) and c) the mean number of lying bouts (no./24 h) from four experimental groups, based on infection level group (LP = low; HP = high) and weaning age (EW = early; LW = late), in grazing lambs exposed to overwintering strongyle larvae at pasture. LP groups (EW-LP and LW-LP) were dewormed with Noromectin® (0.2 mg kg^{-1}) at weaning (EW-LP, n = 13; LW-LP, n = 13), exposing them to a lower parasite challenge compared with HP groups (EW-HP, n = 12; LW-HP, n = 11). Dotted and dashed vertical lines indicate the day of early and late weaning, respectively.

mean weight of LW-HP at day 105, 42.9 ± 6 kg, was lower than the minimum of 45 kg for ram lamb live weight for best payment per kg (HKSCAN, 2022). A rise in FEC from day 63–105 was observed in LW-HP compared with LW-LP. An effect of this rise on the BWG of LW-HP could potentially prolong the time to reach slaughter weight. The increased proportion of *Haemonchus* observed in connection to the increase in EPG could have a larger impact on both the health and BWG, if the animals were to be kept longer on the pasture before slaughter, and needs to be further investigated. Furthermore, to be able to adapt weaning age strategies into future parasite control programs, previously mentioned production perspectives, such as labour, pasture quality and availability as well as ewe health, production and longevity, needs to be taken into account.

Several previous studies have shown that GIN infection affects the activity patterns in lamb and adult sheep and have suggested the potential application of on-animal sensors to detect GIN infection on pasture (Burgunder et al., 2018; Ikurior et al., 2020; Högberg et al., 2021b). However, late weaning (>90 days of age) also generates behavioural changes in both the dam and lamb. In connection to separation, lambs tend to increase the frequency of behaviours displayed to search for the dam and decrease the frequency of feeding and resting behaviours, leading to an initial increase in standing time and walking distance (Freitas-de-Melo and Ungerfeld, 2020). Still, differences in activity patterns between early and late weaned groups were observed during our study. This indicates that the contrasting GIN infection levels generated herein had effects on the activity patterns in lambs and that these could be registered with on-animal sensors. Lambs in EW-HP had a 19% lower Motion Index and 15% shorter lying time than dewormed lambs in EW-LP. In a previous study, we observed that lambs naturally exposed to GIN had a shorter average daily lying time and lower Motion Index from one week before weaning until seven weeks after, compared with continuously dewormed control animals (Högberg et al., 2021b). In contrast, no such differences were observed between LW-HP and LW-LP groups in the present study. It has been suggested that the behaviour responses to GIN infection, in both cattle and lambs, are dose-dependent and linked to, but not limited to, parasitic mucosal damage (Szyszka and Kyriazakis, 2013; Högberg et al., 2019, 2021b). Since FEC in lambs provide an indication of infection level (McKenna, 1981; Sutherland and Scott, 2010), it could be argued that the low FEC also in LW-HP in connection to weaning, and the comparable BWG with LW-LP, indicate that the GIN infection level was not substantial enough to trigger a behavioural response, it could be argued that the infection level in LW-HP in connection to weaning, underpinned by the comparable BWG with LW-LP, was not substantial enough to trigger a behavioural response. This underpins the potential for implementing thresholds for drenching based on behavioural responses. However, the implementation of automated indicators of GIN infection, such as behavioural monitoring to enable a TST approach, needs to build on a user-friendly, affordable and robust system (Vercruysse et al., 2018). It has been suggested that on-animal sensors for use in grazing sheep is currently at the proof-of-concept stage, which will allow a future focus on the commercialisation of sensor technology (Fogarty et al., 2018). Moreover, with the development of sensor systems, the possibilities to combine different types of automated data will increase. A limitation in the interpretation of sickness behaviour is that the behavioural responses tend to be general and occur irrespectively of the underlying disease. It can therefore be a challenge to link the behavioural deviations to a specific health disorder, such as GIN infection. It may therefore be necessary to include other information about the herd, such as production parameters and health records, to be able to determine the cause of deviation (Dittrich et al., 2019). Therefore, more studies regarding specific host responses to different diseases, including different parasite infection levels and nematode compositions, are needed. Before a possible implementation, it is important that such systems are evaluated on farm level, including if they yield individual benefits to animal health, welfare and production, and at the same time decrease the total use of anthelmintics, thereby lowering the risk for the development of AR.

5. Conclusion

This study constitutes an attempt to evaluate the effects on animal growth and performance and how it is influenced by the weaning age (early and late weaning) in lambs naturally exposed to different levels of GIN infection. Activity measurements were recorded post-weaning using commercially available on-animal sensors. The results suggest that a postponed weaning age may reduce the risk of adverse effects of GIN infection on performance under the present production settings. However, our results also indicate that a postponed weaning age may increase the exposure to *H. contortus*, and therefore needs to be considered. Early weaned lambs had lower general activity levels (Motion Index) and shorter lying durations with high parasite exposure compared to low, i.e. treated with anthelminthic. Later weaned lambs did not have these differences between high and low parasite exposure.

Ethics statement

The study was approved by the Committee on Animal Experiments in Gothenburg (registration number 824–2017).

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CRediT authorship contribution statement

Niclas Högberg: Investigation, Conceptualization, Formal analysis, Visualisation, Writing - original draft. **Anna Hessle**: Conceptualization, Project administration. **Lena Lidfors**: Conceptualization, Investigation, Project administration. **Johan Höglund**: Conceptualization, Project administration.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Johan Hoeglund reports financial support was provided by Swedish Research Council Formas. Anna Hessle reports financial support was provided by Foundation for Agricultural Research. Anna Hessle reports financial support was provided by Region Västra Götaland.

Data Availability

Data was not deposited in any official repository.

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