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Short communication

Weight gain and resistance to gastrointestinal nematode infections in two genetically diverse groups of cattle

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ABSTRACT

Body weight gain (BWG) and gastrointestinal nematode challenge (GIN) were investigated in two genetically diverse groups of cattle. Thirty-two dairy calves (D = Swedish Red/Holstein) and 31 dairy \times beef crosses (C =Swedish Red/Holstein \times Charolais) pairwise matched by dam breed and birth dates, were monitored for \approx 20 weeks on a pasture grazed by cattle in the previous year. At turn-out, animals (between 6 and 12 months age) from each genotype were either infected with 5000 third stage (L3) Ostertagia ostertagi (50%) and Cooperia oncophora (50%) larvae (H, high-exposure); or treated monthly with 0.5 mg ivermectin (Noromectin^{*}, Pour-on) per kg bodyweight to remove worms ingested (L, low-exposure). Animals were weighed every fortnight and individual BWG was calculated. Faecal and blood samples were collected every four weeks throughout the experiment for nematode faecal egg counts (FEC) and larvae cultures and serum pepsinogen concentrations (SPC), respectively. Nematode eggs were observed 29 days post turn-out in both H groups. FEC peaked to around 200 eggs per gram (epg) on days 58 and 85 respectively in both H groups. FEC were also observed in the L groups at the same time, but mean epg remained very low (< 20 epg) and constituted exclusively of C. oncophora. Although, there was no significant difference in SPC values in animals of the different genotypes, ten animals of CH showed a SPC > 3.5 IU tyrosine whereas only six DH animals reached similar pepsinogen levels. The level of infection (H and L) significantly affected BWG in both genotypes. Even though there was no statistically significant genotype (C or D) × treatment (H or L) interaction, there was a larger difference in body weight of H and L in C (37 kg) compared to D (17 kg) genotypes at the end of the experiment. Our data collectively support the view crossbred (C) animals experience the impact of gastrointestinal parasitism more severely compared to pure dairy (D) first season grazers. The mechanisms that underpin this remains speculative.

1. Introduction

Gastrointestinal nematodes (GIN) in cattle can have severe negative effects on the overall animal health and welfare, especially in parasite naive growing animals, unless they are effectively controlled (Sutherland and Scott, 2010). Beef production in Sweden is closely linked to dairy by utilising offspring of dairy cows. Using semen from bulls of beef breed on dairy cows results in crossbreds with higher growth potential and better carcass conformation compared to purebred dairy cattle (Keane and Moloney, 2010). At the same time selection for high production traits in animals may have adverse effects on host's resistance.

In a comprehensive review, Rauw et al. (1998) presented > 100

references on undesirable traits associated with selection for high productivity in livestock, some of which related to increased immunological susceptibility to parasitic diseases. Evidence deriving from sheep studies has demonstrated that selecting animals for improved performance, such as increased body weight gain (BWG) and wool growth, has resulted in reduced resistance to nematodes compared to unselected genotypes (Bisset et al., 2001; Simpson et al., 2009; Zaralis et al., 2008, 2009).

Although it has been shown that cattle with diverse genetics vary in their susceptibility to GIN (Oliveira et al., 2009, 2013), which could be mediated via immunity to GIN (Forbes et al., 2008) there is little evidence associating the variation in productivity with differences in resistance to GIN. The lack of such evidence hinders the incorporation of

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such traits in breeding indices in cattle and the use of crossbreeding as means of improving performance and disease resistance into cattle. Indeed, there are reports on estimates of the heritability of faecal egg counts (FEC) among cattle from temperate regions (Leighton et al., 1989); studies on Aberdeen Angus show heritability estimates for FEC, which could be implemented in a breeding programme (Morris et al., 2003). To the best of our knowledge, most EU countries focused on dairy cattle production have not widely implemented (cross) breeding programmes, with pure breeding still being the dominant breeding method (Swalve, 2007).

The aim of this study was to investigate the effects of GIN parasitism on the performance and resistance of purebred dairy and crossbred beef genotypes. Our hypothesis was that the crossbreds may be less resistant to GIN and may experience greater penalties in their performance compared to purebred dairy genotypes.

2. Material and methods

2.1. Experimental design

The trial was conducted on a 28 ha of permanent semi-natural pasture at Götala Beef and Lamb Research Centre, Sweden (58° 42'N, 13° 21'E; elevation 150 m asl.) between May 2nd and September 20th 2016, and had a split-plot design with repeated measures and involved two genetically different groups of animals; pure dairy breed (D) and dairy x beef crosses (C), which were subjected to two levels of parasitic exposure. The high (H) level was generated by infecting animals at turn-out with a mixture of about 5000 infective third stage larvae (L3) of Ostertagia ostertagi and Cooperia oncophora (1:1) and then allowing them to graze on a naturally contaminated experimental pasture with nematodes. The low (L) level was achieved by pouring ivermectin solution (Noromectin[®] Pour-on, 0.5 mg per kg body weight) in the midline of the back of the animals, from shoulder to base of tail at four-week intervals from turn-out to housing, meanwhile they were grazing separated from the H animals, on a pasture contaminated at similar levels as the H level. Half of the D animals were turned out on the L and the other half on the H section, and the same was the case for the C calves. Ethical approval was by the Committee on Animal Experiments in Gothenburg (registration number 187-2014).

2.2. Animals

The study included 63 first season grazing (FSG) steer calves purchased as weanlings at 2–3 months of age from the same commercial farm. Thirty-one animals were of pure dairy breed (D, 12 Swedish Red and 19 Swedish Holstein), whereas 32 animals were crossbreeds between dairy and beef breed (C = 12 Swedish Red × Charolais and 20 Swedish Holstein × Charolais). The D calves descended from 13 different sires (six Swedish Red and seven Swedish Holstein), whereas all C calves from the same sire. Each C calf was paired with a D calve based on the breed (e.g. Swedish Red paired to Swedish Red × Charolais) and on birth date, aiming for this to not differ more than 2.8 ± 2.7 days within each pair. The birth date of the calves ranged from of April 18th 2015 to November 1st 2015.

Average daily weight gain during the pre-experimental period was 1.02 ± 0.13 kg for D calves and 1.06 ± 0.15 kg for C calves. All calves were naive grazers at the start of the experiment.

2.3. Weighing, sampling and parasitological examinations

The body weight (BW) of the animals was recorded at two consecutive days at turn-out (start of experiment) and housing (end of experiment) respectively, and every fortnight in between. Average daily body weight gain (BWG) was calculated with linear regression as kg/ day, throughout the grazing period. Rectal faecal samples were collected at turn-out and then at four-week intervals until housing. Faeces were used for quantification of gastrointestinal nematode faecal egg counts (FEC) according to a modified McMaster technique based on 5 g of faeces and using saturated salt as the flotation medium with a minimum detection level of 20 nematode eggs per gram of host faeces (epg).

An additional 5–10 g of faeces were pooled from all FSG in the same experimental group, mixed with Vermiculite^{\circ} and then cultured for at least 10 days at 20 °C. At the end of the incubation period, Baermanisation retrieved L3 and the percentage of each parasite species in the mixture was determined by qPCR as described by Höglund et al. (2013b).

Every four weeks, 2×5 ml blood samples were taken from the coccygeal vein or artery using tubes equipped with a cannula (Vacutainer^{*}, Becton Dickinson). Serum was separated to determine the pepsinogen concentration (SPC) according to a micro-method (Charlier et al., 2011).

2.4. Statistical analyses

Data were inserted and sorted in Microsoft ^{*} Excel^{*} for Mac (v. 14.4.9), exported for statistical analyses in JMP-ProTM version 12.4 (SAS Institute Inc. Cary, NC, USA), and for graphical illustrations in GraphPad Prism^{*} version 4.0c (San Diego, California, USA). Models were constructed in JMP-ProTM with the dependent response variables BWG, logFEC + 1 and SPC level. Statistical relationships were compared using the Mixed Model option in the fit-model platform with sampling time (1-6 for logFEC and SPC or 1-12 for and BWG) as well as parasite exposure level (H or L) and genotype (C and D) plus their interactions included as fixed factors, while animal identity nested with genotype was considered to be a random factor. The significance level was set at p < 0.05.

3. Results

3.1. Host performance

A 26 kg difference in BW was observed between genotypes at the start of the experiment (Fig. 1a-b). The animals in both dewormed groups (DL and CL) lost 39 \pm 15 kg, while the animals in the H groups lost 41 \pm 15 (DH) and 41 \pm 14 kg (CH) during their first two weeks on pasture. From week 3 onwards, all animals started to significantly increase in BW over time (p < 0.001), but the dewormed L group animals of both genotypes (DL and CL) gained approximately 9% more weight than their counterparts in the H groups. Both dewormed groups returned to their starting weights observed at turn-out, approximately after 43 days on pasture, unlike animals in the H groups (DH and CH) that returned to their initial BW after approximately 58 days. The daily BWG over the whole grazing study (141 days on pasture) in groups DH and DL was 0.43 ± 0.16 kg and 0.59 ± 0.16 kg respectively. The corresponding values in the CH and CL groups were 0.42 \pm 0.19 kg and 0.69 ± 0.23 kg, respectively. Although, there was a significant (p = 0.003) difference in BWG between the two levels of parasite exposure (H and L) throughout the grazing season, there was no difference between the genotypes (p = 0.683). Although there was no significant interaction between parasite exposure level and genotype (p = 0.766) animals of C genotype weighed at housing 389 ± 59 kg and 426 \pm 72 kg when in H and L treatments respectively, while those of the D genotype weighed 365 \pm 55 kg and 382 \pm 75 kg, in H and L treatments respectively. Thus, on an average there was a difference of 17 kg in the BW in D animals which was attributed to parasitism, while a 37 kg difference was present in H vs L animals of the C genotype.

3.2. Nematode egg counts and larval speciation

A total of 378 faecal samples were analysed on six occasions for the



Fig. 1. Weight gains in first season grazing calves of two different genotypes cattle, a) dairy and b) crossbreds, grazed for \approx 20 weeks from May to September in south-western Sweden. One group of each genotype were infected and thereby exposed to a High parasite challenge (in red), whereas the remaining group of each genotype were dewormed with ivermectin (0.5 mg kg⁻¹ topically over the back) at monthly intervals and thus Low exposed (in blue). Both genotypes were grazed together but Low and High exposure groups were grazed in two separated enclosures of similar size. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



Fig. 2. a–b) Gastrointestinal nematode faecal egg counts expressed as eggs per gram, and c–d) serum pepsinogen concentrations in first season grazing calves of two different genotypes, dairy and crossbreds, grazed for ≈ 20 weeks from May to September in south-western Sweden. One group of each genotype were infected and thereby exposed to a High parasite challenge, whereas the remaining group of each genotype were dewormed with ivermectin (0.5 mg kg⁻¹ topically over the back) at monthly intervals and thus Low exposed. Both genotypes were grazed together but Low (in blue) and High (in red) parasite exposure groups were grazed in two separated enclosures of similar size. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

quantification of nematode eggs, which appeared in all groups after the animals had been on pasture for 29 days. The animals of both genotypes in the H exposure groups (DH and CH), showed a highly significant time effect (p < 0.001) (Fig. 2a–b). In DH, the highest FEC was observed on day 58 post turn out, whereas in CH on day 85. Thereafter, FEC started

to decrease in both groups; as a result a significant (p = 0.043) interaction between genotype and time was observed. In contrast, FEC remained low throughout the trial in both L groups, 55 \pm 60 epg (DL), 50 \pm 68 epg (CL).

The qPCR results demonstrated that both O. ostertagi and C.

oncophora were present in the larval cultures from animals in the H groups. The mean copy numbers of internal transcribed spacer region 2 (ITS2) per µl of *C. oncophora* was 4,894,190 copies and ranged between 29,590 and 28,387,213, whereas the mean ITS2 copies for *O. ostertagi* was 5,408,690 and ranged between 539,441 and 33,990,235 copies per µl. Copy numbers followed the same pattern as FEC values, with the highest levels observed between 58–85 days post turn out. The relative proportion of *O. ostertagi* in both the H groups (DH and CH) varied between 47% and 80% in D calves and 17% and 46% in C calves. In contrast, *C. oncophora* were exclusively observed in the two dewormed groups (DL and CL).

3.3. Pepsinogen

The SPC measurements in the H groups (DH and CH) showed a similar and highly significant (p < 0.001) time effect with higher levels of SPC towards the middle of the grazing season (Fig. 2c–d). There were no significant differences in SPC levels in animals of different genotypes, with the arithmetic mean SPC levels in the DH group ranging from 0.65 \pm 0.15 to 2.52 \pm 1.52 IU tyrosin, whereas average SPC levels in the CH group varied from 0.61 \pm 0.22 to 2.96 \pm 1.78 IU tyrosin. However, SPC values larger than 3.5 IU tyrosin, which is indicative of clinical ostertagiosis, were observed in six calves in the DH group and in ten calves in the CH group. The highest SPC levels observed in DH were 6.4 IU tyrosin, while it was 7.9 in CH. Furthermore, four of the CH animals repeatedly had SPC values above this threshold, whereas that was not the case for the DH animals. In contrast, the SPC in the low parasite exposure dewormed groups (DL and CL) remained on an average below 0.67 \pm 0.16 IU tyrosin throughout the experiment.

4. Discussion

In this grazing study we investigated the effects of GIN parasitism on the performance and resistance in two diverse cattle genotypes with different growth potential. Our initial hypothesis was that crossbred animals may be less resistant to GIN and may experience greater penalties in their performance compared to purebred dairy genotypes and the data generated from this study are in support of this hypothesis.

Exposure to GIN parasites in the current study impaired calf growth in both genotypes, as shown by the significant differences in BWG between dewormed (L) and experimentally infected (H) animals, a finding in agreement with previous studies with FSG dairy calves in Sweden (Dimander et al., 2003; Larsson et al., 2007; Höglund et al., 2013a). Although not significant, the penalty of parasitism (L vs H) in the BW of calves was more pronounced in C (39 kg) than in D (24 kg) calves, indicating that the impact of GIN infection on growth may have been more severe in the crossbreds than in the dairy calves. This observation is in agreement with similar studies in sheep, where genotypes selected for high productivity, were more susceptible to GIN than animals selected less intensively (Amarante et al., 2004; Zaralis et al., 2009). The mechanisms that underline these observations are still under debate; genetics differences (Rauw et al., 1998), nutritional constraints (Coop and Kyriazakis, 1999) or variation in feeding behaviour have all been thought to play a role in this.

It was beyond the scope of the present study to collect data on the immunological responses involved in parasite resistance; FEC can serve as a reliable indicator of the level of resistance also in grazing cattle. Although there was no difference in the FEC of DH and CH calves, FEC temporal patterns showed that the shedding of nematode eggs was more persistent in CH compared to DH. Indeed, shedding was extended until day 85 post turn out in the CH calves compared to day 58 in the DH calves. This significant genotype x time interaction is consistent with earlier expression of immunity to GIN (Houdijk and Athanasiadou, 2003), in DH compared to CH calves. The lack of difference in FEC between CH and DH animals may have been attributed to dilution of eggs in the faeces of CH calves, as a consequence of increased feed

intake because of their higher growth potential. Combined our results, i.e. the persistent egg shedding and the increased cases of ostartagiosis in CH compared to DH calves, indicate there is an increased susceptibility of C calves to GIN parasites compared to D calves.

In conclusion, in this study we found evidence for differences in parasite resistance, as measured by FEC and SPC and growth performance, as measured by BWG between two genetically diverse breeds of cattle. Despite the limitations of a grazing study, to the best of our knowledge this is the first experimental evidence of differences in resistance in genetically diverse cattle under grazing conditions.

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