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Research paper

Performance and parasitological parameters of steers sequentially grazed with lambs

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ABSTRACT

In the majority of mixed or sequential gazing studies with sheep, cattle performance remained unaffected. However, the treatment regime of the sheep in these studies was often intense and this may have limited cross-transmission of nematodes from sheep to cattle. We conducted a sequential grazing trial with cattle and sheep with moderate anthelmintic intervention. Twenty first season grazing steers were stratified to 10 couples according to their origin, egg excretion per gram faeces (EPG), metabolic weight and previous weight gain record. Thirty naturally infected ewe lambs were stratified to 5 groups according to metabolic live weight and EPG. Five pairs of the steers were sequentially grazed with the 5 groups of lambs whereas another five pairs of steers served as control. Grazing duration was 70 days with a subsequent indoor period of additional 35 days for the steers. Weight and EPG was recorded 3 days before and 27, 49, 70 and 105 days after trial start. The recorded live-weight of the sequentially grazed steers was 182 ± 14, 191 ± 11, 205 ± 15, 219 ± 15 and 236 ± 18 and the live-weight of the control steers was 180 ± 18, 193 ± 19, 203 ± 21, 217 ± 24 and 234 ± 24 kg respectively. The EPG of the sequentially grazed steers 3 days before grazing start and at day 27, 49, 70 and 105 was 94 ± 100, 95 ± 48, 49 ± 42, 58 ± 41 and 140 ± 73 EPG respectively. The EPG of the control steers at the same dates was 96 ± 82, 98 ± 24, 104 ± 77, 98 ± 71 and 270 ± 287 EPG respectively. The sequentially grazed steer groups did not differ from the control groups with regard to EPG, live weight and daily weight gain. However, the sequentially grazed steers showed elevated pepsinogen levels compared to the control steers (*e.g.* 3.34 ± 1.05 units tyrosine and 1.29 ± 0.50 units tyrosine after 70 days of grazing, respectively). Larval samples from individual steer coprocultures of both groups were tested PCR-positive for *Cooperia oncophora*, *Ostertagia ostertagi* and *Haemonchus contortus*. We conclude that short term sequential grazing of first season grazing steers with lambs excreting mainly eggs of *Haemonchus* spp. did not adversely affect steer performance despite increased pepsinogen values. However, hot and dry conditions may have had a suppressive effect on larval development, migration and finally uptake by the steers.

1. Introduction

The development of anthelmintic resistance of gastrointestinal nematodes (GIN) in small ruminants and increasingly in cattle (Gasbarre, 2014) makes it necessary to look for supportive control measures to reduce and refine anthelmintic treatment. A reduction of chemical control may be also beneficial for dung fauna due to possible nontarget effects of macrocyclic lactones (Finch et al., 2020). One such measure could be a focus on an improved pasture management. As nematode larvae may persist for many weeks in high numbers on pasture after a grazing event (Eysker et al., 2005a), grazing at times with high risk of infection should be avoided. Just leaving the pasture abandoned at times of high infection risk will lead to a forage with lower palatability and nutrient content. Forage conservation can be one way to overcome this problem. If forage conservation is not possible, grazing of livestock species with partly different susceptibilities to the predominant nematodes is often recommended to reduce the infection pressure or improve the performance of at least one of the involved livestock species (Craig, 2018; Nolan and Connolly, 1989; Southcott and Barger, 1975).

Probably the most commonly studied livestock combinations in

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terms of simultaneous or alternating grazing are sheep and lambs with cattle. These mixed cattle/sheep systems often yield higher overall productivity in terms of live weight gain per unit land (d'Alexis et al., 2014). In the majority of studies it was observed, that lambs or sheep benefit from mixed or sequential grazing in terms of improved daily weight gain, whereas cattle performance remained unaffected in many cases (Abaye et al., 1994; Fraser et al., 2007, 2014; Wright et al., 2006). However, Jordan et al. (1988) observed a tendency towards impaired live weight gains of calves when grazed with sheep and lambs. On the other hand, Nolan and Connolly (1989) found improved performance of young cattle when sheep were involved in the grazing system.

Most of the above-mentioned studies do not provide parasitological data and not all do mention the anthelmintic treatment-regime for nematode control. Of the 4 mentioned studies reporting neutral effects on cattle performance, 3 mentioned relatively intense sheep treatments: 4 treatments per season (Fraser et al., 2007), every 3 weeks during the trial (Abaye et al., 1994) or at monthly intervals (Nolan and Connolly, 1989). The intense nematode control in lambs may limit cross transmission of nematodes from lambs to calves and may therefore contribute to improved or neutral effects on live weight gains of young cattle. High frequency treatments without refugia provision may also increase the risk for resistance development in the respective nematode population (Hodgkinson et al., 2019).

Of the 6 above-mentioned studies that included weight gain as a parameter, only Jordan et al. (1988) provided parasitological data and it has been shown that calves grazed with sheep did harbour more nematodes and had a tendency towards impaired weight gain compared to controls. Anthelmintic treatments were given restrained, with no treatment of lambs in the first year of the study and only one treatment for the lambs during the second and third year of the study.

A high treatment frequency is also not feasible in organic husbandry systems, as according to the IFOAM standards veterinary medicines should be used very restrained but management practices that are preventive in nature should be included (IFOAM, 2014). The aim of this study was therefore, to assess the effect of sequential grazing of lambs treated moderately with anthelmintics and steers in their first grazing season on weight gain and parasitological parameter in the steers.

2. Materials and methods

All animal-related procedures were performed following the Swiss animal welfare act, the animal welfare ordinance as well as the animal experimentation ordinance with approval from the Cantonal Veterinary Office, Aargau, Switzerland, and permission No. 75713.

2.1. Experimental pastures

The grazing trial was carried out in 2018 for 70 days from August 9 to October 18, with a subsequent indoor period of 35 days. Two pastures in close distance to each other (air-line distance 1.5 km; 47° 29' N; 7° 59' E) with areas of 1.97 (Pasture A, Fig. S1) and 1.42 (Pasture B, Fig. S2) hectares were used for the trial. The experimental pastures were divided into 15 equivalent plots. Pasture A was a natural, herb rich pasture and each of the 15 plots had a size of 0.11 ha. Pasture B was a sown ryegrass/clover (80/20) pasture and each plot had a size of 0.10 ha. Both pastures had not been grazed previously for over 3 years by livestock. Each plot was provided with a shelter, water trough and separate mineral as well as salt block for *ad libitum* intake. Average temperature (°C), precipitation (mm) and air humidity (%) before and during the study were obtained from a public meteorological station located next to pasture A (<300 m).

2.2. Animals and preparations for the experimental grazing period

2.2.1. Steers

Twenty castrated male animals of the Swiss Fleckvieh breed, aged 173 ± 16 days and with average live weight of 171 ± 15 kg 3 days before grazing start (D-3) were used. The steers originated from two different organic farms. The steers had pasture access for 100 days before trial start (D-100) on their farms of origin. Each steer, irrespective of farm, received in total 700 kg milk until weaning. Number of nematode eggs per gram faeces (EPG) was individually surveyed on farms of origin 48 days prior to the start of the trial (D-48) to get information on the parasitological status and to obtain faeces for larval culture. Thirty-seven days before the start of trial (D-37), the steers were transferred, merged and drenched (12 mg Triclabendazole and 7.5 mg Levamisole hydrochloride per kg body weight) on a third commercial organic farm. After drenching, the steers were kept indoors for 5 days and then allowed approx. 6 h pasture access per day until the trial started. These pastures were not the trial pastures and they were grazed by other yearling steers of the commercial farm in the spring of the trial year and throughout the years before and were considered moderately infective. EPG was checked 14 days post treatment *i.e.* 22 days before experimental grazing started (D-22). Two days later (D-20), steers were infected with a single dose of about 300 and 3000 infective third stage larvae of *Ostertagia ostertagi* and *Cooperia* spp., respectively, per 100 kg body weight. The total number of larvae was comparable to Hoglund et al. (2018), but the proportion of *O. ostertagi* was lower and *Cooperia* spp. proportion higher in our study. Common grazing, drenching and subsequent infection was done to equalize possible parasitological differences between the animals from the different farms. The steers were introduced to experimental pasture A on trial day 1 (D1). The time from D-37 until the start of experimental grazing (day1; D1) is referred to as preparatory period. The timeline of the trial is summarised in Fig. 1.

2.2.2. Lambs

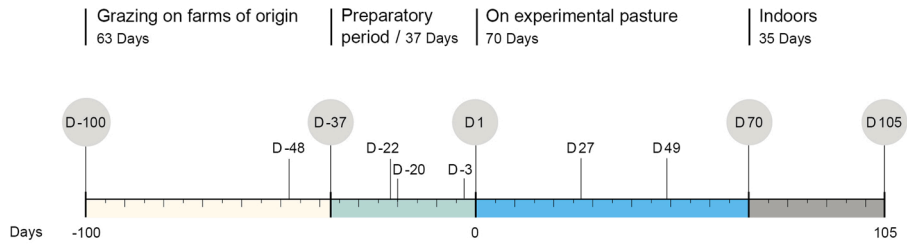
Thirty female Lacaune ewe lambs, raised pasture based on an organic farm with known *Haemonchus* spp. history, were used for the study. The lambs had a mean live weight of 42 ± 5 kg 5 days before experimental grazing (D-5), were naturally infected and never treated with anthelmintics. The lambs arrived on D-17 at the experimental station and were kept on a separate, moderately infective pasture until trial start. On D-5 the lambs were weighted and faecal sampled.

2.3. Group allocation

Twenty steers were stratified to the respective groups according to their origin (farm A or B), daily weight gain record from D-37 to D-3, metabolic live weight ($kg^{-0.75}$) and EPG at D-3. The initial plan was to use 10 steers from each farm to form pairs consisting of a steer from each farm. Some steers originating from farm B, however, showed elevated temperatures and diarrhea (diagnosis without any specific findings) several days during the preparatory period. Therefore, we decided to use surplus steers from farm A to replace the sick animals from farm B. The surplus animals received the same treatment as all other animals and were run with the other animals throughout the preparation period from the beginning. The trial was then realised with 12 steers from farm A and 8 steers from farm B. The lambs were stratified according to their metabolic live weight ($kg^{-0.75}$) and EPG at D-5 to 5 groups consisting of 6 lambs each. Each group of lambs was allocated to the respective steer treatment-group in order to get balanced groups with regard to weight and EPG. Five pairs of steers served as control and 5 pairs of steers served as treatment-groups (sequential-grazing with lambs). Start configuration with parasitological data, farm of origin, weight and previous live weight gains is shown in Table 1.

2.4. Experimental design

The configuration on the pastures with each having 15 plots was as follows: plot 1 was assigned to one pair of steers (control group), plot 2 was assigned to a second pair of steers (treatment group) and plot 3 was assigned to a group of lambs. This formation was repeated in order to



D-100 Start of grazing on farms of origin.
D-48 Eggs per gram faeces (EPG) and larval cultivation for PCR analysis.
D-37 Merging of steers, recording of weight and treatment with Levamisole & Triclabendazole (Endex®).
D-22 Efficacy test after anthelmintic treatment.
D-20 Superinfection with *Ostertagia ostertagi* and *Cooperia oncophora*.
D-3 Recording of weight, sampling of blood and faeces. Stratify animals according farm of origin, weight, weight gain and EPG.
D 1 Start of experimental grazing.
D27, D49 Recording of weight, sampling of blood and faeces.
D70 Recording of weight, sampling of blood and faeces. Steers moved indoors and kept in one group for another 35 days.
D105 Last recording of weight, sampling of blood and faeces. End of trial.

Fig. 1. Timeline of the experiment.

Table 1
Start configuration, EPG history and mean daily weight gain of steers as well as metabolic live weight of steers and lambs per plot.

Farm of origin (steers)	EPG D-42 (steers)	EPG D-22 (steers)	EPG D-3 (steers)	Steer daily weight gain D-37 to D-3 (g) per plot	Steer $LW^{0,75}$per plot	sheep kg $^{0.75}$ per plot	Cattle plots	According sheep plots
A	800	0	37.5	684	96.8	–	1	–
B	0	50	187.5					
A	800	50	112.5	735	98.1	96.2	2	3
B	50	0	50					
A	0	0	25	691	97.9	–	4	–
A	950	50	62.5					
A	400	0	50	662	97.7	97.3	5	6
A	0	0	25					
A	0	50	50	603	97.5	–	7	–
B	150	50	262.5					
A	650	50	87.5	618	98.3	98.5	8	9
B	50	0	350					
A	50	0	75	728	100.0	–	10	–
A	0	50	50					
A	400	50	37.5	647	100.5	100.4	11	12
A	50	50	25					
B	50	50	37.5	566	99.5	–	13	–
B	200	0	175					
B	200	0	37.5	610	100.9	101.5	14	15
B	50	50	162.5					

have 5 replicates of these experimental subunits on a pasture. In summary the steers on plots 1, 4, 7, 10 and 13 served as controls, whereas the steers on plots 2, 5, 8, 11 and 14 (treatment groups) regularly changed with the sheep on plots 3, 6, 9, 12, and 15 when animals were transferred from pasture A to pasture B and vice versa. Steers in control groups grazed on plots that had only been grazed by themselves before. The pastures were changed at intervals of 1–2 weeks (Table 2). After 70 days on the pastures, the 20 steers were moved indoor and maintained in one group for another 35 days until day 105 (D-105). The ewe lambs were returned to their farm of origin at the time when the steers were moved indoor.

2.5. Weighing, faecal sampling and coproculture procedures

2.5.1. Steers

The individual faecal samples of the steers from the sampling event at D-48 on farms of origin were used for individual McMaster analysis and remaining faeces was pooled per farm and incubated at 22 °C for 14 days to obtain larvae from coproculture for later Polymerase Chain Reaction (PCR).

During the experimental period, the body weight and EPG of the steers was recorded on D-3, D27, D49, D70 and D105. On each occasion, individual coprocultures were incubated at 22 °C for 14 days to obtain third stage larvae for later PCR identification. The faecal samples were collected in polythene bags, directly from the rectum, and transported to the laboratory within a maximum of 4 h after collection. EPG were determined using a modified McMaster technique described by Schwarz et al. (2020) on a detection level of 12.5 eggs per gram faeces for the steer from D-3 to D105. All McMaster procedures before D-3 were done similarly, but at a lower detection level of 50 EPG. None of the steers was drenched during the experimental period from D-3 to day 105.

Table 2
Days of the experimental animals spend on the respective pasture.

Pasture	A	B	A	B	A	B	A	Total days
days	14	7	7	7	14	14	7	70

2.5.2. Ewe lambs

The faecal samples of the ewe lambs were taken and processed at D-5, D20 and D61 as described for the steers, but throughout with the lowest detection level being 50 EPG. At the two sampling events at D20 and D61, faeces of the 6 individually sampled lambs were pooled per plot, with proportionally same amount of faeces from each lamb. Per pool, a total of 150–200 g faeces was incubated at 22 °C for 14 days. On D34, 50 % of the lambs with highest EPG were drenched with Monepantel (2.5 mg/kg live weight), to avoid health problems in the lambs, but to allow further contamination of the plots with GIN.

2.6. Larval identification

2.6.1. Steers

Larvae were obtained from pooled coprocultures at the farms of origin on D-48, and subsequently from individual coprocultures. Forty larvae each were stored in deionised water in 1.5 mL Eppendorf tubes at −20 °C for identifying the species using PCR. Genomic DNA was isolated i) from two pools of larvae samples (each 40 larvae) from the two farms of steer-origin and ii) 40 L3 larvae obtained per individual steer at the dates D27, D49, D70 and D105 (Kawasaki, 1990). The obtained larvae from D-3 were discarded by mistake, and were therefore not available for analysis. Briefly, the larval samples were subjected for digestion each in 100 µL of extraction buffer containing 10 mM Tris HCl (pH 8.0), 1 mM Na_2EDTA and 0.5 % Tween 20 with 50 µg/mL Proteinase K at 50 °C for 2 h. Proteinase K was inactivated by heating at 95 °C for 10 min followed by centrifugation at 10,000 *g* for 5 min. Quality and quantity of the extracted genomic DNA in the supernatant was estimated through UV–vis Spectrophotometer (Nanodrop, Thermo Scientific), and stored at −20 °C until use.

PCR was carried out to amplify ITS2 regions of *Cooperia oncophora, Ostertagia ostertagi* and *Haemonchus contortus*, using the primer sets mentioned in Table 3 (Demeler et al., 2013). PCR reactions were performed in CFX^{96} Touch Real-Time PCR System (Bio-Rad, Hercules) for 40 cycles in a total volume of 12 µL per reaction containing each 6 µL of Kapa SYBR® Fast qPCR Kit Master Mix (2X) Universal (Merck/Sigma-Aldrich), 1.2 µL of primer pair mix (3 µM each), 2.8 µL of milli-Q water and 2 µL of template DNA. Cycling conditions were 98 °C of initial denaturation for 3 min followed by 39 cycles of 98 °C denaturation for 10 s, primer specific annealing temperatures for 30 s and 72 °C extension. Fluorescence was recorded with excitation at 470 nm and emission at 510 nm after each elongation step at 72 °C. The specificity and efficiency of each primer pair was verified using DNA in different 10-fold dilutions from known laboratory isolates of *Haemonchus contortus*, *C. oncophora* (both Frei Universität Berlin) and *O. ostertagi* (University of Ghent, Belgium) following the same DNA isolation protocol. PCR reaction with Cq values < 30 were scored as positive for the respective parasite species.

2.6.2. Lambs

One-hundred third stage larvae per plot and sampling event (D20 and D61) were subjected to microscopic differentiation on the recommendations of Deplazes et al. (2013) and van Wyk et al. (2004).

2.7. Sward surface height and animal feeding

Pastures at both places were evaluated for sward height each time before animals were introduced to the pastures by using a rising plate meter (Handley EC09). The pastures were scanned for known plants containing usually anthelmintic compounds. However, no detailed analysis concerning plant composition was carried out. The summer of 2018 was hot and dry (MeteoSchweiz, 2018) and that severely affected the pasture growth before and during the trial. To compensate for the limited pasture growth, the steers and lambs additionally received increasing amounts of hay in the course of the experiment. Additionally, steers and sheep also received 250 g of concentrates per group per day to allow better handling of the animals. During the subsequent indoor period, the steers had *ad-libitum* access to hay and grass/clover silage with a feeding space ratio of 1:1.

2.8. Blood sampling and processing

To compensate for the possibly limited significance of the FEC as a proxy for pasture and animal infection level in steers with developing immunity (Ploeger et al., 1994), we also estimated the serum pepsinogen values of the steers. Steer blood samples were collected by jugular venipuncture on D-3, D27, D49, D70 and D105 for estimation of serum pepsinogen using the micro method described by Dorny and Vercruysse (1998).

2.9. Statistical analyses

All analyses were performed in R (version 4.0.5; (R Core Team, 2021). Linear and generalised linear mixed effect models were applied using the functions lmer and glmer.nb from the lme4 package (Bates et al., 2015). Outcome variables were EPG, weight, weight gain and pepsinogen concentration of the steers. Fixed effects were treatment and date as well as their interaction. For EPG and weight gain, date was included as natural cubic spline with three degrees of freedom to capture the non-linear course. Random effects were animal within pair as nested random effect and farm of origin as crossed random effect. The amount of hay provided and sward height on the respective plots were a priori excluded from the models, as a visual assessment as well as a Mann-Whitney-U-test did not show any differences between treatments. Model assumptions were tested by graphically inspecting model residuals for deviances from normality or homogeneity of variance. Because deviances from model assumptions were detected, the outcome variables EPG (negative binomial, log link function), weight and pepsinogen (both log function) were transformed accordingly. For pepsinogen the last measurement was excluded from the model, as the concentration of pepsinogen was clearly affected by the dislocation of the animals from pasture into the barn after the measurement at D70.

For model comparisons, we used sum contrasts for fixed effects, which were coded as dummy variables. P-values were obtained by comparing the full model to models reduced by one fixed effect or interaction. This was done by applying a parametric bootstrap approach with the function PBmodcomp from the pbkrtest package and 1000 simulations (Halekoh and Hojsgaard, 2014). The advantage of using sum contrasts and comparing reduced models to the full model is that it provides interpretable main effects, even in presence of a significant interaction. Model estimates and confidence intervals for the full model were obtained with 1000 simulations of parametric bootstrap using the function bootMer from the lme4 package. Significance level was set to p=0.05.

3. Results

3.1. Daily weight gain and live weight of steers

Body weight increased during the trial period ($p < 0.001$; Fig. 2.). Daily weight gain decreased in the beginning, then increased until the

Table 3
PCR primers used along with primer specific annealing temperatures.

Species	Name of primers	Anneal. Temp.	Primer sequence	Product size
Cooperia oncophora	Coop-SH-for2 Coop-SH-rev2	62 °C	ATGGCATTTGTCTACATCTGTTT AAATGATAACGAATACTACTATCTCCA	192 bp
Ostertagia ostertagi	Ost.ost-SH-ror Ost.ost-SH-rev	50 °C	TAACATTGTTAACGTTACTGAATGATACGT ATATAAATGATACATCGAATATACAATAC	124 bp
Haemonchus contortus	Hc-SH-for Hc-SH-rev	62 °C	CCATATACTACAATGTGGCTAATTTC TACAAATGATAAAAGAACATCGTCGC	226 bp

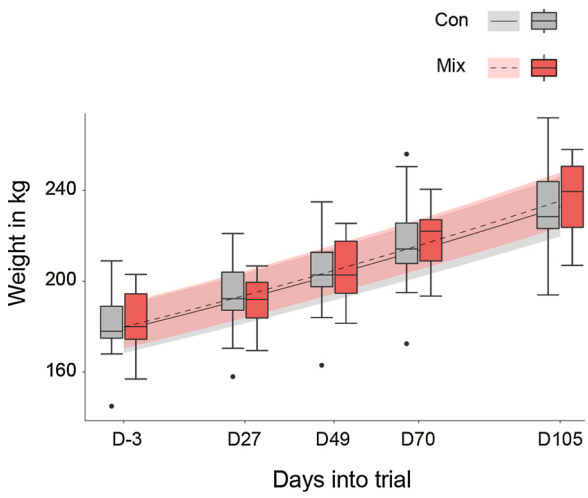


Fig. 2. Development of live weights (kg) during the total trial period. Boxplots show the measured values, solid lines and shaded areas represent model estimates and 95 %-confidence intervals (treatment p = 0.85, date p = 0.001 and treatment*date p = 0.73).

end of the grazing period and was lower again at D105 (p < 0.001; Fig. 3). There were no differences in the development of the body weight (p = 0.83) and daily weight gain (p = 0.82) of the steers between treatments. No interaction between treatment and date was detected.

3.2. Faecal egg counts

3.2.1. Steers

The drenching of the steers with Levamisole on D-37 could not fully eliminate egg excretion. Eleven steers showed EPG-values of 50 at check after drenching on D-22 (Table 1). Steers from both farms of origin were tested positive (Farm A: 7 of 12 steers and farm B: 4 of 8 steers). At D-3 all steers were tested positive for nematode egg excretion (Table 1).

During the trial period, the EPG of the steers was low and balanced between groups until D70. Towards D105 the EPG rose to 270 ± 287 in control steers, with one animal showing a maximum at 975 EPG, whereas the mixed steers had a mean EPG of 140 ± 70 with a maximum of 287.5 (effect of date p < 0.001; Fig. 4). No difference between

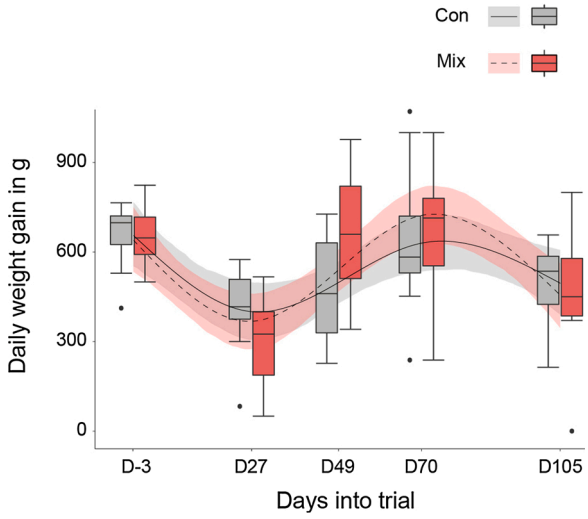


Fig. 3. Daily weight gains (g) during the total trial period. Boxplots show the measured values, solid lines and shaded areas represent model estimates and 95 %-confidence intervals (treatment p = 0.84, date p < 0.001 and treatment*date p = 0.60).

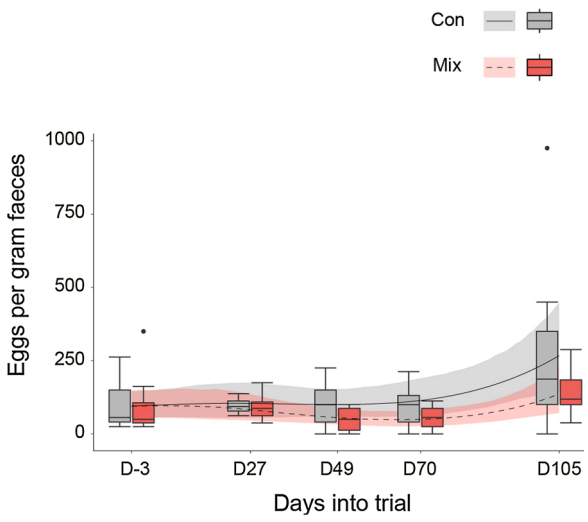


Fig. 4. Development of the nematode eggs per gram faeces of treatment and control steers over the total trial period. Boxplots show the measured values, solid lines and shaded areas represent model estimates and 95 %-confidence intervals (treatment p = 0.96, date p < 0.001 and treatment*date p = 0.38).

treatment and control (p = 0.95) and no interaction between treatment and date (p = 0.38) was found across the whole trial period.

3.2.2. Faecal egg counts of ewe lambs

The FEC of the sheep was moderate at grazing start (D-3), rose at the measurement at D20 to maxima of 400–800 EPG and declined to low levels (D61) after drenching the animals with highest EPG at D34 (Table 4). The threshold for treatment per group varied between 600 and 1050 EPG.

3.3. Larval identification

3.3.1. Steers (PCR)

Larval samples from pooled coproculture collected at farms of origin (D-48) were tested PCR-positive for *Cooperia oncophora* and *Ostertagia ostertagi*, but negative for *Haemonchus contortus* at both places. During experimental grazing, *C. oncophora* positive animals increased from 60 % (D27) to 100 % (D105) in the control group. In the mixed grazing group, proportion of *C. oncophora* positive animals varied from 80 to 100 % during the trial. A steer proportion of 90 % was tested positive for *O. ostertagi* at D27, in both treatment and control group. For the mixed group, this proportion was stable throughout and it was always a different animal that tested negative on different dates. After D27, 100 % of the control steers were tested positive for *O. ostertagi*. In both the groups, steers were tested positive for *H. contortus* with variable shares from 20 to 50 % in the mixed group and 30 to 80 % in the control group (Table 5).

3.3.2. Ewe lambs

Microscopic differentiation of cultured larvae revealed that on both occasions *Haemonchus* spp. was the dominant genus. In all sheep groups and on both occasions (D20 and D61) the *Haemonchus* spp. proportion

Table 4
Eggs per gram faeces of the sheep (mean per plot) over the trial.

Plot	FEC day -3	FEC day 20	FEC day 61
3	240 ± 216	681 ± 579	67 ± 121
6	275 ± 129	796 ± 721	217 ± 299
9	175 ± 82	458 ± 254	67 ± 98
12	529 ± 427	408 ± 441	133 ± 209
15	300 ± 197	625 ± 401	250 ± 387

Table 5
Proportion of animals tested (PCR) positive for *C. oncophora*, *O. ostertagi* and *H. contortus* eggs in the course of the trial in percent.

Date	Control: *C. oncophora*	Mixed: *C. oncophora*	Control: *O. ostertagi*	Mixed: *O. ostertagi*	Control: *H. contortus*	Mixed: *H. contortus*
D27	60	90	90	90	60	40
D49	70	80	100	90	80	50
D70	80	90	100	90	60	50
D105	100	100	100	90	30	20

across all groups was at least 84 % (Table 6).

3.4. Serum pepsinogen

Before grazing started (D-3), mean pepsinogen levels were 0.38 ± 0.24 U Tyr and 1.06 ± 0.34 U Tyr for the control and mixed grazing steers, respectively. Levels reached a maximum on D70 for both the groups with 1.29 ± 0.50 U Tyr and 3.34 ± 1.05 U Tyr for control and mixed grazing steers, respectively. Then U Tyr values declined during the indoor feeding (Fig. 5). Considering the grazing period until day 70, the pepsinogen level of the mixed steers were higher when compared to the control steers ($p < 0.001$). Pepsinogen level of mixed steers was already higher shortly before the start of grazing, but increased more than in the control group, indicated by an interaction between date and treatment ($p < 0001$).

3.5. Pasture and hay supplementation

The average sward height (Fig. 6) of both pastures declined over the experimental grazing period and did not differ between the treatments. The consumption of hay increased with the decrease in the average sward height. No differences of the hay consumption could be observed between groups. Mean amount of hay consumed per plot was 2.5 kg ± 0.3 kg from D1 to D28, 7.2 kg ± 0.0 kg from D29 to D50 and 9.7 kg ± 0.2 kg from D50 to D70. When screening the pastures for plants with possible anthelmintic effects, only rarely single plants of *Lotus corniculatus* were found. The proportion of *L. corniculatus* was estimated to be less than 0.1 percent of the total green mass.

3.6. Precipitation and temperature

The temperature in Switzerland in 2018 was 2 °C above and precipitation 30 % below the long-term average (MeteoSchweiz, 2018). The experimental sites were also affected with longer periods of low precipitation, especially during the 5 week period before the start of grazing, but also during the trial (Fig. 7).

4. Discussion

This study was carried out to get more information on effects on young steers when sequentially grazed with ewe lambs and restrained anthelmintic treatment.

In contrast to the work of Nolan and Connolly (1989), we could not find improvement in the live weight gain in our sequentially-grazed steers. Nolan and Connolly (1989) observed a significant improvement in steer-performance in a mixed grazing situation at a balanced proportion with lambs but did not report parasitological data. However, the insignificant difference in the weight gain between steer treatments in our study is in line with a number of other studies in a mixed grazing situation with cattle and sheep (Abaye et al., 1994; Fraser et al., 2007, 2014; Wright et al., 2006). On the other hand, unlike Jordan et al. (1988) we did not observe negative effects on the weight development of our steers by sequentially grazing them with sheep despite the high *Haemonchus* spp. proportion of the ewe lambs.

Just as with the weights, we could also not find any differences of EPG between the steer groups. This is in agreement with Brito et al. (2013) and Bairden et al. (1995), who could not find EPG differences of cattle that was mixed or sequentially grazed with sheep. Ploeger et al. (1994) found significant correlations between larval exposure and EPG in first season calves. However, this was only evident in the first half of the grazing season and the positive correlation disappeared during the

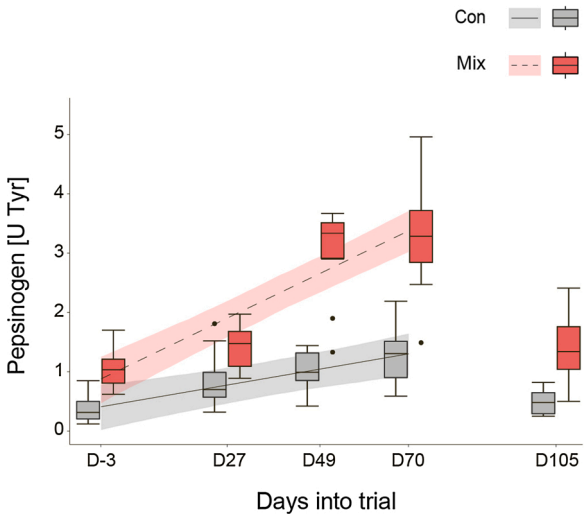


Fig. 5. Course of the pepsinogen levels (U Tyr) over the trial period. Boxplots show the measured values, solid lines and shaded areas represent model estimates and 95 %-confidence intervals. Measurements on day 105 were not included in the model due to the strong effect of dislocation of the animals into the barn, which happened on day 70 (treatment $p < 0.001$, date $p < 0.001$ and treatment*date $p < 0.001$).

Table 6
Trichostrongylid larvae found in percent in coprocultures from sheep at day 20 (D20) and day 61 (D61) after grazing start.

	Plot	Haemonchus spp.	Teladorsagia spp./Trichostrongylus spp.	Chabertia/Oesaophagostomum
Copro-culture D20	3	98	2	–
	6	84	8	2
	9	94	2	4
	12	94	4	2
	15	96	4	–
Copro-culture D61	3	86	14	–
	6	93	7	–
	9	98	2	–
	12	93	7	–
	15	100	–	–

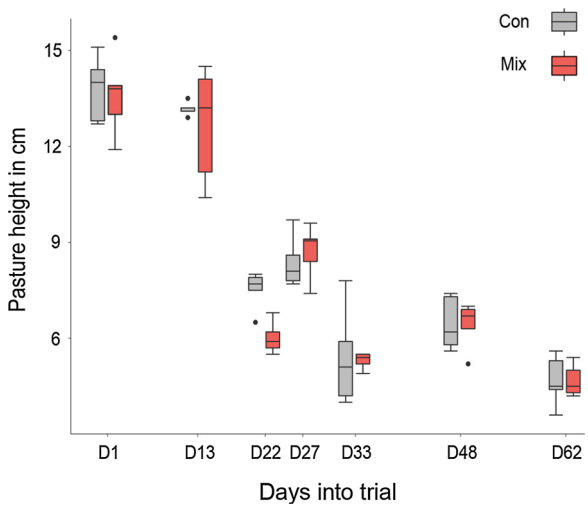


Fig. 6. Sward height in cm at the day the animals accessed the respective pasture.

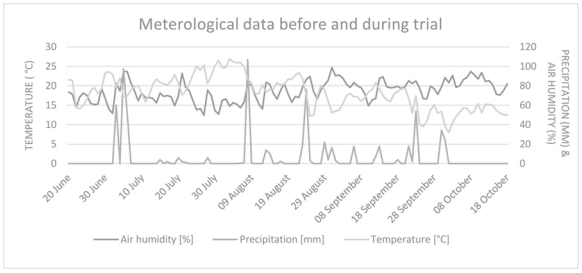


Fig. 7. Precipitation, average temperature and air humidity from April to the end of the experimental period.

second half of the season. The authors assumed that the developing immunity of the calves might be responsible for the absent correlation in the second half of the grazing season. As our experimental animals were grazed since the beginning of the year starting on their farms of origin already 100 days before start of the experimental grazing, it is likely that the EPG towards the end of the trial may not correctly reflect the worm burden or pasture infection pressure in our study.

In contrast to EPG, serum pepsinogen usually correlates well with infection levels of *O. ostertagi* (Eysker and Ploeger, 2000). In our study, the pepsinogen values were low to moderate in the beginning of the experimental grazing until and including the measurement on D27. This fits well to the expected low infection level of the pastures, as it was not grazed by livestock for at least 3 years, and infection pressure had to build up first. At the measurements at D49 and D70, the pepsinogen levels of the steers were increased, in particular in the mixed steers. In general, it was not surprising that pepsinogen levels rose at D49 and D70, as other studies showed high larval recoveries 4–7 weeks (Hertzberg et al., 1992) and 4–10 weeks (Eysker et al., 2005a) after faeces deposition on pasture. Rather surprising was that the steers sequentially grazed with lambs did show significantly higher pepsinogen levels compared to control steers. Even though pepsinogen values were already higher at grazing start for the mixed steers at D-3, the increase of pepsinogen values in the mixed steers was clearly steeper than in control steers, indicated by the significant interaction.

It seems rather unlikely, that higher numbers of *O. ostertagi* of the mixed steers compared to the control steers have caused the difference in pepsinogen values, as the FEC did not differ between steer treatments and the number of *O. ostertagi* positive animals identified by PCR were comparable between steer treatments. Also, it is unlikely that the sheep acted as multiplier for *Ostertagia* spp., as we could not identify larvae of this genus from the coprocultures obtained from sheep. Also in other studies where cattle with *O. ostertagi* infections were co- or sequentially grazed with lambs, no or very limited cross infection of *O. ostertagi* occurred from cattle to sheep (Arundel and Hamilton, 1975; Jordan et al., 1988).

It could be that the elevated pepsinogen levels of the mixed-steers were caused by *Haemonchus* spp. infection. *Haemonchus* spp. larvae were identified in abundance from the coprocultures of sheep accounting for at least 84 % at both the measurement time points. Shoo and Wiseman (1986) have demonstrated that calves can show elevated pepsinogen levels after *H. contortus* infection. Also Simpson et al. (1997) pointed out that infection with *H. contortus* might provoke elevated secretory products including pepsinogen, even though this was in sheep. Thresholds above which clinical parasite induced gastroenteritis is assumed, do vary between 3 and 5 U tyrosine (Eysker and Ploeger, 2000). The mean pepsinogen values of the mixed steer group at day 49 and 70 were at 3.0 and 3.34 U tyrosine. As the repeatability of pepsinogen values between labs is rather poor (Charlier et al., 2011), it is difficult to determine a clinical infection level from pepsinogen values alone. Since the daily weight gains of the mixed-steers at the dates of high pepsinogen values were numerically above those of the control steers, that showed low to moderate pepsinogen values, we consider pepsinogen values of the mixed-steers as high, but not necessarily an indicator for a clinical parasite induced gastroenteritis.

Steers of both treatments were tested positive (PCR) for *H. contortus*. Considering the entire trial period, the mean of *H. contortus* positive steers was 57.5 % *vs.* 40 % in the control and mixed steer groups, respectively. The lower proportion of *H. contortus* positive steers in the mixed-group may contradict the assumption that *Haemonchus* spp. may be the reason for the elevated pepsinogen values. But the link between infection and egg excretion seems to be weak, as Flores-Perez et al. (2019) found that in only 38 % of feedlot-calves infected with 4000 infective larvae of *H. contortus* eggs were found in the faeces. Beyond that the number and proportion of larvae for PCR analysis depends on the FEC of the steers and the conditions during coprocultures. As coproculture may yield varying proportions of developing larvae when larval recovery rates were low (Eysker and Ploeger, 2000), as it was the case in our study, the proportion of *H. contortus* positive animals may not be of much help to conclude whether the higher pepsinogen values in mixed steers may have been caused by Haemonchus or not. This is also true for the above mentioned use of PCR results to estimate the proportion of *O. ostertagi* positive animals. A shortcoming of our work is the lack of information on pasture larval counts including larval identification. This would have allowed using larval numbers and proportion of *Haemonchus* spp. to draw a better conclusion on the origin of the elevated pepsinogen values in the steers. We did not include PCR tests for *H. placei*, as this preliminary cattle nematode seems to occur rarely in temperate climate (Deplazes et al., 2013).

Looking at the steers tested positive for *H. contortus* in both, the mixed and the control groups, it raises the question on where they acquired the infection. Unfortunately, the larvae obtained from the first coproculture of the steers at D-3 intended for PCR-analysis, were discarded by mistake. This made D27 to be the first event after the start of experimental grazing for PCR based identification of cattle nematodes. With *H. contortus* having a prepatency period of only 18–19 days (Deplazes et al., 2013), acquisition from the experimental pastures is well possible. It is just as possible that the steers acquired the *H. contortus* infection on the pastures during the preparatory period. However, none of the pastures were grazed by small ruminants in the years before. But at both locations (experimental pastures and preparatory pastures) sheep were grazing close. All pastures were situated in a densely forested area and the edges of the forest were directly bordering with the experimental pasture A as well as the preparatory pasture. Deer (*Capreolus capreolus*) is very common in the area where the experiment took place and it may be that deer have introduced *H. contortus* to the pastures (Megyesi et al., 2020). An error, such as a mix-up the plots, is very

unlikely; this would have been noticed, latest at the next sampling and weighting event.

Eysker et al. (2005b) described reduced numbers of larvae migrating onto herbage under dry and hot conditions. We assume that this was also the case in our trial, however, our conditions were not as extreme as reported by Eysker et al. (2005b) with only 9 mm rainfall during August. The total precipitation of 69 mm in August in our study (which refers to day 1–22 of the trial), as well as the following precipitation should have allowed the development of a proportion of the deposited eggs and larval migration onto herbage.

The amount of hay consumed from D50 to D70 matched the Swiss national feeding recommendations *i.e.* 4.7 kg dry matter per day for 225 kg live weight with 600 g daily live weight gain per animal for a dual-purpose breed. Therefore, the ingested amount of roughage from pasture in this phase of the trial must have been small. However, the animals were frequently observed grazing if not lying and ruminating. We assume that larvae were ingested by the steers when grazing the sprouting grass close to the soil, which was indicated by the increased pepsinogen levels. It is known that plant species *e.g.* rich in condensed tannins can show effects on parasitological parameters. However, *L. corniculatus* was the only plant with known anthelmintic properties occurring on the pastures. As it was found very rarely only, the potential effect was considered negligible, as the effect is usually dose dependent in tannin containing forage (Brunet et al., 2007; Novobilský et al., 2011).

5. Conclusion

We conclude that short term sequential grazing of first season grazing steers with ewe lambs did not affect steer performance despite of moderate anthelmintic intervention in the ewe lambs. The higher pepsinogen values of the mixed-steers may indicate a higher infection pressure on the shared pastures with lambs. It seems that sequential grazing of first season grazing cattle can be recommended even if anthelmintic intervention in the lambs is restrained and *Haemochus* spp. the dominant nematode genus of the sheep. However, these results must be taken with caution, as rather dry weather conditions may have limited larval development and migration onto grass. In addition, the reduced roughage intake from pasture may have limited the larval uptake.

CRediT authorship contribution statement

Joken Bam: writing – original draft & investigation. Susann Thüer: investigation. Mirjam Holinger: methodology, formal analysis & visualisation. Thomas Oberhänsli: methodology, Markus Leubin: investigation. Florian Leiber: supervision, methodology and editing. Steffen Werne: Writing – original draft & review and editing, investigation, project administration, funding acquisition & methodology.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.vetpar.2021.109645.

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