Contents lists available at ScienceDirect

# Veterinary Parasitology: X

journal homepage: www.journals.elsevier.com/veterinary-parasitology-x

# Potentials of using milk performance data and FAMACHA score as indicators for Targeted Selective Treatment in Lacaune dairy sheep in Switzerland

Katharina Schwarz<sup>a,b</sup>, Beat Bapst<sup>c</sup>, Mirjam Holinger<sup>a</sup>, Susann Thüer<sup>a</sup>, Inga Schleip<sup>b</sup>, Steffen Werne<sup>a,\*</sup>

<sup>a</sup> Research Institute of Organic Agriculture (FiBL), Ackerstrasse 113, 5070 Frick, Switzerland

<sup>b</sup> Eberswalde University for Sustainable Development, Schicklerstrasse 5, 16225 Eberswalde, Germany

<sup>c</sup> Qualitas AG, Chamerstrasse 56, 6300 Zug, Switzerland

## ARTICLE INFO

Keywords: Gastrointestinal nematodes Dairy sheep Milk yield Targeted selective treatment Organic farming Anthelmintic resistance

# ABSTRACT

Targeted Selective Treatment (TST) is one approach to slow down the development of anthelmintic resistance. Its success is closely linked to the correct identification of animals in need of treatment. In dairy goats it has been proposed to use milk yield as TST indicator and to focus treatments on high yielding dairy goats. In dairy sheep the relationship between milk performance and infection with gastrointestinal nematodes (GIN) is not well known. The aim of this study was to investigate the relationship between milk yield and GIN infection in dairy sheep and based on this, to evaluate milk performance data as a potential TST indicator. Overall 1159 Lacaune ewes of 15 dairy sheep farms in Switzerland were included in the study. The ewes were phenotyped once between August and December 2019, when they were at least 70 days in milk (DIM). Individual faecal samples were taken from every ewe to determine the nematode egg concentration per gram faeces (EPG). In addition, the clinical parameters FAMACHA score and packed cell volume (PCV) were measured. Linear mixed models were used to analyse the effects of the collected parameters on EPG. EPG increased significantly with increasing test day milk yields (P = 0.002), indicating high yielding ewes to be less resistant to GIN infections than low yielding ewes. The effect was most pronounced in earlier lactation but remained within a moderate range. Overall, our results indicated the potential of using milk yield data of rather early lactation as TST indicator in dairy sheep. On farms with predominantly H. contortus the combination with FAMACHA might improve the correct identification of highly infected ewes, as FAMACHA was correlated with EPG (r = 0.37, P < 0.001).

# 1. Introduction

In sheep farming, gastrointestinal nematode (GIN) infections are among the most important diseases, causing high economic losses due to reduced performance and animal health problems (Morgan et al., 2013). As a result of improper use and high treatment frequencies, the worldwide increase of resistance to anthelmintics has become a central challenge in sheep farming. Since the 1970s, numerous studies of anthelmintic resistance (AR) in GIN have been published worldwide (Kaplan, 2004). Increasingly, multiple-drug resistant GIN are also occurring in Europe (Scheuerle et al., 2009; Voigt et al., 2012; Traversa and von Samson-Himmelstjerna, 2016). This highlights the urgent need to develop sustainable strategies for parasite control in small ruminants. In dairy systems, the pressure to act is increased by the limitation to few anthelmintic drugs, which are allowed to use during lactation, if milk is intended for human consumption.

The impact of parasitism on productivity in dairy sheep has been explored in previous studies and there seems to be a general agreement that GIN infections reduce milk production in dairy ewes. A metaanalysis quantified the mean loss in milk yield to 22 % in infected compared with uninfected dairy sheep (Mavrot et al., 2015). Effective anthelmintic treatment of infected dairy ewes appears to lead to longer milking periods (Suarez et al., 2009), improved lactation persistence (Fthenakis et al., 2005) and lower somatic cell counts (Arsenopoulos et al., 2019), whereas effects of GIN infections on milk components are not consistent across studies. While Sechi et al. (2010) did not find

\* Corresponding author at: Department of Livestock Sciences, Research Institute of Organic Agriculture FiBL, Ackerstrasse 113, 5070 Frick, Switzerland. *E-mail address:* steffen.werne@fibl.org (S. Werne).

https://doi.org/10.1016/j.vpoa.2020.100030

Received 29 April 2020; Received in revised form 31 August 2020; Accepted 1 September 2020 Available online 2 September 2020 2590-1389/© 2020 Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).







effects of GIN infection on milk components, Cruz-Rojo et al. (2012) reported lower total milk protein contents.

Usually, dairy sheep farmers try to keep anthelmintic treatments of ewes during lactation to a minimum, as the subsequent withholding periods for milk will have negative impacts on their returns. This particularly affects organic farming, where legally required milk withdrawal times usually double after anthelmintic treatment (Bio Suisse, 2020). It may be possible that dairy sheep farmers tend to treat all ewes in the dry period in order to avoid milk withdrawal periods and animal welfare problems during lactation. If all ewes were treated in the dry period, this would conflict with refugia-based methods, which are considered essential to slow down the development of AR. The phenomenon of refugia is based on leaving a part of the parasite population unexposed to anthelmintic treatment in order to maintain genotypes of parasites still susceptible to the anthelmintic drug (Van Wyk, 2001). Whole herd anthelmintic treatments during the dry period, especially during winter, may kill all susceptible GIN. Thus, after lambing and at the beginning of the grazing period, only eggs of resistant GIN would be excreted on the relatively clean pastures in spring, which might select strongly for AR. However, the described pattern may differ between farms, as lambing periods can vary depending on farm management and dairy sheep breeds. Targeted selective treatment (TST) implements the refugia-based approach as TST is aiming to treat only animals in need of treatment and/or animals which are responsible for most of pasture contamination, while aiming to sustain animal productivity (Kenyon et al., 2009). Usually, only few individuals within a flock are responsible for most of the egg excretion on pasture (Sréter et al., 1994). The success of TST is therefore closely linked to identifying these animals within a flock based on corresponding parameters. In order to integrate TST into field conditions, especially on large farms, it is necessary to keep the costs and effort required to collect these parameters to a minimum (Besier, 2008).

Monthly milk recordings provide dairy farmers with easily accessible data for each individual animal. In contrast to other used TST indicators, such as eggs per gram of faeces (EPG) (Cringoli et al., 2009; Gallidis et al., 2009), anthelmintic treatment based on milk performance data would therefore involve only minimum additional effort or costs for data collection and analysis. However, this is only applicable to farms which are already included in official milk recording schemes. In dairy goats, milk yield and lactation number has been reported to be possible indicators for TST. In the studies of Hoste and Chartier (1993) and Hoste et al. (1999, as cited in Hoste et al., 2002) goats with high milk yield and first lactating goats caused the highest egg excretions in a herd. TST of these animal groups allowed an efficient control of GIN infections while maintaining animal productivity (Hoste et al., 2002). In dairy sheep, there are still very few studies on this topic and the relationship between milk performance and EPG is not well known. Previous studies using milk yield as TST indicator in dairy sheep did not investigate the correlation between EPG and milk yield, but they relied on the assumption of the same relationship between milk performance and EPG in sheep as in goats (Cringoli et al., 2009; Gallidis et al., 2009). However, Hoste et al. (2006) could not find a significant difference in egg excretion between high and low yielding dairy ewes. Regarding the lactation number a significant effect on egg excretion was found, as first lactating dairy ewes had higher EPG than dairy ewes with higher lactation numbers.

The FAMACHA score has been proposed to be a reliable indicator to identify sheep, which are in need of treatment due to infections with *H. contortus* (Bath and Van Wyk, 2001). This has been shown in several studies, where significant correlations between FAMACHA and packed cell volume (PCV) have been found (Kaplan et al., 2004; Papadopoulos et al., 2013; Chylinski et al., 2015). As TST indicator, the FAMACHA score should also be able to reflect *H. contortus*-related EPG, but findings are not consistent across studies. Kaplan et al. (2004); Burke et al. (2007) and Notter et al. (2017) found significant correlations between FAMA-CHA and EPG ranging from 0.21 to 0.44, whereas in other studies no

relationship was identified (Chilinsky et al., 2015; Moors and Gauly, 2009). All of these studies investigated mixed GIN infections, except for Chylinski et al. (2015), where ewes were artificially infected with *H. contortus*. As most studies have been performed in non-dairy sheep, results of using the FAMACHA score in dairy sheep breeds is limited.

The aim of this study was therefore (i) to investigate the general relationship between milk performance and GIN infections in a Swiss Lacaune dairy sheep subpopulation and based on this, to evaluate milk performance data as a potential TST indicator for dairy sheep farms. Furthermore, (ii) the application of the FAMACHA score in Lacaune dairy ewes as additional TST indicator was to be tested.

## 2. Materials and methods

All animal-related procedures were 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, permission No. 75730.

# 2.1. Study design and animals

Overall data of 1159 lactating Lacaune dairy sheep were used in the study. The data were collected from August to December 2019 on 15 commercial dairy sheep farms in Switzerland. Selection criteria for dairy sheep farms included: (i) a herd size of at least 30 animals, (ii) breeding pure-bred Lacaune dairy sheep (female sheep of at least 87.5 % Lacaune blood), (iii) all ewes have access to pasture during the vegetation period, (iv) availability of milk performance data and (v) no anthelmintic treatment was given during the current lactation period. Of the included dairy sheep farms 14 were managed according to the organic farming standards in Switzerland (Bio Suisse, 2020).

All farms were visited once for data collection, where all ewes were sampled which were at least 70 days in milk (DIM). Only one farm was visited twice to increase the number of ewes meeting the requirement of  $\geq$  70 DIM. To achieve a high degree of temporal agreement between all parameters, there were no more than 3 days between official milk recordings and farm visits, except for one farm, where the difference between milk recording and farm visit was 10 days.

# 2.2. Faecal egg counts, larval culture and identification

Faecal samples were taken directly from the rectum of each animal. The samples used for EPG determination were transported in cool boxes and stored at approximately 5 °C for 1 to maximal 4 days in the laboratory until further examination. For EPG determination, a modified McMaster egg-counting technique by Wetzel (1951) was used: 4 g of homogenised faeces were mixed with approximately 10 ml of flotation suspension (saturated salt solution; density 1.2 g/cm<sup>3</sup>). The mixture was sieved in a measuring cylinder and the flotation suspension was added up to a total volume of 60 ml. The suspension was mixed and immediately filled into two counting fields of a McMaster counting chamber. After 10 min the chambers were examined at 100x magnification with one counted egg representing 50 EPG.

On each farm, 2–5 pooled samples of at least 10 % randomly selected ewes were taken to conduct larval culture. For small herds faecal samples were taken from at least 10 animals. The faecal samples used for larval cultures were transported and stored at room temperature. The cultures were incubated at 25 °C and 80 % humidity for 10–14 days. One-hundred 3rd stage larvae from every pooled sample were then differentiated under the microscope in *H. contortus* and other GIN of the order Strongylida according to keys provided by Deplazes et al. (2013). The mean value of the proportion of *H. contortus* was calculated from the pooled samples for each farm.

# 2.3. Performance data

Performance data of all ewes were provided by the Swiss Dairy Sheep Farming Cooperative. For each animal the results of test day milk yield (kg), test day milk protein (%), lactation number and the date of lambing of the current lactation were obtained. DIM was calculated for each animal as the difference between lambing date and date of the farm visit. For ewes sampled at  $\geq$  200 DIM, data of the 200-day milk yield (kg) and the mean 200-day milk protein content (%) were also obtained.

# 2.4. Faecal egg count reduction test (FECRT)

FECRTs were performed to evaluate the efficacy of moxidectin (Cydectin® 0.1 %, oral suspension) and a combination of levamisole and triclabendazole (Endex® 8.75 %, oral suspension) on 4 and 3 of the monitored farms, respectively. For every FECRT a group of 10-15 sheep was selected at the end of lactation with EPG > 300 as recommended by Cabaret and Berrag (2004), based on the last EPG results obtained. Where this was not feasible, animals with lower EPG were also included. Only on one farm the ewelambs were sampled for FECRT. The ewelambs grazed on the same pastures as the lactating ewes throughout the year. The anthelmintic products were dosed according to the manufacturer's instructions (Cydectin® 0.1 %: 0.2 mg moxidectin per kg live body weight; Endex® 7.5 %: 10 mg triclabendazole and 7.5 mg levamisole per kg of live body weight respectively). Faecal samples were collected before drenching and 10-14 days post-treatment. For FECRT EPG-determination, the above-mentioned method was slightly adapted to achieve a higher sensitivity: for 4 g of faeces 3 McMaster slides (or 6 chambers) were counted instead of 1 slide (or 2 chambers, see equation below), with otherwise identical conditions. This resulted in a minimum detection level of 16.7 EPG.

of *H. contortus*, whereas the farm was considered again as random effect. For M2 the dataset was reduced to only the animals that were sampled at  $\geq 200$  DIM. Residuals of both models were graphically evaluated to ensure the model assumptions of normality and homoscedasticity. For both models EPG was transformed to meet the assumption of normality of residuals by using the transformation logEPG = log(EPG + 40). Spearman correlation coefficients were calculated to estimate the correlations between EPG and test day milk yield and of the FAMACHA score with PCV and EPG. FECRTs and corresponding 95 % credible intervals (CI) were determined using the function fecr\_stan from the R-package eggCounts 2.3 (Wang et al., 2018). For calculations, a paired design model with individual efficacy was used. As recommended by Wang et al. (2018) the 2.5 % and 97.5 % percentiles as the 95 % CI and the median as the summary statistics were used. Observations with zero EPG pre-treatment were excluded prior to the analysis.

# 3. Results

# 3.1. Descriptive statistics and data handling

Overall data of 1159 lactating ewes were collected in the present study. The number of sampled ewes per farm varied between 17 and 199 animals with a mean of 77 animals per farm. On one farm  $\leq$  30 ewes were sampled, as only 17 ewes met the requirement of  $\geq$  70 DIM at the time of data collection. Table S1 shows the descriptive statistics of all variables collected in the study. It was not possible to obtain a complete data set with all parameters from all ewes, because of insufficient faecal material or incomplete milk performance records. That was the case for 33 and 47 ewes, respectively. In addition, there were 25 observations with missing test day milk protein, due to insufficient milk quantities at milk recordings, which were handled as missing values. One outlier for

 $EPG = \frac{\text{counted eggs } \times \text{ used amount of flotation suspension (ml)}}{\text{used faces (g) } \times \text{grid size (cm<sup>2</sup>)} \times \text{chamber hight (cm)} \times \text{number of chambers}}$ 

## 2.5. PCV and FAMACHA score

Blood samples were taken from jugular vein puncture in 2 ml EDTA vacutainer tubes for subsequent PCV determination and were kept at 5 °C until one hour before the examination to bring the samples to room temperature. PCV was determined within 24 h using the microhematocrit method. Therefore, blood samples were filled into microhematocrit tubes and centrifuged at 9600g for 5 min (Heraus, Pico 17 Centrifuge). The FAMACHA score was used to classify the ocular mucosa colour of each animal into one of the five categories (1–5) as described by Van Wyk and Bath (2002).

# 2.6. Statistical analyses

All statistical analyses were performed in R 3.6.1 (R Core Team, 2019). Two linear mixed effect models were calculated to assess the impact of the collected parameters on EPG, using the lmer function from the lme4 package (Bates et al., 2015). In Model 1 (M1) test day milk yield, test day milk protein, lactation number, DIM, the proportion of *H. contortus* and an interaction between test day milk yield and DIM were included as fixed effects. The farm was considered as a random effect to correct for any dependencies between ewes within the same farm. In Model 2 (M2) the fixed effects were the 200-day milk yield, 200-day milk protein content, DIM, lactation number and the proportion

EPG was removed, because of very high EPG and  $<2\,g$  of faeces. Therefore, M1 and M2 were calculated based on sample sizes with  $n_1=1053$  and  $n_2=505.$ 

# 3.2. Linear mixed models

M1 revealed a relationship between the test day milk yield and logEPG (P = 0.002), showing increasing logEPG with increasing milk yields. Further, there was an interaction between test day milk yield and DIM (P = 0.022), which is presented in Fig. 1 for 70 and 200 DIM. The relationship between test day milk yield and logEPG was most pronounced at the earliest time of lactation covered in our study (70 DIM) and decreased towards the end of lactation. All other fixed effects were not statistically significant, but the lactation number and the proportion of *H. contortus* showed values tending towards significance (P = 0.067 and P = 0.064, respectively). Fig. 2 shows the relationship between the proportion of *H. contortus* and logEPG. Equation 1 was developed to predict back transformed EPG from all included fixed effects in M1:

$$EPG = \exp(4, 242 + 0, 4845x_1 + (-0, 001695)x_2 + 0, 03342x_3 + 0, 08284x_4 + 0, 01628x_5 + (-0, 002114)x_1x_2) - 40$$
(1)

where  $x_1$  = test day milk yield (kg),  $x_2$  =DIM,  $x_3$  = lactation number,  $x_4$  = test day milk protein (%),  $x_5$  = percentage of *H. contortus*.

Exemplary calculations using Equation 1 for ewes with high (3 kg) and low milk yields (1 kg) at 70 DIM, while keeping all other predictors adjusted to their mean, resulted in about 310 EPG higher egg excretions



**Fig. 1.** Scatterplot of test day milk yields (kg) versus log(EPG + 40) for all animals included in the calculations of M1 (n = 1053). Regression lines with confidence intervals predicted from M1 for 70 and 200 DIM.

in the high yielding ewes. Towards the end of lactation (200 DIM) the same calculations resulted in a difference of 26 EPG, with higher EPG in high yielding ewes. The correlation coefficient between EPG and test day milk yield was in a moderate range (r = 0.31, P < 0.001).

In M2 the lactation number was the only fixed effect that showed a relationship with logEPG (P = 0.018), indicating increasing EPG with higher lactation numbers (Fig. 3). However, the relationship between



Veterinary Parasitology: X 4 (2020) 100030



Fig. 3. Scatterplot of lactation number versus log(EPG + 40) for all animals sampled  $\geq$  200 DIM (n = 505). Regression line and confidence interval predicted from M2.

EPG and lactation numbers  $\geq 8$  was difficult to estimate due to the low number of ewes with high lactation numbers. Neither the 200-day milk yield nor the 200-day milk protein content had an effect on logEPG (P = 0.886 and P = 0.91, respectively).

# 3.3. Larval culture and identification

The proportion of *H. contortus* varied on the farms between 4.3 and 97.3 %, with a mean of 43.3 %. Fig. 4 reports the prevalence of *H. contortus* and other GIN of the order Strongylida for each farm.

# 3.4. PCV and FAMACHA score

The FAMACHA score was correlated with PCV (r = -0.32; P < 0.001) and EPG (r = 0.15; P < 0.001). Including only farms in the analysis, where *H. contortus* was the predominant parasite ( $\geq$  50 %), the correlation coefficient of FAMACHA and PCV increased to r = -0.48 (P < 0.001) and of FAMACHA and EPG to r = 0.37 (P < 0.001). The relationship of FAMACHA and EPG for farms with  $\geq$  50 % *H. contortus* is shown in Fig. 5.

# 3.5. FECRTs

According to the World Association for the Advancement of Veterinary Parasitology guidelines (WAAVP) (Coles et al., 1992) and according to Levecke et al. (2018) the results of the FECRTs indicated the occurrence of AR against moxidectin on one farm (farm 5), as the mean reduction percentage was  $\leq$  95 % and the lower CI  $\leq$  90 %. AR against levamisole was suspected on two farms (farm 1 and 5), based on the classification criteria of either the mean reduction percentage or the lower CI is  $\leq$  95 % and  $\leq$  90 %, respectively. More detailed information of the results of the FECRTs can be found in Table S2.

### 4. Discussion

**Fig. 2.** Scatterplot of the proportion of *H. contortus* versus  $\log(EPG + 40)$  for all animals included in the calculations of M1 (n = 1053). Regression line with confidence intervals predicted from M1.

#### TST is one approach to reduce the selection pressure for AR. It relies



Fig. 4. Percentage of 3<sup>rd</sup> stage larvae of *H. contortus* and other gastrointestinal nematodes (GIN) of the order Strongylida found in coprocultures of the 15 dairy sheep farms.

on the correct identification of animals that are in need of treatment due to GIN infections (Kenyon et al., 2009). The aim of this study was therefore to investigate the general relationship between milk performance and GIN infection and to evaluate the potential of milk performance data as an indicator for TST in Swiss Lacaune dairy sheep. Overall, this study showed that higher test day milk yields were associated with higher EPG. This relationship was most pronounced during



**Fig. 5.** Scatterplot of the FAMACHA score versus EPG of all animals on farms with predominantly *H. contortus* ( $\geq$  50 %) (n = 410). A random jitter was added to the data points to visualise their density.

the earliest stage of lactation covered in this study.

In the present study the ewes were sampled when they were at least 70 DIM. This cut-off date was chosen because this study was performed within a project that was designed to investigate the possibility of breeding for improved resistance to GIN infections in sheep. In this context it was necessary to have a phenotype with data of at least two milk recordings available for each ewe at the time of faecal analysis. In Lacaune dairy sheep the lactation peak occurs between the 27<sup>th</sup> and 48<sup>th</sup> day postpartum depending on the lactation number. Until the 90<sup>th</sup> day, 50 % of the total milk yield is usually produced (Elvira et al., 2013). Thus, in this study we covered only the two last thirds of the lactation period where milk yield is already decreasing.

It has been postulated that immune functions compete for scarce nutrients with other body functions during high nutritional demand periods, e.g. late pregnancy and lactation, whereas the reproductive functions are prioritised over immune functions (Coop and Kyriazakis, 1999). Consequently, during the time of highest nutrient requirements for milk production, the penalisation of immune functions would be expected to be most pronounced. This is supported by findings of Notter et al. (2017), where ewes with two or more lambs were less resistant to GIN infections than single-rearing ewes from lambing to 60 days postpartum. With decreasing milk yields towards the end of the lactation period, it can be assumed that the competition for scarce nutrients between milk production and immune functions is not as strong anymore. This would account for the observed decreasing relationship between test day milk yield and EPG towards the end of lactation in M1. Our calculations for 200 DIM have shown a small difference of 26 EPG between high (3 kg) and low (1 kg) yielding ewes. At 70 DIM the difference was more apparent (310 EPG) but remained within a moderate range. This seems to be in accordance with Houdijk et al. (2003), suggesting that there is no total absence of immune functions against GIN in sheep, even in times of scarce nutrients.

The effect of test day milk yield on EPG observed in our study is in contrast to findings of Hoste et al. (2006), who didn't observe a relationship between the milk production level and EPG in dairy sheep. The differing results might be due to the low mean infection level (< 200 EPG) in the study of Hoste et al. (2006). Our analyses were based on a large sample size, which allowed to detect even small effect sizes, but

only a single measurement of EPG was determined per animal between 70 and 385 DIM (Table S1). We are aware that repeated measurements of EPG within the same ewes would have allowed an improved estimation of the susceptibility to GIN infections of the ewes. However, we assume that a single EPG determination may be sufficient to classify an ewe being rather resistant or susceptible to GIN infections. This is supported by the study of Notter et al. (2017), who found correlations between EPG determined at different times of lactation within the same ewes, although this study covered only the time of the periparturient period until the 60<sup>th</sup> day postpartum.

Cringoli et al. (2009) and Gallidis et al. (2009) have already used milk yield data as a TST indicator in dairy sheep but did not investigate the general relationship between milk yield and EPG. Cringoli et al. (2009) treated all ewes with milk yields above the flock mean, based on the assumption, that high yielding ewes are more susceptible to GIN infections than low yielding ewes. However, no conclusions of the effect of TST on milk performance and the parasitological status of the ewes could be drawn from this study. Gallidis et al. (2009) did not evaluate the impact of TST on milk performance, but in regard to the parasitological status, a comparable control of GIN infection of a systematically treated group and treatment of high yielding ewes only (> 2 litres), has been observed. The positive relationship between test day milk yield and EPG observed in our study supports the approach to focus anthelmintic treatment on high yielding ewes. However, the potential of milk yield data to estimate the GIN infection level was indicated to be dependent on the lactation stage of the ewes. Milk yield records from late lactation stage did not seem to be suitable to identify ewes in need of treatment due to the weak relationship with EPG in this stage of lactation. This suggests the limitation of milk yield data as TST indicator to early and mid-lactation. Further studies would be necessary to explore these relationships and the effects of TST (based on milk yield data) in terms of the parasitological status and milk performance of ewes. Research is also needed to investigate, if the association between milk yield data and EPG might be improved by using milk yield data of < 70 DIM.

The effect of milk protein content on EPG was evaluated, because of the demand of the immune system for metabolisable protein and its sensitivity to protein scarcity (Houdijk, 2012). In our analyses no effect of milk protein content was found in either model. This is supported by results of Sechi et al. (2010) who did not find differences in milk protein contents between different infection levels in ewes. In contrast, findings of Cruz-Rojo et al. (2012) suggested a lower milk protein content in dairy ewes due to parasitic infection. Based on our results and due to the conflicting findings of other studies, we suggest that milk protein content is not suitable as an indicator for TST in dairy sheep.

Both our models indicated a positive relationship between lactation number and EPG, as with increasing lactation numbers also EPG increased. The absolute differences of EPG between different lactation numbers varied in a low range, as calculations with Equation 1 predict an increase of approximately 7 EPG per lactation number. These results do not support findings of previous studies, which indicated primiparous ewes to be less resistant to GIN infections compared to multiparous ewes (Hoste et al., 2006; Sechi et al., 2010). Hoste et al. (2006) suggested to focus anthelmintic treatment on first lactating ewes and hypothesised, that the low immune response of primiparous ewes might be due to the lack of or the little previous exposure to GIN. Most farms in our study were managed according to the organic farming standards in Switzerland (Bio Suisse, 2020), where access to pasture is mandatory. Therefore, primiparous ewes might have already been sufficiently exposed to GIN as lambs and were able to develop a good immune defence to GIN infections before the first lactation. Another explanation of the discrepancy between this study and the one of Hoste et al. (2006) could be the diet, as nutrient supply and quality can have an effect on host resistance (Houdijk, 2012). However, as we did not collect data on nutritional supply, no conclusion could be drawn here. Our results suggest that the lactation number is not a suitable indicator for ewes in need of treatment. Instead, it may be considered to combine daily milk

yield data of rather early stages of lactation with other already evaluated TST indicators. The FAMACHA score might improve the precision of identifying ewes with high GIN infection levels, as FAMACHA showed a moderate correlation with EPG in our study (r = 0.37). However, this applies only to farms where *H. contortus* is the predominant GIN, as the correlation between FAMACHA and EPG was low (r = 0.15) when farms with < 50 % *H. contortus* were included in the analysis. Burke et al. (2007) and Notter et al. (2017) have reported similar results, indicating the FAMACHA score to be a valuable tool to support TST decisions if *H. contortus* is present in relevant proportions. The combination with the body condition score (BCS) might be a further possibility, as BCS has been suggested to be a suitable TST indicator in sheep and does not require high effort for its implementation (Cornelius et al., 2014; Calvete et al., 2020).

The timing of anthelmintic treatment in dairy farms is of particular concern, as the required withholding periods of anthelmintic drugs for milk must be taken into account. Most farmers participating in this study did not treat any ewes during lactation, with few exceptions for individual animals when clinical signs appeared (e.g. bottle jaw). If farmers used anthelmintic drugs, they usually treated all ewes in the dry period prior to lambing to avoid salvage treatments during subsequent lactation. Performance data, which is closely time related to the dry period of the ewes is the 200-day milk yield, since the average lactation length in Lacaune dairy sheep is around 224 days (Elvira et al., 2013). In M2 we tested therefore the potential of the 200-day milk yield as TST indicator in dairy sheep for anthelmintic treatments. However, the 200-day milk performance does not seem to be suitable to identify animals in need of treatment at the end of lactation.

In Switzerland, *H. contortus* appears to have a high prevalence (Rinaldi et al., 2015), which is in accordance with our results. The proportion of *H. contortus* was included in the models to account for its high fecundity compared with other trichostrongylids (Cabaret et al., 1998; Saccareau et al., 2017). As expected, with an increasing proportion of *H. contortus* an increase of EPG was observed.

To ensure the efficacy of TST, it is essential to use effective anthelmintics for treatment. Recent information of AR in dairy sheep farms in Switzerland is very limited. The results of the present study showed that the occurrence of AR is also present on Swiss dairy sheep farms. This situation has become especially visible on farm 5, where according to the WAAVP guidelines (Coles et al., 1992) the efficacy of moxidectin was reduced and AR against levamisole was suspected.

#### 5. Conclusion

Our results showed a positive relationship between daily milk yield and EPG in Swiss Lacaune dairy sheep, indicating high yielding ewes to be less resistant to GIN infections than low yielding ewes. The effect was most pronounced in the earlier stage of lactation, suggesting the potential of using milk yield data of earlier lactation as indicator for TST. In farms with predominantly *H. contortus*, treatment decisions could be supported by the application of the FAMACHA score.

# **Declaration of Competing Interest**

The authors report no declarations of interest.

# CRediT authorship contribution statement

Katharina Schwarz: Writing - original draft, Investigation, Formal analysis, Visualization, Software. Beat Bapst: Resources, Software, Investigation. Mirjam Holinger: Methodology. Susann Thüer: Investigation. Inga Schleip: Writing - review & editing. Steffen Werne: Supervision, Methodology, Conceptualization, Investigation, Writing review & editing.

#### Acknowledgements

This work has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No 772787 (SMARTER). We gratefully thank all sheep farmers who participated in the study and the Swiss Dairy Sheep Farming Cooperative (Schweizerische Milchschafzucht Genossenschaft) for the provision of milk recording results. Many thanks also to A. Steiner, E. Perler and M. Leubin, S. Dörig and T. Manser for their support in field and laboratory work.

# 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.vpoa.2020.100030.

#### References

- Arsenopoulos, K., Gelasakis, A., Delistamatis, V., Papadopoulos, E., 2019. Evaluation of the pour-on administration of eprinomectin on milk yield and somatic cell counts in dairy ewes naturally infected with gastrointestinal nematodes. Vet. Parasitol. 2 https://doi.org/10.1016/j.vpoa.2019.100016.
- Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting Linear Mixed-Effects Models Using Ime4. J. Stat. Softw. 67 https://doi.org/10.18637/jss.v067.i01.
- Bath, G.F., Van Wyk, J.A., 2001. Using the FAMACHA system on commercial sheep farms in South Africa. Proceedings of the 5th International Sheep Veterinary Congress, 22-25 January 2001.
- Besier, B., 2008. Targeted treatment strategies for sustainable worm control in small ruminants. Trop. Biomed. 25, 9–17.
- Bio Suisse, 2020. Richtlinien für die Erzeugung, Verarbeitung und den Handel von Knospe-Produkten (Accessed 23 April 2020). https://www.bio-suisse.ch/media/ VundH/Regelwerk/2020/DE/bio\_suisse\_richtlinien\_2020\_d\_gesamt.pdf.
- Burke, J.M., Kaplan, R.M., Miller, J.E., Terrill, T.H., Getz, W.R., Mobini, S., Valencia, E., Williams, M.J., Williamson, L.H., Vatta, A.F., 2007. Accuracy of the FAMACHA system for on-farm use by sheep and goat producers in the southeastern United States. Vet. Parasitol. 147, 89–95. https://doi.org/10.1016/j.vetpar.2007.03.033.
- Cabaret, J., Berrag, B., 2004. Faecal egg count reduction test for assessing anthelmintic efficacy: average versus individually based estimations. Vet. Parasitol. 121, 105–113. https://doi.org/10.1016/j.vetpar.2004.01.020.
- Cabaret, J., Gasnier, N., Jacquiet, P., 1998. Faecal egg counts are representative of digestive-tract strongyle worm burdens in sheep and goats. Parasite 5, 137–142. https://doi.org/10.1051/parasite/1998052137.
- Calvete, C., González, J.M., Ferrer, L.M., Ramos, J.J., Lacasta, D., Delgado, I., Uriarte, J., 2020. Assessment of targeted selective treatment criteria to control subclinical gastrointestinal nematode infections on sheep farms. Vet. Parasitol. 277, 109018 https://doi.org/10.1016/j.vetpar.2019.109018.
- Chylinski, C., Cortet, J., Neveu, C., Cabaret, J., 2015. Exploring the limitations of pathophysiological indicators used for targeted selective treatment in sheep experimentally infected with *Haemonchus contortus*. Vet. Parasitol. 207, 85–93. https://doi.org/10.1016/j.vetpar.2014.10.029.
- Coles, G.C., Bauer, C., Borgsteede, F.H.M., Geerts, S., Klei, T.R., Taylor, M.A., Waller, P. J., 1992. World Association for the Advancement of Veterinary Parasitology (W.A.A. V.P.) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. Vet. Parasitol. 44, 35–44. https://doi.org/10.1016/0304-4017(92) 90141-U.
- Coop, R.L., Kyriazakis, I., 1999. Nutrition–parasite interaction. Vet. Parasitol. 84, 187–204. https://doi.org/10.1016/S0304-4017(99)00070-9.
- Cornelius, M.P., Jacobson, C., Besier, R.B., 2014. Body condition score as a selection tool for targeted selective treatment-based nematode control strategies in Merino ewes. Vet. Parasitol. 206, 173–181. https://doi.org/10.1016/j.vetpar.2014.10.031.
- Cringoli, G., Rinaldi, L., Veneziano, V., Mezzino, L., Vercruysse, J., Jackson, F., 2009. Evaluation of targeted selective treatments in sheep in Italy: effects on faecal worm egg count and milk production in four case studies. Vet. Parasitol. 164, 36–43. https://doi.org/10.1016/j.vetpar.2009.04.010.
- Cruz-Rojo, M.A., Martínez-Valladares, M., Álvarez-Sánchez, M.A., Rojo-Vázquez, F.A., 2012. Effect of infection with *Teladorsagia circumcincta* on milk production and composition in Assaf dairy sheep. Vet. Parasitol. 185, 194–200. https://doi.org/ 10.1016/j.vetpar.2011.10.023.
- Deplazes, P., Eckert, J., Von Samson-Himmelstjerna, G., Zahner, H., 2013. Lehrbuch der Parasitologie f
  ür die Tiermedizin, 3rd ed. Enke Verlag Stuttgart.
- Elvira, L., Hernandez, F., Cuesta, P., Cano, S., Gonzalez-Martin, J.-V., Astiz, S., 2013. Accurate mathematical models to describe the lactation curve of Lacaune dairy sheep under intensive management. Animal 7, 1044–1052. https://doi.org/ 10.1017/S175173111200239X.
- Fthenakis, G., Papadopoulos, E., Himonas, C., 2005. Effects of three anthelmintic regimes on milk yield of ewes and growth of lambs. J. Vet. Med. A Physiol. Pathol. Clin. Med. 52, 78–82. https://doi.org/10.1111/j.1439-0442.2004.00687.x.
- Gallidis, E., Papadopoulos, E., Ptochos, S., Arsenos, G., 2009. The use of targeted selective treatments against gastrointestinal nematodes in milking sheep and goats in Greece based on parasitological and performance criteria. Vet. Parasitol. 164, 53–58. https://doi.org/10.1016/j.vetpar.2009.04.011.

- Hoste, H., Chartier, C., 1993. Comparison of the effects on milk production of concurrent infection with *Haemonchus contortus* and *Trichostrongylus colubriformis* in high- and low-producing dairy goats. Am. J. Vet. Res. 54, 1886–1893.
- Hoste, H., Le Frileux, Y., Pommaret, A., Gruner, L., Van Quackebeke, E., Koch, C., 1999. Importance du parasitisme par des strongles gastrointestinaux chez les chèvres laitières dans le Sud Est de la France. INRA Prod. Anim. 12, 377–389. https://doi. org/10.1016/S0304-4017(02)00084-5 cited in Hoste, H., Le Frileux, Y., Pommaret, A., 2002. Comparison of selective and systematic treatments to control nematode infection of the digestive tract in dairy goats. Vet. Parasitol. 106, 345–355.
- Hoste, H., Le Frileux, Y., Pommaret, A., 2002. Comparison of selective and systematic treatments to control nematode infection of the digestive tract in dairy goats. Vet. Parasitol. 106, 345–355. https://doi.org/10.1016/S0304-4017(02)00084-5.
- Hoste, H., Rulie, A.C., Prevot, F., Bergeaud, J.P., Grisez, C., De La Farge, F., Jacquiet, P., Dorchies, P., 2006. Differences in receptivity to gastrointestinal infections with nematodes in dairy ewes: influence of age and of the level of milk production. Small Rumin. Res. 63, 150–155. https://doi.org/10.1016/j.smallrumres.2005.02.025.
- Houdijk, J.G.M., 2012. Differential effects of protein and energy scarcity on resistance to nematode parasites. Small Rumin. Res. 103, 41–49. https://doi.org/10.1016/j. smallrumres.2011.10.017.
- Houdijk, J.G.M., Kyriazakis, I., Jackson, F., Huntley, J.F., Coop, R.L., 2003. Is the allocation of metabolisable protein prioritised to milk production rather than to immune functions in *Teladorsagia circumcincta*-infected lactating ewes? Int. J. Parasitol. 33, 327–338. https://doi.org/10.1016/S0020-7519(02)00284-9.
- Kaplan, R.M., 2004. Drug resistance in nematodes of veterinary importance: a status report. Trends Parasitol. 20, 477–481. https://doi.org/10.1016/j.pt.2004.08.001.
- Kaplan, R.M., Burke, J.M., Terrill, T.H., Miller, J.E., Getz, W.R., Mobini, S., Valencia, E., Williams, M.J., Williamson, L.H., Larsen, M., Vatta, A.F., 2004. Validation of the FAMACHA© eye color chart for detecting clinical anemia in sheep and goats on farms in the southern United States. Vet. Parasitol. 123, 105–120. https://doi.org/ 10.1016/j.vetpar.2004.06.005.
- Kenyon, F., Greer, A., Coles, G.C., Cringoli, G., Papadopoulos, E., Cabaret, J., Berrag, B., Várady, M., Van Wyk, J., Thomas, E., Vercruysse, J., Jackson, F., 2009. The role of targeted selective treatments in the development of refugia-based approaches to the control of gastrointestinal nematodes of small ruminants. Vet. Parasitol. 164, 3–11. https://doi.org/10.1016/j.vetpar.2009.04.015.
- Levecke, B., Kaplan, R.M., Thamsborg, S.M., Torgerson, P.R., Vercruysse, J., Dobson, R. J., 2018. How to improve the standardization and the diagnostic performance of the fecal egg count reduction test? Vet. Parasitol. 253, 71–78. https://doi.org/10.1016/ j.vetpar.2018.02.004.
- Mavrot, F., Hertzberg, H., Torgerson, P., 2015. Effect of gastro-intestinal nematode infection on sheep performance: a systematic review and meta-analysis. Parasit. Vectors 8, 557. https://doi.org/10.1186/s13071-015-1164-z.
- Moors, E., Gauly, M., 2009. Is the FAMACHA© chart suitable for every breed? Correlations between FAMACHA© scores and different traits of mucosa colour in naturally parasite infected sheep breeds. Vet. Parasitol. 166, 108–111. https://doi. org/10.1016/j.vetpar.2009.07.040.
- Morgan, E., Charlier, J., Hendrickx, G., Biggeri, A., Catalan, D., von Samson-Himmelstjerna, G., Demeler, J., Müller, E., van Dijk, J., Kenyon, F., Skuce, P., Höglund, J., O'Kiely, P., van Ranst, B., de Waal, T., Rinaldi, L., Cringoli, G., Hertzberg, H., Torgerson, P., Wolstenholme, A., Vercruysse, J., 2013. Global change and helminth infections in grazing ruminants in Europe: impacts, trends and sustainable solutions. Agriculture 3, 484–502. https://doi.org/10.3390/ agriculture3030484
- Notter, D.R., Burke, J.M., Miller, J.E., Morgan, J.L.M., 2017. Association between FAMACHA scores and fecal egg counts in Katahdin lambs. J. Anim. Sci. 95, 1118. https://doi.org/10.2527/jas2016.1248.
- Papadopoulos, E., Gallidis, E., Ptochos, S., Fthenakis, G.C., 2013. Evaluation of the FAMACHA© system for targeted selective anthelmintic treatments for potential use in small ruminants in Greece. Small Rumin. Res. 110, 124–127. https://doi.org/ 10.1016/j.smallrumres.2012.11.019.
- R Core Team, 2019. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
- Rinaldi, L., Catelan, D., Musella, V., Cecconi, L., Hertzberg, H., Torgerson, P., Mavrot, F., de Waal, T., Selemetas, N., Coll, T., Bosco, A., Biggeri, A., Cringoli, G., 2015. Haemonchus contortus: spatial risk distribution for infection in sheep in Europe. Geospat. Health 9. https://doi.org/10.4081/gh.2015.355.
- Saccareau, M., Sallé, G., Robert-Granié, C., Duchemin, T., Jacquiet, P., Blanchard, A., Cabaret, J., Moreno, C.R., 2017. Meta-analysis of the parasitic phase traits of Haemonchus contortus infection in sheep. Parasit. Vectors 10, 201. https://doi.org/ 10.1186/s13071-017-2131-7.
- Scheuerle, M.C., Mahling, M., Pfister, K., 2009. Anthelminthic resistance of *Haemonchus contortus* in small ruminants in Switzerland and Southern Germany. Wien. Klin. Wochenschr. 121, 46. https://doi.org/10.1007/s00508-009-1235-2.
- Sechi, S., Giobbe, M., Sanna, G., Casu, S., Carta, A., Scala, A., 2010. Effects of anthelmintic treatment on milk production in Sarda dairy ewes naturally infected by gastrointestinal nematodes. Small Rumin. Res. 88, 145–150. https://doi.org/ 10.1016/j.smallrumres.2009.12.022.
- Sréter, T., Molnár, V., Kassai, T., 1994. The distribution of nematode egg counts and larval counts in grazing sheep and their implications for parasite control. Int. J. Parasitol. 24, 103–108. https://doi.org/10.1016/0020-7519(94)90063-9.
- Suarez, V.H., Cristel, S.L., Busetti, M.R., 2009. Epidemiology and effects of gastrointestinal nematode infection on milk productions of dairy ewes. Parasite 16, 141–147. https://doi.org/10.1051/parasite/2009162141.
- Traversa, D., von Samson-Himmelstjerna, G., 2016. Anthelmintic resistance in sheep gastro-intestinal strongyles in Europe. Small Rumin. Res. 135, 75–80. https://doi. org/10.1016/j.smallrumres.2015.12.014.

## K. Schwarz et al.

Van Wyk, J., 2001. Refugia – overlooked as perhaps the most potent factor concerning the development of anthelmintic resistance. Onderstepoort J. Vet. Res. 68, 55–67.

- Van Wyk, J.A., Bath, G.F., 2002. The FAMACHA system for managing haemonchosis in sheep and goats by clinically identifying individual animals for treatment. Vet. Res. 33, 509–529. https://doi.org/10.1051/vetres:2002036.
  Voigt, K., Scheuerle, M., Hamel, D., 2012. Triple anthelmintic resistance in
- Voigt, K., Scheuerle, M., Hamel, D., 2012. Triple anthelmintic resistance in *Trichostrongylus spp.* in a German sheep flock. Small Rumin. Res. 106, 30–32. https:// doi.org/10.1016/j.smallrumres.2012.04.017.
- Wang, C., Torgerson, P.R., Kaplan, R.M., George, M.M., Furrer, R., 2018. Modelling anthelmintic resistance by extending eggCounts package to allow individual efficacy. Int. J. Parasitol. Drugs Drug Resist. 8, 386–393. https://doi.org/10.1016/j. ijpddr.2018.07.003.
- Wetzel, E., 1951. Verbesserte McMaster-Kammer zum Auszählen von Wurmeiern. Tieraerztliche Umschau 6, 209–210.