Veterinary and Animal Science 19 (2023) 100278

Contents lists available at ScienceDirect

Veterinary and Animal Science

journal homepage: www.elsevier.com/locate/vas

Relationships between sheep nematode infection, nutrition, and grazing behavior on improved and semi-natural pastures

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ARTICLE INFO

Keywords:
Gastrointestinal nematode
Livestock blood properties
Grazing behavior
Sheep parasite
Livestock performance
Semi-natural pasture

ABSTRACT

Gastrointestinal nematodes (GINs) are key parasites of grazing sheep worldwide. To understand the factors influencing GIN infections, we examined the relationships among infection and nutrition, foraging behavior, and animal performance. Further, the parasitism and nutrition of sheep between improved and semi-natural pastures in Japan were compared. Sheep were grazed for 1 month each, first on an improved and then on a semi-natural pasture. Afterward, vegetation surveys, forage analyses, and (plant) nematode larval counts were conducted in both pastures, and fecal egg counts, biochemical analyses, and bite counts were completed for each sheep. The semi-natural pasture had diverse plant species, though it contained less crude protein, and nematode larvae were rarely observed on bamboo. Consequently, fecal egg per gram decreased after grazing on the semi-natural pasture. White blood counts, hematocrit, and glucose also decreased and body weight increased after grazing on this pasture. Principal component and correlation analyses revealed a significant relationship between GIN infection and behavior, but not between nutrition and either behavior or infection. As parasitized animals may become more aggressive feeders to compensate for their reduced nutritional uptake, grazing sheep on semi-natural pastures may facilitate more stable performance due to the lower risk of nematode infection from wild plants.

Abbreviations
GIN Gastrointestinal Nematodes

1. Introduction

Globally, infection by gastrointestinal nematodes (GINs) is an important health issue in grazing sheep such as weight loss, anemia, diarrhea and severe protein loss (Mavrot et al., 2015). While anthelmintic drugs are used to control GIN infections, anthelmintic resistance is an escalating problem in most sheep-raising countries (Papadopoulos, 2008). Thus, alternative and more sustainable treatments for preventing and controlling GIN infections, including animal and pasture management practices, are needed (Mederos et al., 2012).

Animals infected with gastrointestinal parasites display careful and selective foraging behaviors aimed at reducing further parasite ingestion. Consequently, a trade-off arises between nutrient intake and fecal avoidance in herbivore foraging decisions (Hutchings et al., 1999). For example, cattle with elevated levels of parasitism are more likely to avoid contaminated areas than animals with lower rates of infection (Seó et al., 2015). Parasitized sheep take briefer bites, have lower bite masses and depths (Hutchings et al., 1998), and move greater distances (Falzon et al., 2013), resulting in reduced nutrient intake (Hutchings et al., 2001). Despite these negative effects on the foraging behaviors of ruminants, there remains little evidence regarding the effect of infection on body weight (Forbes et al., 2004; Ikurior et al., 2020). Thus, examining the mechanism(s) underlying the relationships between infection and the nutrition, foraging behavior, and performance of grazing sheep is critical.

As is the case elsewhere, livestock grazing in Japan is associated with a risk of contracting parasites (Yoshihara et al., 2022). The typical vegetation in a Japanese grazing pasture is artificially sown, which augments the existing vegetation, thereby improving its productivity. However, creating and maintaining such improved pastures requires large amounts of fertilizer, exotic grass seeds, and higher costs for routine sward renovation. Thus, grazing on semi-natural pastures that support various native plant species has received increased attention as an alternative, more sustainable option (Yoshihara et al., 2014). For

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https://doi.org/10.1016/j.vas.2022.100278

Available online 5 December 2022

many years, it has been recognized that both sward structure and species composition play important roles in determining the levels of nematode parasitism in grazing lambs due to differences in plant leaf morphologies and the related vertical migration (and resistance thereto) of nematodes (Gazda et al., 2009; Moss and Vlassoff, 1993; Niezen et al., 1998; Silangwa, 1964). Thus, we hypothesized that nematode parasitism and nutrition would differ between sheep grazed on improved versus semi-natural pastures due to differences in sward structures and species compositions.

2. Materials and methods

2.1. Study site and pasture management

Our experimental study was conducted at the Charo Sheep Farm, Hokkaido, in north-eastern Japan. The climate is subarctic, with a mean temperature of 16.0 °C and an annual rainfall of 132 mm, as measured in July from 1991 to 2020. The farm consists of two grazing areas, separated by a distance of ~40 km. The main (improved) grazing pasture is a flat, 2.9 ha area that comprises distinct stations. Compost is applied to this pasture every year before grazing. A separate, 4.0 ha grazing area (i.e., the semi-natural pasture) sits on a mountain slope that is covered with short and wild grasses and trees, and was excluded from pasture management (e.g., fertilization) during the study year.

Rotational grazing with 87 fat sheep (Suffolk × Poll Dorset, aged 1–2 years) was undertaken at 10 distinct stations for 1 month in June 2021. Fifty of the sheep grazing on the improved pasture were then moved to the semi-natural pasture and grazed for an additional month. Thus, the grazing densities (sheep/ha) of the improved and semi-natural pastures in the first and second months were 30.0 and 12.5, respectively. Anthelmintic drugs for GIN infections were not administered to the study sheep.

2.2. Field sampling

After the sheep grazed, we established ten 1 m^2 quadrats within each pasture. All plant species in each quadrat were identified and clipped to a height of 1 cm. The collected samples were then oven-dried at 70 °C for 48 h to determine their dry weights. Samples were taken to quantify the number of nematode larvae on each plant species in the improved and semi-natural pastures at the same time. In each pasture, four separated study plots were randomly established, from which 10 individuals of the dominant plant species (orchard grass, Kentucky blue grass, white clover, and bamboo) were collected from 1 cm above ground level. Nematode larvae were counted immediately by direct observation with a microscope. After counting the number of larvae, the dry matter (DM) weights of the samples were obtained.

The nutritional and health statuses of the study sheep were measured by changes in body weight and blood properties, and by parasite loads. We weighed the 20 sheep after 1 month of grazing in each pasture. Blood was collected from the jugular veins of the sheep using two types of collection tubes—with and without coagulant—to diagnose anemia and assess nutritional status. Blood serum was obtained by centrifugal separation and stored in a freezer (−20 °C). We collected fecal samples directly from the rectum of each sheep and examined for an internal parasite check. A neckband bite counter was attached to a collar on all sheep from June to the August to count the jaw movements every 10 min for 48 h.

2.3. Laboratory experiments

To determine the nutrient composition of the forage, the collected plant samples were chemically analyzed. The neutral detergent fiber (NDF) content was assessed without treatment with heat-stable amylase and its expression in the residual ash was determined using the detergent method after grinding the dried sample (Udén et al., 2005). The crude protein (CP) content was measured by determining the amount of N using the combustion method (Elementar, Germany). A factor of 6.25 was used for the conversion of N into CP.

Table 1
Results from vegetation survey and nutrition analysis.

Pasture	Plant species	Biomass (g/m^2)	CP (%)	NDF (%)
Improved	Orchard grass, Kentucky blue grass, white clover	144.7	20.3*	69.3
Semi-natural	Orchard grass, Kentucky blue grass, White clover, Cerastium fontanum, Sasa kurilensis, Agrostis gigantea, Equisetum arvense, Leucanthemum sp., Juncus effusus, Geum japonicum, Cirsium kamtschaticum, Achillea millefolium, Phleum sp.	762.3*	11.8	63.7

Note: Asterisks indicate a statistically significant difference ($p < 0.05$) between the pastures.

Blood serum protein, glucose, and cholesterol levels were determined using a Spotchem™ SP-4430V Autodry Chemistry Analyzer (Arkray, Inc., Japan). The blood collected with coagulant was immediately used for blood cell analysis at Fujifilm Monolith Co., Ltd., Tokyo, Japan. The blood properties examined included the white blood cell (WBC) and red blood cell (RBC) counts, hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume, mean cell hemoglobin, and mean corpuscular hemoglobin concentration. Fecal egg counts were performed on individual fecal samples using a McMaster concentration method (Hansen and Perry, 1990). The flotation fluid was saturated sodium chloride (NaCl).

2.4. Data analysis

The bite rates and grazing times of each sheep were calculated using measured bite counts. Grazing time was defined as the total time over which there were >10 bites taken per unit of time (10 min). A paired *t*-test was employed to test for statistically significant differences in animal body weight and blood properties between pastures, and a *t*-test was used assess the relationship between plant biomass and the CP and NDF contents of the grass. A one-way analysis of variance was also used to determine if significant differences existed in nematode larval counts among the plant species in each season and pasture. When necessary, data were log-transformed to meet assumptions of normality and similarity of variance. All differences among comparisons with p-values < 0.05 were considered statistically significant, and values such as $0.05 < p < 0.1$ were considered different in tendency. Our analyses were performed using Statistica v. 12 (Dell Technologies, Inc., USA).

To extract the dominant features from the measured variables, we used principal coordinate analysis (PCOA) based on the means of two times for each grazing behavior (i.e., bite rate, grazing time), nutrition (i.e., body weight, glucose, protein, and cholesterol in the blood), and infection-related measurements (i.e., EPG of nematode, WBC, RBC, HGB, and HCT). The relationships among the infection, grazing behavior, and nutrition of sheep were then visualized as a tri-plot using the PCOA scores of the first axis (PC-1). Pearson's correlation coefficients were also calculated between each of the variables if a significant relationship was observed.

3. Results

The total DM biomass of the improved pasture was 144.7 g/m^2 and included only three species (Table 1). On the contrary, that of the semi-natural pasture was significantly higher (762.3 g/m^2, $p = 0.015$, Table 1) and included many different plant species. Rather than the NDF ($p = 0.231$), it was the CP of the plants in the semi-natural pasture that was lower than that in the improved pasture ($p = 0.059$).

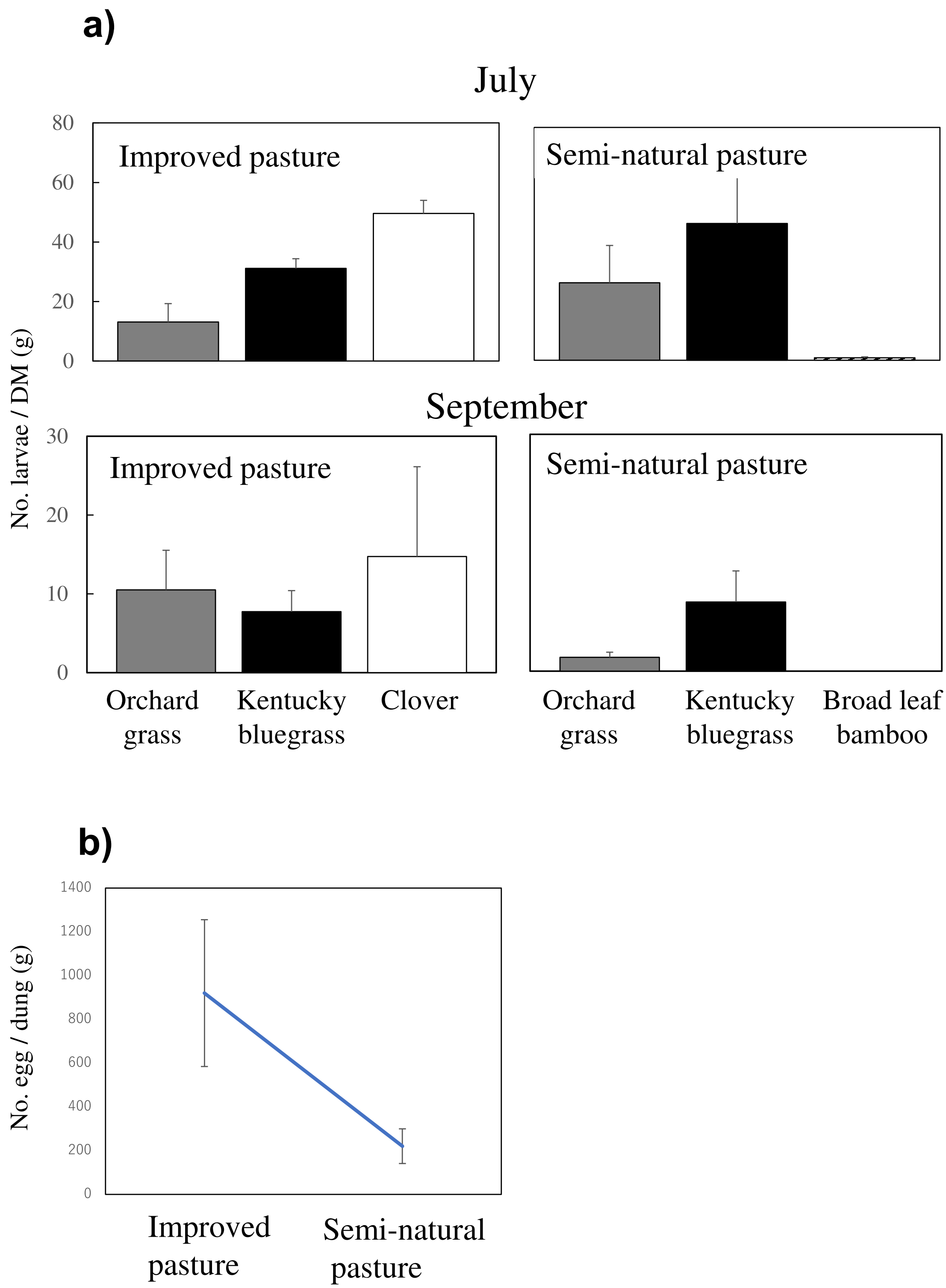

Fig. 1. Nematode larval density (± S.D.) found on main plant species (a) and nematode egg density from each sheep dung (b) in each pasture after grazing.

An mean of 27.9 and 7.3 nematode larvae per DM sample were recorded from the main plant species in July and September 2021, respectively (Fig. 1), from both improved and semi-natural pastures. In both pastures, the density of nematode larvae differed significantly between plant species in July ($p = 0.001$ and 0.046 on the improved an semi-natural pastures), but not differed in September ($p = 0.798$ and 0.056 on the improved an semi-natural pastures). The EPG detected from the dung decreased from mean of 920 – 220 after grazing on the improved and semi-natural pastures ($t = 2.34$, $p = 0.029$).

White blood cell counts ($t = 2.43$, $p = 0.029$), HCT ($t = 2.61$, $p = 0.020$), and glucose ($t = 3.18$, $p = 0.006$) concentrations decreased after grazing in the semi-natural pasture; however, other measured blood values were not significantly different between the pastures ($p > 0.05$). The body weights of the sheep increased after grazing in the semi-natural pasture (Fig. 2, $t = 2.47$, $p = 0.022$). Over an observation period of 48 h, the mean bite rate and time spent grazing were 158.4 bites/min and 52.2%, respectively.

Our PCOA results are shown in Fig. 2 and the contributions of the

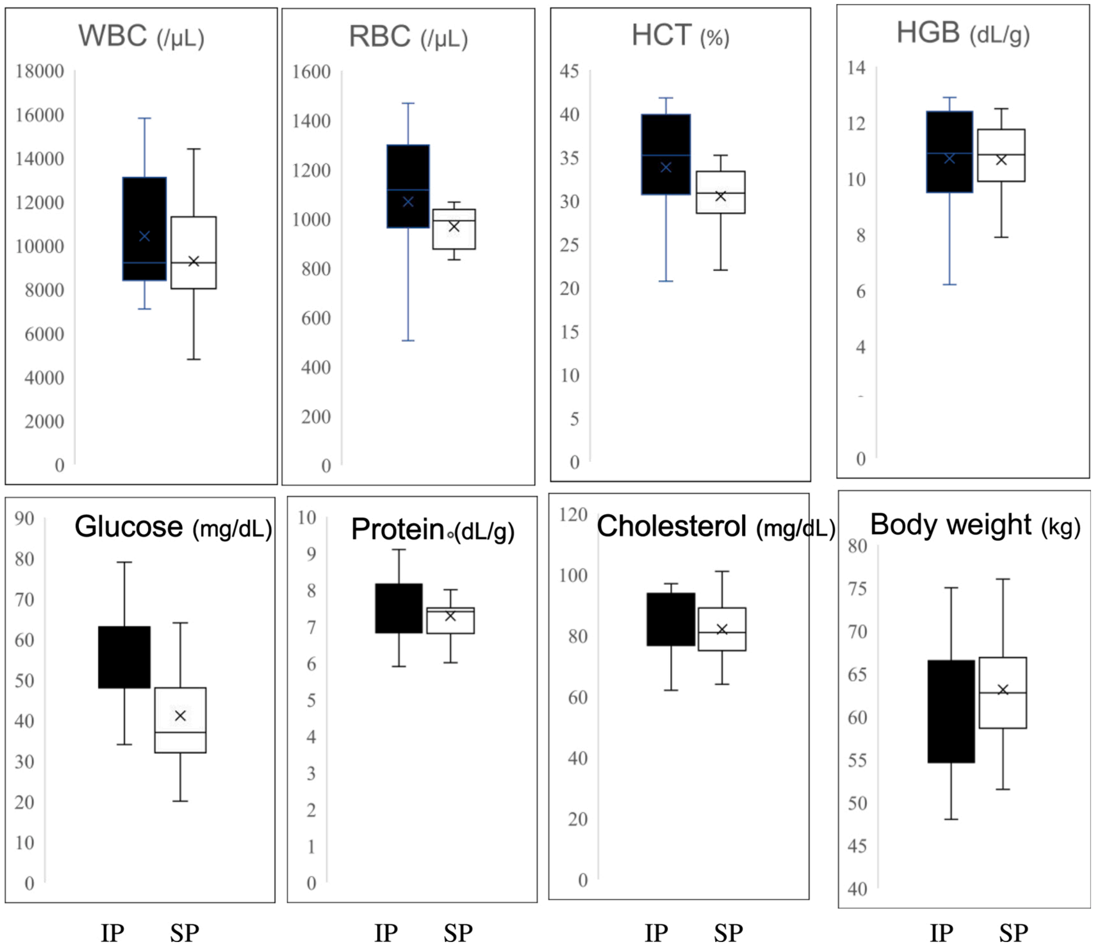

Fig. 2. Changes in the blood properties of 20 sheep after grazing in the improved (IP) and semi-natural (SP) pastures with respect to parasite infection (upper row) and nutrition and body weight (lower row).

first axis were relatively high (infection: 64.0%; nutrition: 39.5%; behavior: 60.6%). Blood cell values (WBC, RBC, HCT, and HGB) changed in same direction, although EPG exhibited the opposite trend. Similarly, the changes in the nutrient components showed roughly the same trend as those in the blood properties.

A tri-plot of sheep infection, nutrition, and behavior is shown in Fig. 3. The correlation coefficients between infection and nutrition, infection and behavior, and nutrition and behavior were 0.253, −0.027, 0.369, respectively. A significant relationship was only found between infection and behavior ($p = 0.026$), but not between infection and nutrition ($p = 0.136$) or between nutrition and behavior ($p = 0.871$). Therefore, further detailed analyses of the correlations between proxies of infection (i.e., blood values) and behavior were conducted (Table 2). Significant relationships were subsequently observed between the bite rate and nematode EPG, and between the grazing time and HGB and HCT.

4. Discussion

Based on the results of nematode EPG counts, sheep infection status improved from severe to moderate after grazing on the semi-natural pasture, although some blood properties (e.g., hematocrit) related to infection had not yet improved. Grass nutrient values were lower on the semi-natural pasture compared with the improved pasture, according to the lower CP content. Nevertheless, the blood protein and cholesterol of the sheep was not worsened by grazing there; rather, body weight improved, indicating that grazing on a semi-natural pasture did not hinder animal performance.

With regard to why the GIN infection risk was reduced under semi-natural pasture grazing, we deduce that the abundance of wild bamboo, upon which nematode larval densities are quite low, on the semi-natural pasture limited the rate of exposure to this parasite when compared to the improved pasture. As the leaves of bamboo grow at higher positions along their vertically elongated stems, it is likely difficult for the larvae of GINs to be deposited upon the grass blade (usually higher than 5 cm) and thus to reach the leaves for ingestion by the sheep (Peregrine et al., 2010). Additionally, the lower grazing density of the semi-natural pasture might have reduced the infection risk. However, the influence of sampling date on nematode infection between the pastures would not negligible, according to the seasonal change of GINs in Canadian sheep farms. (Mederos et al. 2010)

According to our PCOA results, a significant relationship was observed between the infection and behavior of grazing sheep. Although negative effects on the foraging behavior and instead increase of laying time of parasitized sheep are dominant (Hutchings et al., 1998, 1999, 2001; Seó et al., 2015; Falzon et al., 2013; Högberg et al. 2021; Williams et.al. 2022), the aggressive grazing of infected sheep in this study was consistent with the findings of Forbes et al. (2007), who reported a significant increase in the bite rate and decrease in the bite mass of dairy heifers with sub-clinical parasitic gastroenteritis when compared to those on anthelmintic treatments. Young dairy heifers given anthelmintic treatments grazed for 105 min longer than their untreated counterparts (Forbes et al., 2000) and lactating heifers and cows treated with eprinomectin grazed for 50 and 47 min longer than the controls, respectively (Forbes et al., 2004).

The effect of nematode infection on sheep activity is dependent on

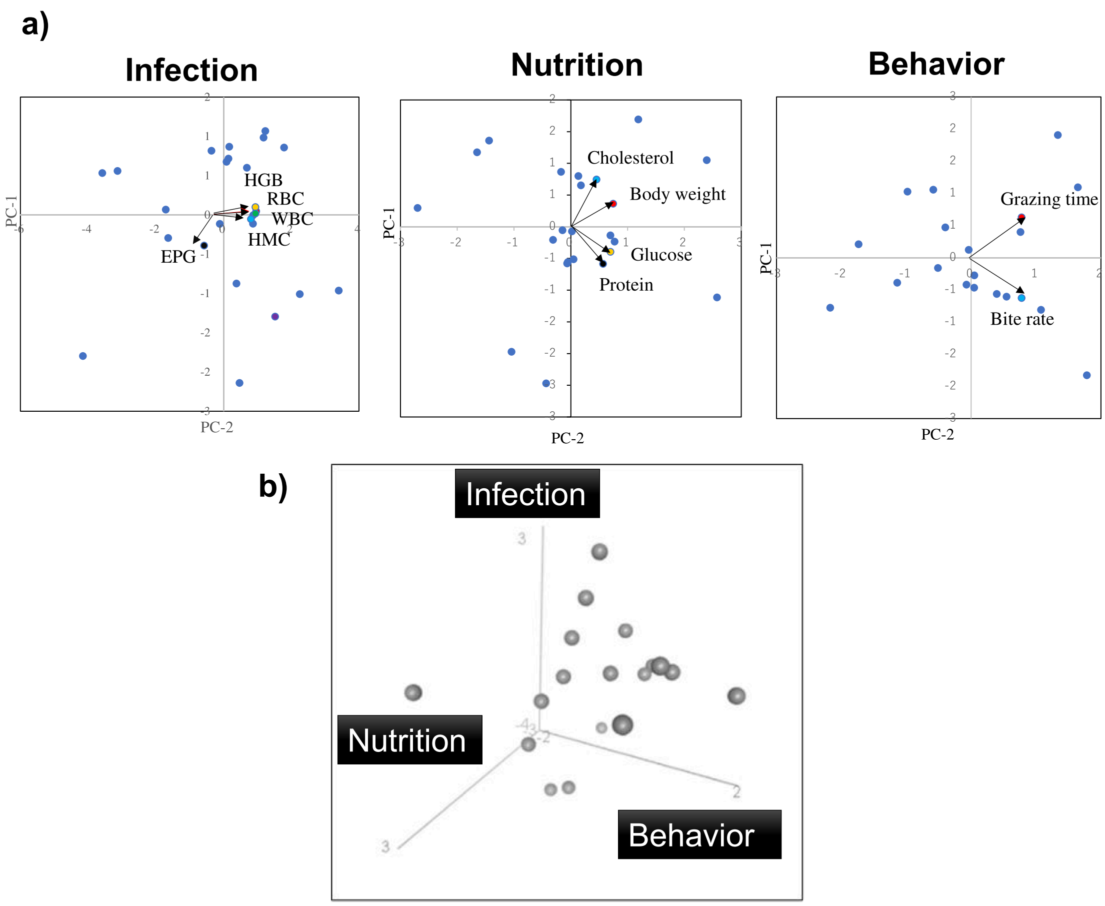

Fig. 3. (a) Principal components (PCs) of measured variables for individual sheep (dots); the direction and scale of each value is shown as an arrow. (b) Tri-plot showing the PC-1 scores of infection-, nutrition-, and behavior-related measurements; dots represent individual sheep.

Table 2
Pearson's correlation coefficients of the measured values between infection and grazing behavior. Significant relationships were shown in bold.

Behavior	WBC	RBC	HGB	HCT	EPG (nematode)
Bite rate	−0.150	−0.239	−0.208	−0.137	**0.426**
Grazing time	0.130	0.375	**0.470**	**0.471**	0.190

body weight (Ikurior et al., 2020). Based on the number of nematode eggs recorded in this study, the sheep began recovering from infection-induced nutritional damage while grazing on the semi-natural pasture. We therefore hypothesized that the parasitized animals may have grazed more aggressively when on the improved pasture to compensate for their reduced nutritional status and thus, increased parasitism resulted from aggressive foraging behavior (i.e., due to increased larval dispersion). Additional research will be needed to test these hypotheses in the future.

In conclusion, GIN infection and behavior of the grazing sheep is related as parasitized animals may become more aggressive feeders to compensate for their reduced nutritional uptake. Grazing sheep on semi-natural pastures may facilitate more stable performance due to the lower risk of nematode infection from wild plants.

Animal welfare statement and Ethics approval

All experimental procedures were approved by Mie University as acceptable standard care of and experimentation with animals. All efforts were made to minimize animal suffering.

Declaration of Competing Interest

The authors declare that there is no conflict of interest associated with the paper.

Acknowledgements

We thank H. Muto, and S. Kamata of Charo sheep farm and Prof. H. Inokuma of university Tokyo for their kindness in helping with the field survey and other aspects of the study. This research is financially supported by a Grant-in-Aid for Scientific Research provided by the Japan Society for the Promotion of Science (JSPS).

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