Immune Responses of Cattle to Theileria orientalis Infection and Seasonal Change

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KEY WORDS: Cattle, Grazing, Immune, Summer, *Theileria orientalis*

ABSTRACT

The application of a grazing-based system is still challenging in the cattle industry. Little is known about immune responses of cattle to grazing-associated risks. Therefore, the aim of study was to investigate the effects of grazing-associated *Theileria orientalis* infection and seasonal temperature on immune changes in cattle. Blood samples were collected from cattle before and after grazing, or in the spring and summer. The levels of interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and insulin-like growth factor-1 (IGF-1) in the serum were measured. Also, the presence of *T. orientalis* in the blood was investigated using PCR. Cattle that were positive for *T. orienta-*

lis were lower than those that were negative for *T. orientalis* in the level of IFN-γ ($p < 0.05$). Grazing triggered IFN-γ and IGF-1 secretion in cattle in the absence of *T. orientalis* ($p < 0.05$). Interestingly, cattle chronically infected with *T. orientalis* showed a decrease in IGF-1 level after grazing ($p < 0.05$). Moreover, summer heat resulted in increased IL-6 in cattle. In conclusion, *T. orientalis* infection and summer heat may disturb cattle grazing activity. These results provide information on the efficient utilization of grazing system in terms of immune activity in cattle.

INTRODUCTION

Grazing-based systems in grassy areas are generally beneficial to animal welfare and cost efficient in the cattle industry (Barkema et al., 2015). However, the application of a grazing-based system is still challenging because of energy and nutritional imbalance caused by a lack of available feed, uncontrolled disease, and continuously changing environmental conditions (Arnott et al., 2017; Knaus, 2016).

Disease and heat stress from outdoor grazing can affect immune responses (Min et al., 2016; Yamaguchi et al., 2010). Exposure to ticks and tick-borne diseases are one of the major concerns for cattle grazing on grassy mountainous areas (Kamio et al., 1990; Onuma et al., 1998). *Theileria orientalis* is a widespread non-lymphoproliferative tick-borne pathogen in the Republic of Korea (ROK) (Kim et al., 2017a), which causes anemia and associated chronic wasting diseases (Yamaguchi et al., 2010). Unlike the lymphoproliferative form of theileriosis (Dobbelaere et al., 2004), immune responses to *T. orientalis* are rarely investigated besides its role in hemolytic anemia. Recently, only one report shows that *T. orientalis* can be related to immune response by indicating an increase in cytokine-producing T-cells in *T. orientalis*-infected Holstein cattle (Yamaguchi et al., 2010).

Generally, heat stress weakens immune function and causes an inflammatory response in livestock (Inbaraj et al., 2016; Wenz et al., 2010). The detrimental effect of heat stress on dairy cows has been reported in several studies (Carroll et al., 2013; Wenz et al., 2010). Cattle are often at pasture in summer with no shade (Veissier et al., 2018). Exposure to summer heat during grazing may suppress immune function, resulting in high susceptibility of cattle to various diseases (Veissier et al., 2018).

Despite the risks, most studies have discussed the utility of grazing dairy cows in terms of the economic efficacy of milk production. Little is known about the endogenous responses of cattle to grazing-associated risks. Therefore, this study was to examine the effects of grazing system considering *T. orientalis* infection and seasonal temperature on immunological responses and growth-mediated hormone in Korean native cattle (Hanwoo) in ROK. Changes in the immune system would help to establish strategies to ameliorate the risks to cattle health during grazing.

MATERIAL AND METHODS

All procedures were carried out according to ethical guidelines for the use of animal samples, as approved by the National Institute of Animal Science (institutional animal care and use committee [IACUC] decision No. NIAS-2017017). The study was conducted from 2014 to 2015 using Hanwoo cattle from two farms located in Jeju island and Jeongeup in ROK. Jeju island, which has a subtropical climate (annual average temperatures ranged from 12.9 to 18.9 °C over the last 30 years) and is located on the southern Korean peninsula, was selected for cattle grazing. Healthy cattle with no abnormal physical conditions were used. Ten cattle that were maintained indoors were grazed on grassy mountains from spring (April) to summer (August) on Jeju island. Meanwhile four cattle raised in Jeongeup were kept indoors from spring to summer (average summer temperature ranged from 21.8 to 29.7 °C) to examine the effect of seasonal temperature on immune responses. Blood samples were collected from cattle before and after grazing, or in the spring

and summer. The levels of interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and insulin-like growth factor-1 (IGF-1) in the serum were measured using enzyme-linked immunosorbent assay (ELISA) kits (Cusabio Biotech Co., Ltd., Hubei, PR China) according to the manufacturer's instructions. The levels were compared before and after grazing or between spring and summer for indoor cattle.

Meanwhile, PCR was used to detect *T. orientalis* in blood samples, as described previously (Kim et al., 2017b). Cattle were divided into three groups based on the presence of *T. orientalis* infection before and after grazing.

- Group I were cattle that were not infected with *T. orientalis* in both before and after grazing
- Group II were cattle that were not infected with *T. orientalis* before grazing, but infected with *T. orientalis* after grazing
- Group III were cattle that were infected with *T. orientalis* in both before and after grazing.

SPSS 24.0 software (SPSS, Chicago, IL, USA) was used to analyze the data. The Shapiro-Wilk test was used to test the normality of the data. A t-test or Mann–Whitney U test was used to compare levels of IFN-γ, TNF-α, IL-6, and IGF-1 in relation to *T. orientalis* infection, grazing, and seasonal temperature. Data are expressed as mean ± standard deviation (SD) and $p < 0.05$ was considered significant.

RESULTS

Figure 1 shows the effect of *T. orientalis* infection on inflammatory reaction and growth-related hormone production in each housing (indoor) and grazing cattle. Grazing cattle that were positive for *T. orientalis* showed lower level of IFN-γ than grazing cattle that were negative for *T. orientalis* ($p = 0.045$), whereas housing cattle did not show significant difference in the IFN-γ level between *T. orientalis*-negative and positive groups. Meanwhile, IGF-1 level in housing cattle was increased in *T. orientalis*-positive group compared to *T. orientalis*-negative group ($p = 0.006$). Conversely, grazing cattle did not show a significant difference in the level of IGF-1 between *T. orientalis*-negative and positive groups. These results suggest that *T. orientalis* infection differently influences on IFN-γ and IGF-1 levels depending on growth type such as housing or grazing system.

A subsequent experiment confirmed the additive effect of grazing and *T. orientalis* on cytokines and IGF-1 by tracking the changes in immune responses and presence of *T. orientalis* before and after grazing in individual cattle (Fig. 2). Group I cattle that were not infected with *T. orientalis* in both before and after grazing showed increases in IFN-γ and IGF-1 concentrations after grazing, compared to before grazing ($p < 0.05$). Conversely, IGF-1 in Group III cattle that were infected with *T. orientalis* in both before and after grazing was decreased after grazing compared to before grazing ($p = 0.049$). This result

Figure 1. *Effects of T. orientalis infection on IFN-γ, TNF-α, IL-6, and IGF-1. Housing cattle that were negative (n = 7) and positive (n = 3) for T. orientalis; Grazing cattle that were negative (n = 5) and positive (n = 5) for T. orientalis. *p < 0.05.*

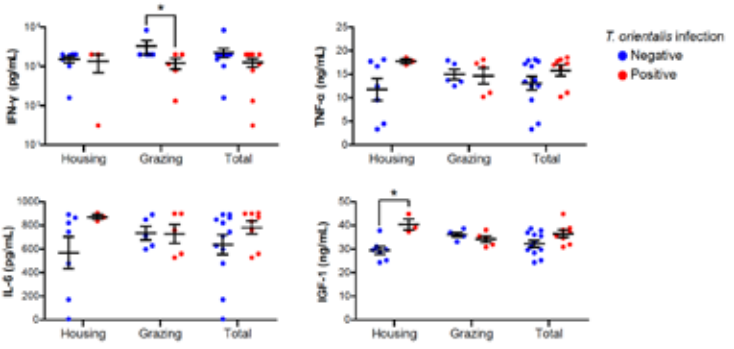
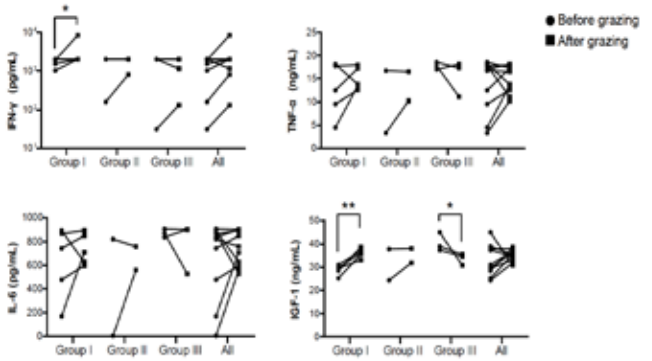


Figure 2. *Changes in IFN-γ, TNF-α, IL-6, and IGF-1 according to the presence of T. orientalis before and after grazing. Group I (n = 4): cattle that were not infected with T. orientalis both before and after grazing; Group II (n = 2): cattle that were infected with T. orientalis only after grazing; Group III (n = 3): cattle that were infected with T. orientalis both before and after grazing. *p < 0.05, **p < 0.005.*



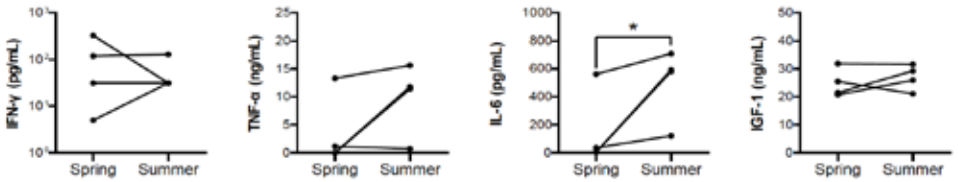
demonstrated that grazing had an inductive effect on immunity and growth activity in the absence of *T. orientalis* infection. Meanwhile, the positive effect of grazing may be counteracted or diminished by *T. orientalis* infection in cattle.

Finally, we investigated the effect of seasonal temperature on changes in cytokines and IGF-1. Cytokines and IGF-1 concentrations in indoor cattle were compared between spring and summer to determine if heat stress affected immune function and growth hormone production in cattle (Fig. 3). The level of IL-6 was elevated in summer compared to spring ($p = 0.016$), but no significant differences were observed in the levels of IFN-γ, TNF-α, or IGF-1 between spring and summer ($p > 0.05$). It indicated that IL-6 was a cytokine that responds to high temperature of summer season in cattle.

DISCUSSION

The effect of grazing on cattle health and productivity may vary according to geographic area, grass formation, and environmental conditions (Knaus, 2016). Despite the controversial results of cattle grazing due to various outdoor conditions, this is the first report to show the positive effects of grazing and the negative effects of *T. orientalis* infection on immunity and growth in cattle. This study provides useful information for establishing measures to maximize

Figure 3. *Seasonal changes in IFN-γ, TNF-α, IL-6, and IGF-1 in housing cattle without T. orientalis infection (n = 4). *p < 0.05.*



the advantages of a grazing-based system and minimize an interference by a grazing-associated risks.

Activation of macrophages by IFN-γ contributes to the elimination of parasites through phagocytosis and toxic metabolites, which are involved in cell-mediated immunity (Yamaguchi et al., 2010). In this study, *T. orientalis* infection suppressed the level of IFN-γ in the grazing, but not housing cattle. A combination of *T. orientalis* infection and grazing appeared to affect the immune response of IFN-γ, which may be involved in protecting against *T. orientalis* or other parasitic infections in cattle (Yamaguchi et al., 2010). *T. orientalis* is considered to be an important factor affecting cattle immune function under a grazing-based system. Therefore, monitoring for *T. orientalis*-infected cattle should be required during grazing. In addition, a preventive method for *T. orientalis* infection should be performed in grazing-based cattle systems.

IGF-I is important for growth potential and metabolism in beef cattle (Tuggle et al., 1996). Unexpectedly, IGF-1 level were higher in *T. orientalis*-positive cattle than *T. orientalis*-negative cattle in housing cattle. It was not clear why IGF-1 showed increased response to *T. orientalis* infection in housing cattle, but it might have been due to the degree and duration of the *T. orientalis* infection. Housing cattle that were positive for *T. orientalis* may indicate a chronic and latent infection (Kim et al., 2017a). The increase in IGF-1 might have been a defense or compensatory mechanism in cattle to cope with chronic disease status.

To control a variable by *T. orientalis* infection before grazing and clarify grazing effect on immunity and growth activity, we monitored the changes in cytokines, IGF-1, and *T. orientalis* infection in individual cattle before and after grazing. Interestingly, this study demonstrated that grazing could have a positive effect on cattle health via enhanced production of IGF-1 and IFN-γ, as evidenced by the changes in Group I cattle. However, immune and growth system of grazing cattle may be suppressed or inhibited under certain unfavorable conditions, such as continuous *T. orientalis* infection.

TNF-α and IL-6 were reported to be pro-inflammatory cytokines that are related to inflammatory responses in dairy cows (Esposito et al., 2014). Similar to previous studies of dairy cows (Carroll et al., 2013; Min et al., 2016), seasonal temperature altered the level of IL-6, which showed higher concentrations in indoor cattle in summer compared to spring. Unlike a previous report (Min et al., 2016), there was no significant difference in TNF-α between spring and summer in indoor cattle in this study. IL-6 may be a sensitive inflammatory protein that is related to heat stress in hot temperatures. Although not all cattle showed an increase in IL-6 after grazing, IL-6 may be useful for health monitoring of cattle grazed in hot summer conditions.

In conclusion, grazing systems may have an influence on cattle health by inducing the production of IFN-γ and IGF-1. Infection and heat stress can be a factor that disturbs growth and immune responses during grazing. The regulation of risk factors associated with grazing should be considered for the successful application of a grazing system to cattle.

ACKNOWLEDGEMENTS

This work was carried out with the support of "Cooperative Research Program for Agriculture Science & Technology Development (Project title: Research on the health and welfare management condition in the mountain grazing cattle, Project No. PJ01009202)" Rural Development Administration, Republic of Korea.

STATEMENT OF AUTHORSHIP

The authors hereby certify that all work contained in this article

CONFLICT OF INTEREST

We declare that there are conflicts of interest.

AUTHOR'S CONTRIBUTION

CYC, YHJ, JGY, and JP designed the study

and provided consultation. CC, AC, MGK, YJD, SIO, and HSK participated in the collection of materials and animal samples. CC, DHY, JP, KSC, and SK conducted the analyses and CC and SK drafted the manuscript. All the authors contributed to the interpretation of the data. All authors have read and approved the final manuscript.

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