Potential impact of botanically diverse pasture on the nutritional, physiological, and immunological status of grazing cows

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Keywords:	diverse pasture, immunology, mineral intake, nutrition, physiology

Abstract:	The aim of this study was to reveal the potential impact of botanically diverse pasture on the nutritional, physiological, and immunological status of grazing cattle using multifaceted indices. Ten Japanese black beef cows (325.5 ± 40.6 kg of BW, 7.9 ± 3.8 years of age) were used in this experiment. Five of them grazed on a 1.8 ha grassland (botanically diverse pasture: DP) that was composed of sown grassland and grazable forestland (approximately 34 plant species). The other five cows grazed on 1.0 ha of sown grassland with only a few plant species (botanically monotonous pasture: MP, approximately 5 plant species) for two months. Blood samples were collected approximately every two weeks. In DP cows, the hemoglobin (HGB) concentration, hematocrit (HCT) ratio, and superoxide dismutase (SOD) activity increased linearly after the start of grazing, as did plasma sodium (Na), calcium (Ca), and potassium (K) concentrations. Urea nitrogen (UN) levels were higher in DP than in MP cows throughout the grazing period, whereas in MP cows, the red blood cell (RBC) count, HGB concentration, and HCT ratio decreased quadratically after the start of grazing. The DP cows gained more BW than the MP cows throughout the grazing period. Thus, the increased intake and/or the change in the ingested plant species in DP cows might have promoted the increase in the plasma UN concentration. In summary, the present study showed that grazing in botanically diverse pasture improved the nutritional and physiological status of cows; however, it aggravated the imbalance of protein and energy intake induced by grazing within two months.

Potential impact of botanically diverse pasture on the nutritional, physiological, and immunological status of grazing cows

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Abstract

The aim of this study was to reveal the potential impact of botanically diverse pasture on the nutritional, physiological, and immunological status of grazing cattle using multifaceted indices. Ten Japanese black beef cows (325.5 ± 40.6 kg of BW, 7.9 ± 3.8 years of age) were used in this experiment. Five of them grazed on a 1.8 ha grassland (botanically diverse pasture: DP) that was composed of sown grassland and grazable forestland (approximately 34 plant species). The other five cows grazed on 1.0 ha of sown grassland with only a few plant species (botanically monotonous pasture: MP, approximately 5 plant species) for two months. Blood samples were collected approximately every two weeks. In DP cows, the hemoglobin (HGB) concentration, hematocrit (HCT) ratio, and superoxide dismutase (SOD) activity increased linearly after the start of grazing, as did plasma sodium (Na), calcium (Ca), and potassium (K) concentrations. Urea nitrogen (UN) levels were higher in DP than in MP cows throughout the grazing period, whereas in MP cows, the red blood cell (RBC) count, HGB concentration, and HCT ratio decreased quadratically after the start of grazing. The DP cows gained more BW than the MP cows throughout the grazing period. Thus, the increased intake and/or the change in the ingested plant species in DP cows might have promoted the increase in the plasma UN concentration. In summary, the present study showed that grazing in botanically diverse pasture improved the nutritional and physiological status of cows; however, it aggravated the

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KEYWORDS

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1 | INTRODUCTION

Grazing is assumed to contribute to cattle health. It diversifies behavioral patterns, reduces abnormal behavior, improves the quality of rest (Higashiyama, Nashiki, Narita, & Kawasaki, 2007; O'Connell, Giller, & Meaney, 1989; Redbo, 1993), and decreases the incidence of disorders such as lameness (Arnott, Ferris, & O'Connell, 2017; Haskell, Rennie, Bowell, Bell, & Lawrence, 2006; Washburn, White, Green, & Benson, 2002). However, some previous reports have shown that grazing occasionally causes an energy shortage and an imbalance in energy and protein intake (Butler, 2014), which induces a decrease in major blood components, such as red blood cells and lymphocytes (Nakajima, Doi, Tamiya, & Yayota, 2017). This pattern implies that grazing does not necessarily contribute to cattle health.

The effect of grazing on cattle health depends on the pasture and on management practices. In particular, grazing in botanically diverse pasture may have beneficial effects on cattle health because cattle can ingest various native grasses, shrubs, and tree leaves. These plants often contain pharmacological agents, including some plant secondary metabolites (PSMs) and high total antioxidant capacity (Haga et al., 2014, 2016; Han, Lo, Choi, Kim, & Baek, 2004; Provenza & Villalba, 2010; Villalba, Beauchemin, Gregorini, & MacAdam, 2019). Grazing on native grass, shrubs, and tree leaves in forestland enhances total antioxidant capacity (Haga et al., 2016). Ingesting various PSMs from different plant species may also positively affect the immunity of grazing animals (Egea, Hall, Miller, Spackman, & Villalba, 2014; Kayser, Kiderlen, & Croft, 2003; Min & Hart, 2003; Provenza & Villalba, 2010). For example, tannins and alkaloids in a plant possess anti-parasitic activity (Provenza & Villalba, 2010). Galactomannans, which are found in legume and grass seeds, stimulate macrophage function (Provenza & Villalba, 2010; Tizard, Carpenter, McAnalley, & Kemp, 1989). In addition, mannan oligosaccharide, which is common in vascular plants with fungi and yeasts, improves the immune response to a virus (Franklin, Newman, Newman, & Meek, 2005). Plant-species-rich vegetation improves nutrient balance and promotes mineral intake (Ohlson & Staaland, 2001; Provenza, Villalba, Dziba, Atwood, & Banner, 2003). Thus, if the botanical diversity in a grassland increases, this nutritional, physiological, and immunological potential of plants may beneficially affect the health of grazing cattle. However, little information is available on the effect of pasture botanical diversity on the health of grazing cattle from the perspective of physiological, immunological, and nutritional status.

The objective of the present study was to identify whether grazing in botanically diverse pasture improves cattle physiological, nutritional, and immunological indices and contributes to cattle health. We hypothesized that grazing in botanically diverse pasture improves the balance and quantity of minerals, proteins, and energy status and enhances the immunity and physiological status of cattle.

2 | MATERIALS AND METHODS

2.1 | Animals, housing, grazing, and diets

All animal experimental procedures were approved by the Committee for Animal Research and Welfare of Gifu University (#17032). This study was conducted on Minokamo livestock farm, Gifu field science center, Gifu University (longitude 137°03'57"E; latitude 35°26'44"N), from July to September 2017. The maximum and minimum temperatures and humidity outside the cowshed during grazing were 38°C, 21.6°C, 83 % and 47 %, respectively. Ten Japanese black cows (325.5 ± 40.6 kg of body weight (BW), 7.9 ± 3.8 years of age, beef cattle, non–lactating, non–pregnant) with no clinical signs of illness and no external injury at the start of the experiment were used for this experiment. For two weeks prior to the study, all cows were kept in an 8 m x 7.3 m indoor pen and tethered to tie stalls. They were fed 11.3 kg/d of Italian ryegrass silage and 1.6 kg/d of concentrate feed on an as-fed basis at 08.00 h and 16.00 h according to the Japanese Feeding Standard for beef cattle (NARO, 2008). Each cow was tethered with a rope but was able to engage in social interaction with neighboring individuals. The pen included a concrete floor covered with sawdust bedding. Then, five of the ten cows grazed on a 1.8 ha pasture composed of sown pasture (1.0 ha) and a grazable forestland (0.8 ha) with diverse vegetation (botanically diverse pasture: DP) continuously for two months. The remaining cows grazed on a 1.0 ha pasture composed of sown pasture with a few plant species (botanically monotonous pasture: MP) continuously for two months. Grazing commenced at the same time in both treatments. The two grazing areas were adjacent to each other. All experimental cows had grazing experience. The grazing cows were outside all day and were allowed to eat only herbages in the pasture; however, they were given access to water and a mineral salt block (rich salt: sodium chloride, phosphoric acid, calcium carbonate, calcium fatty acid, baker's yeast, magnesium oxide, iron sulfate, copper sulfate, zinc carbonate, manganese carbonate, cobalt carbonate, calcium iodate, and 0.3 ppm selenium, Onoda Chemical Industry Co., Ltd, Tokyo, Japan) to ensure a minimum amount of mineral intake. The same number of mineral salt blocks were placed in both treatment areas. All grazing cows were able to engage in social interaction with other cows in each group. The cows were allocated to the treatments according to their age and body weight.

2.2 | Herbage composition, herbage mass, and chemical composition

The herbage composition and mass in the pasture were measured at 19 plots in the DP (10 quadrats in the grazable forested land and 9 quadrats in the sown grassland) and at 9 plots in the MP using a 50 cm × 50 cm quadrat before the start of grazing. The plant species in the quadrats were identified, and the herbage was cut with hand shears at the ground level. The collected herbages were weighed and mixed, and a representative sample from each quadrant was separated into green leaves, stems, and dead material. The green leaf and stem samples were oven dried at 60°C for 48 h, ground to pass through a 1-mm screen and then analyzed for dry matter (DM), crude ash (Ash), crude protein (CP), and acid detergent fiber (ADFom) according to the AOAC method (AOAC, 2007: 930.15). Neutral detergent fiber (aNDFom) was analyzed according to the method of Van Soest et al. (Van Soest, Robertson, & Lewis, 1991). The herbage mass in the pasture and the chemical compositions of each herbage sample are shown in Table 1. Before grazing, the sown pasture and the grazable forestland in DP were composed of approximately 24 and 10 plant species, respectively; whereas, the sown pasture in MP was composed of approximately 5 plant species (Table S1). The pre-grazing herbage masses in the sown pasture and grazable forestland in DP and in the sown pasture in MP were 4.6, 1.3, and 5.7 Mg DM/ha, respectively. The DM contents of the herbage in the sown pasture and forestland in DP and in the sown pasture in MP before grazing were 239 g/kg fresh matter, 567 g/kg fresh matter, and 227 g/kg fresh matter, respectively. The plant species, herbage mass, and chemical composition after grazing are shown in Tables 1 and S2.

2.3 | Body weight measurement, Blood collection and analysis

Body weight (BW) was measured before the start of grazing and every two weeks after the start of grazing (Table 2). Blood samples were collected on day 0 (0 week) before the start of grazing and every two weeks, i.e., 2 (7 to 21 days), 4 (22 to 36 days), 6 (37 to 51 days), and 8 (52 to 66 days) weeks after the start of grazing. Blood samples were collected via the jugular vein using a vacuum collection tube containing heparin or EDTA (Venoject II vacuum blood collection tube, TERUMO Co., Ltd., Tokyo, Japan) at approximately 08.00 h. Blood samples containing EDTA were used for complete blood cell counts and leukocyte differentiation. Heparinized blood samples were centrifuged at 1,000 × g at 4°C for 10 min to collect blood plasma. The plasma samples were stored at −80°C until biochemical analysis and oxidative stress marker analysis.

A complete blood cell count was conducted within 24 h after blood collection. The number of red blood cells (RBCs), white blood cells (WBCs) and platelets (PLTs) were counted, and the hematocrit (HCT) ratio and hemoglobin (HGB) concentration were determined. Then, the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated using an automated hematology analyzer (Celltac α, Nihon Kohden Co., Ltd., Tokyo, Japan).

Thin blood smears were air dried and stained with May−Grunwald and Giemsa stain solution. Two hundred cells on each slide, including neutrophils, basophils, eosinophils, monocytes, and lymphocytes, were counted with a microscope at 400 × magnification. Each leukocyte was classified into the above 5 types, and the numbers of each type were calculated.

Plasma samples were used for biochemical analysis. The concentrations of urea nitrogen (UN), glucose (Glu), total cholesterol (TCHO), magnesium (Mg), inorganic phosphorus (IP), calcium (Ca), chlorine (Cl), sodium (Na), and potassium (K) were determined using a dry chemistry method with a commercial kit (Fuji dry chem slide, Fujifilm Co., Ltd., Tokyo, Japan).

Superoxide dismutase activity (SOD), total antioxidant capacity (TAC) and glutathione peroxidase activity (GPx) were determined in plasma samples using commercial kits (SOD Assay Kit−WST, Dojindo laboratories Co., Ltd., Kumamoto, Japan; PAO, Nikken SEIL Co., Ltd, Tokyo, Japan; Glutathione peroxidase assay kit, Northwest Life Science Specialties, LLC, Washington, USA, respectively).

2.4 | Statistical analysis

We calculated statistical power using G*Power version 3.1.9.2 (two−way ANOVA with repeated measure, $\alpha = 0.05$, $(1-\beta) = 0.8$, University of Dusseldorf, Dusseldorf, Germany) to determine sample size. Considering the cost and availability of experimental cows, the sample size was decided based on a high effect size setting. The statistical unit in this experiment was not a grazing herd; rather, the unit was an individual animal. This unit was chosen because the grazing period lasted two months and because it was difficult to recreate several replications in each treatment due to the limitations of pasture and herd size and the relatively long study period. The adequacy of this approach was supported by some other studies (Connolly, 2018; Robinson, Wiseman, Udén, & Mateos, 2006).

All data were analyzed statistically using the lmerTest package (Kuznetsova, Brockhoff, & Christensen, 2014) in R (version 3.0.2: R core team, 2013). The normality test for all data was conducted using the Shapiro-Wilk test before analysis. Data that did not match parametric assumptions were transformed by a square–root transformation before statistical analysis. Then, the data were analyzed with a Gaussian distribution using a generalized linear mixed model (GLMM) with repeated measurements using the data from 2, 4, 6 and 8 weeks to compare the treatments. The treatments (DP *vs.* MP) were considered fixed effects, and each individual animal was considered a random intercept effect. The treatment effect through time was tested by polynomial contrasts. Differences were considered significant at $P < 0.05$, and $0.05 < P < 0.1$ was considered indicative of a trend. All statistical results are shown in the Supporting Information (Table S3).

3 | RESULTS

3.1 | Complete blood cell count

Significant differences were not found in WBCs between the treatment groups (treatment: $P = 0.817$). The number of WBCs in the DP cows increased linearly from the start of grazing ($P = 0.034$; Figure 1A and Table S3). There was no difference in RBC numbers between treatments ($P = 0.483$). The number of RBCs in the MP cows exhibited a quadratic decreasing tendency ($P = 0.076$), whereas those in the DP cows did not change during the experimental period (Linear: $P = 0.545$, Quadratic: $P = 0.386$). Although HCT and HGB did not differ between the treatments, those in cows on DP increased linearly after the start of grazing (HCT: $P < 0.01$, HGB: $P < 0.01$). With respect to MCV, MCH, and MCHC, there were no significant differences between the treatments (Figure 1F, 1G, 1H and Table S3), though these parameters increased linearly (MCV in both: $P < 0.01$, MCH in both: $P < 0.01$) in both treatments.

3.2 | Leukocyte differentiation

No significant difference in leukocyte differentiation was detected between the treatments. The number of neutrophils in cows on DP increased linearly or showed a quadratic trend after the start of grazing. The number of neutrophils in cows on MP increased linearly and quadratically after the start of grazing (linear: DP: $P < 0.01$, MP: $P = 0.026$, quadratic: DP: $P = 0.083$, MP: $P = 0.017$, Figure 2B and Table S3). The number of monocytes in cows on DP increased quadratically ($P = 0.027$, Figure 2D and Table S3). Basophils were not found throughout the experiment (data not shown).

3.3 | Biochemical analysis for metabolic profiles

The Glu and TCHO did not differ between the treatments, whereas there was a significant difference between DP and MP in the UN concentrations ($P = 0.015$). The concentrations of TCHO in both treatments increased linearly ($P < 0.01$), and the concentrations of UN in both treatments increased after the start of grazing (linear: $P < 0.01$, quadratic: $P < 0.01$, Figure 3B, 3C and Table S3). A significant difference in IP was found between treatments (P = 0.023, Figure 3D and Table S3). Although the concentrations of Ca and Na did not differ between the treatments (Ca: P = 0.414, Na: P = 0.518), those in cows on DP increased linearly after the start of grazing (Ca: P < 0.01, Na: P = 0.014, Figure 3F, 3G and Table S3). A significant difference was found in the concentration of K (P = 0.025) between the treatments. The K concentration in cows on DP showed linear and quadratic trends (linear: P < 0.01, quadratic: P < 0.01) and that in cows on MP decreased linearly (P = 0.016, Figure 3H and Table S3).

3.4 | Oxidative stress maker analysis

There was no significant difference in SOD (P = 0.432), GPx (P = 0.816), and TAC (P = 0.344) between treatments. However, the activity of SOD in DP cows increased linearly (P < 0.01, Figure 4A and Table S3). The GPx activity in cows on MP decreased quadratically (P = 0.055), while that in DP cows did not change throughout this experiment (Figure 4B and Table S3). The TAC concentrations in both treatments increased linearly and quadratically during the experimental period (linear: P < 0.01, quadratic: P < 0.01, Figure 4C and Table S3).

4 | DISCUSSION

Before grazing, the DP, which included sown pasture and grazable forestland, was composed of approximately 34 plant species, while the MP was composed of approximately 5 plant species. The nutritional composition of the pasture, including the CP, NDF, and ADF, was more varied in DP than in MP. The sward characteristics, such as plant height and species, in DP varied widely compared with those in MP. After grazing, the plant composition in DP decreased to approximately 20 plant species, and the variation of the nutritional composition in DP reduced compared with that in DP before grazing, implying that cattle selectively ingested various plant species. In addition, cows on DP likely ingested more plant species than cows on MP based on a meta-analysis of the forage selection and foraging behavior of cattle in species-rich, Japanese native grasslands (Ogura, 2011). Thus, these changes suggest that cows on DP could ingest various nutritional components by eating a wide variety of species. The cows in DP likely choose to consume certain plant species and thereby ingest more nutritional components than are suggested by the measured herbage values (Mayes, & Dove, 2000).

The ingestion of a wide variety of plant species on DP improved the mineral status in cows. Cattle consume more plant species when more species are available (Ogura, 2011). A highly diverse pasture improves the mineral balance of grazing cattle by allowing the ingestion of a wide variety of plant species (Ohlson & Staaland, 2001; Yoshihara, Mizuno, Ogura, Sasaki, & Sato, 2013). In fact, the plasma Ca, Na and K contents in DP cows increased after the start of grazing, although cows in both treatment groups had free access to mineral salt blocks.

Furthermore, the DP cows gained more body weight than the MP cows throughout the grazing period (326.8 kg to 382.2 kg for MP, 324.2 kg to 406.0 kg for DP). The canopy structure of vegetation affects their bite frequency; cows take more bites from layers with more available leaves (Ogura, 2011). The frequency of bites related to the canopy structure of plants could be higher in DP than that in MP. Moreover, several studies have shown that plant diversity increases the foraging motivation of herbivores and their daily gain (Feng et al., 2016； Yang, Wang, Yuan, Wang, & Wang, 2017). Thus, a botanically diverse pasture could increase the amount of mineral intake via increased herbage intake. The present results support these previous findings and emphasize that grazing in plant species-rich vegetation affects the balance and/or amount of mineral intake and improves the mineral status of animals. In contrast, with regard to CP and energy status, the concentration of UN, which is one of the components of protein and energy balance, increased in both treatments after the start of grazing. Moreover, the concentration of UN was higher in DP than that in MP after the start of grazing. Hirata et al. (2012) reported that cattle can select and ingest plants with a high CP concentration. The cows in DP gained more BW than the cows in MP throughout the grazing period. Thus, the increased plant intake and/or the change in the ingested plant species in cows in DP might promote the upregulation of the UN concentration in the plasma.

The protein and mineral status of the cows affected blood component concentrations. Grazing often decreases protein absorption due to an energy shortage (Boken, Staples, Sollenberger, Jenkins, & Thatcher, 2005; Bulter, 2014; Kay, Roche, Kolver, Thomson, & Baumgard, 2005). The reduced protein absorption leads to a reduction in RBCs and lymphocytes (Lewicki, Lewicka, Kalicki, Kłos, Bertrandt, & Zdanowski, 2014; Sasaki, Ohnota, Yanagawa, & Chiba, 1985; Stangl, Schwarz, Müller, & Kirchgessner, 2000). The present results showed that the values of RBCs in MP decreased and remained at low levels as UN increased after the start of grazing. Thus, the decrease of this circulating blood cell number may be due to low protein absorption with a shortage of energy intake in cows on MP, as supported by the concentration of UN. However, the values of HCT and HGB increased linearly in cows on DP after the start of grazing. This change may be due to improved mineral intake and balance. The injection or feeding of vitamin B12, which involves cobalt as an essential constituent, increases the values of HCT, HGB, and lymphocytes (Girard & Matte, 2005; Lewicki et al., 2014; Stangl et al., 2000). Takamizawa et al. (2016) reported that grazing in diverse vegetation enhanced uptake of cobalt. Furthermore, trace minerals, such as iron and copper, are also involved in blood cell production. Yoshihara et al. (2013) reported that beef cattle potentially experience deficiencies in iron and copper when they graze pastures with few plant species. Namely, the improvement of these trace mineral intakes in cows on pastures with diverse vegetation might enhance the values of HCT and HGB.

Even if no significant difference was detected in the number of neutrophils between treatments, the number of neutrophils in both treatments increased linearly, and the number of monocytes in DP increased quadratically. This finding reflects the change of the autonomic nervous system via the change in feeding condition from confinement conditions before the study to grazing conditions. Grazing increases the number of circulating neutrophils (Nakajima et al., 2017). When the sympathetic nervous system is activated, the number of neutrophils increases in the blood because neutrophils are under the control of the sympathetic nerve (Abo & Kawamura, 2002). Moreover, the automatic nervous system affects the number of monocytes in the blood (Suzuki et al., 1997). Grazing may activate the automatic nervous system through exercise (Fu & Levine, 2013) and direct exposure to the outside environment (Nakajima, Doi, Tamiya, & Yayota, 2019). Thus, the present study emphasized that grazing itself has a positive impact on circulating neutrophils. In addition, grazing in botanically diverse pasture might have a positive impact on circulating monocytes via automatic nervous system modulation.

In this study, grazing improved the concentration of TAC in plasma regardless of the pasture vegetation. A previous study reported that grazing in a grazable forestland increased TAC more than did grazing in a sown pasture (Haga et al., 2014). The present study is inconsistent with this previous study. In the present study, the dominant plant species in the grazable forestland was *Pleioblastus argenteostriatus* (Regel) Nakai f. glaber (Makino) Murata (approximately 69 % of the coverage), indicating that the plant diversity in the grazable forestland was less than that reported in Haga et al. (2014). Moreover, some previous studies have reported that the soil conditions in a pasture also affect the antioxidant content in plants (Andrés, Jimenez, Mane, Sánchez, & Barrera, 1997). Thus, not only the plant diversity in grazable forestland but also the soil condition in the pasture might cause a difference between the studies. The present study also showed that the activity of SOD in DP increased linearly. Heat stress and intake of minerals such as zinc often affect the activity of SOD (Bernabucci, Ronchi, Lacetera, & Nardone, 2002; Olin, Golub, Gershwin, Hendrickx, Lonnerdal, & Keen, 1995). In the present study, the grazing area in both treatments had a shade forest, implying that cows were able to avoid direct sunlight. Thus, it might be speculated that the improvement of mineral intake by grazing in botanically diverse pasture enhanced the activity of SOD.

In addition, some plants such as forbs, shrubs, and tree leaves contain various plant secondary metabolites and minerals. The ingestion of these secondary metabolites enhances immunity and physiological status via a reduction in oxidative stress (Beck, & Gregorini, 2020; Provenza, & Villalba, 2010; Villalba, Beauchemin, Gregorini, & MacAdam, 2019). Improvement in mineral ingestion also enhances antioxidant enzyme activity and hematological status (Olin et al., 1995; Stangl et al., 2000). Thus, the ingestion of balanced minerals and potential secondary metabolites on DP may improve physiological and oxidative conditions in cows. As we expected, the herbage mass and plant species in the pasture changed throughout the experimental period. These vegetational changes during the grazing period likely affected the dynamics of the nutritional and health status of the grazing cattle. This dynamic relationship should be further investigated.

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TABLE 1 Herbage mass and chemical composition of botanically diverse pasture (DP) and monotonous pasture (MP) before grazing

		DP†		MP‡
	Before and after grazing	Sown pasture	Forest land	Sown pasture
Herbage mass (Mg DM / ha)	Before	4.64	1.33	5.74
	After	1.89	1.32	1.72
Grass height (cm)	Before	54 ± 14.7	40 ± 11.8	54 ± 8.4
	After	17 ± 9.0	35 ± 9.7	9.6 ± 3.2
DM (g/ kg fresh matter)	Before	239 ± 32	567 ± 60	227 ± 56
	After	358 ± 62	795 ± 58	450 ± 111
CP (g/ kg DM)	Before	139 ± 52	154 ± 11	136 ± 15
	After	165 ± 16	156 ± 14	147 ± 30
Ash (g/ kg DM)	Before	117 ± 16	122 ± 16	104 ± 16
	After	119 ± 17	169 ± 10	116 ± 14
ADFom (g/ kg DM)	Before	376 ± 46	353 ± 14	385 ± 17
	After	294 ± 16	328 ± 19	340 ± 20
aNDFom (g/ kg DM)	Before	576 ± 103	701 ± 22	635 ± 12
	After	473 ± 83	643 ± 18	542 ± 73

† DP: 1.8 ha pasture composed of sown pasture and grazable forestland with diverse vegetation.

‡ MP: 1.0 ha pasture composed of sown pasture with few plant species.

CP: crude protein, Ash: crude ash. ADFom: acid detergent fiber expressed exclusive ash. aNDFom: neutral detergent fiber assayed with a heat–stable amylase and expressed exclusive ash.

TABLE 2 Body weight of cows in botanically diverse pasture (DP) and monotonous pasture (MP) before and after grazing

		Before grazing	After grazing (weeks)			
			2	4	6	8
MP‡	Mean (kg)	326.8	356.6	366.4	380.4	382.2
	Sd § (kg)	49.0	44.4	43.2	46.1	50.1
DP†	Mean (kg)	324.2	360.2	375.8	392.6	406.0
	Sd (kg)	36.1	28.0	27.0	26.5	20.1

‡ MP: 1.0 ha pasture composed of sown pasture with few plant species.

† DP: 1.8 ha pasture composed of sown pasture and grazable forestland with diverse vegetation.

§Sd: Standard deviation.

Figure legends

FIGURE 1 Comparison of complete blood cell counts in grazing cows on botanically diverse pasture (□) and on botanically monotonous pasture (■).

The horizontal axis shows the week after the start of grazing (day 0). Blood samples were collected on day 0 (0 week) before the start of grazing and 2 (7 to 21 days), 4 (22 to 36 days), 6 (37 to 51 days), and 8 (52 to 66 days) weeks after the start of grazing. The data are presented as the mean ± SEM. A) WBC = white blood cell, B) RBC = red blood cell, C) HGB = hemoglobin, D) HCT = hematocrit, E) PLT = platelet, F) MCV = mean corpuscular volume, G) MCH = mean corpuscular hemoglobin, H) MCHC = mean corpuscular hemoglobin concentration. T = Treatment: a significant difference between treatments (botanically diverse pasture *vs.* botanically monotonous pasture), L = Linear time effect of grazing in botanically diverse pasture and monotonous pasture, Q = Quadratic time effect of grazing in botanically diverse pasture and monotonous pasture. Differences were considered significant at $P < 0.05$. Tendency was considered $0.05 < P < 0.1$.

FIGURE 2 Comparison of leukocyte differentiation in grazing cows on botanically diverse pasture (□) and on botanically monotonous pasture (■).

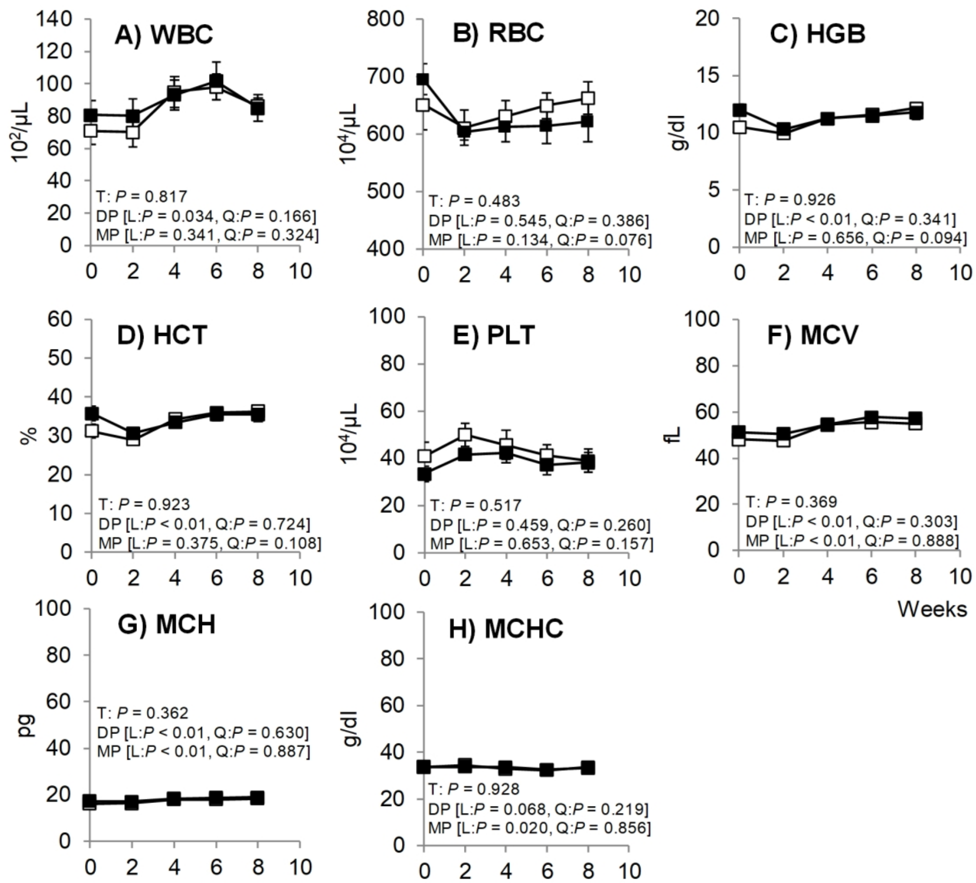
The horizontal axis shows the week after the start of grazing (day 0). Blood samples were collected on day 0 (0 week) before the start of grazing and 2 (7 to 21 days), 4 (22 to 36 days), 6 (37 to 51 days), and 8 (52 to 66 days) weeks after the start of grazing. The data are presented as the mean ± SEM. T = Treatment (botanically diverse pasture *vs.* monotonous pasture), L = Linear time effect of grazing in botanically diverse pasture and monotonous pasture, Q = Quadratic time effect of grazing in botanically diverse pasture and monotonous pasture. Differences between means with $P < 0.05$ were considered statistically significant differences, and $0.05 < P < 0.1$ was considered indicative of a trend.

FIGURE 3 Comparison of metabolic profiles in grazing cows on botanically diverse pasture (□) and on botanically monotonous pasture (■).

The horizontal axis shows the week after the start of grazing (day 0). Blood samples were collected on day 0 (0 week) before the start of grazing and 2 (7 to 21 days), 4 (22 to 36 days), 6 (37 to 51 days), and 8 (52 to 66 days) weeks after the start of grazing. The data are presented as the mean ± SEM. A) Glu = glucose, B) TCHO = total cholesterol, C) UN = urea nitrogen, D) IP = inorganic phosphorus, E) Mg = magnesium, F) Ca = calcium, G) Na = sodium, H) K = potassium, I) Cl = chlorine. T = Treatment (botanically diverse pasture *vs.* monotonous pasture), L = Linear time effect of grazing in botanically diverse pasture and monotonous pasture, Q = Quadratic time effect of grazing in botanically diverse pasture and monotonous pasture. Differences between means with $P < 0.05$ were considered statistically significant differences, and $0.05 < P < 0.1$ was considered indicative of a trend.

FIGURE 4 Comparison of oxidative stress markers in grazing cows on botanically diverse pasture (□) and on botanically monotonous pasture (■).

The horizontal axis shows the week after the start of grazing (day 0). Blood samples were collected on day 0 (0 week) before the start of grazing and 2 (7 to 21 days), 4 (22 to 36 days), 6 (37 to 51 days), and 8 (52 to 66 days) weeks after the start of grazing. The data are presented as the mean ± SEM. A) SOD = superoxide dismutase activity, B) GPx = glutathione peroxidase activity, C) TAC = total antioxidant capacity. T = Treatment (botanically diverse pasture *vs.* monotonous pasture), L = Linear time effect of grazing in botanically diverse pasture and monotonous pasture, Q = Quadratic time effect of grazing in botanically diverse pasture and monotonous pasture. Differences between means with $P < 0.05$ were considered statistically significant differences, and $0.05 < P < 0.1$ was considered indicative of a trend



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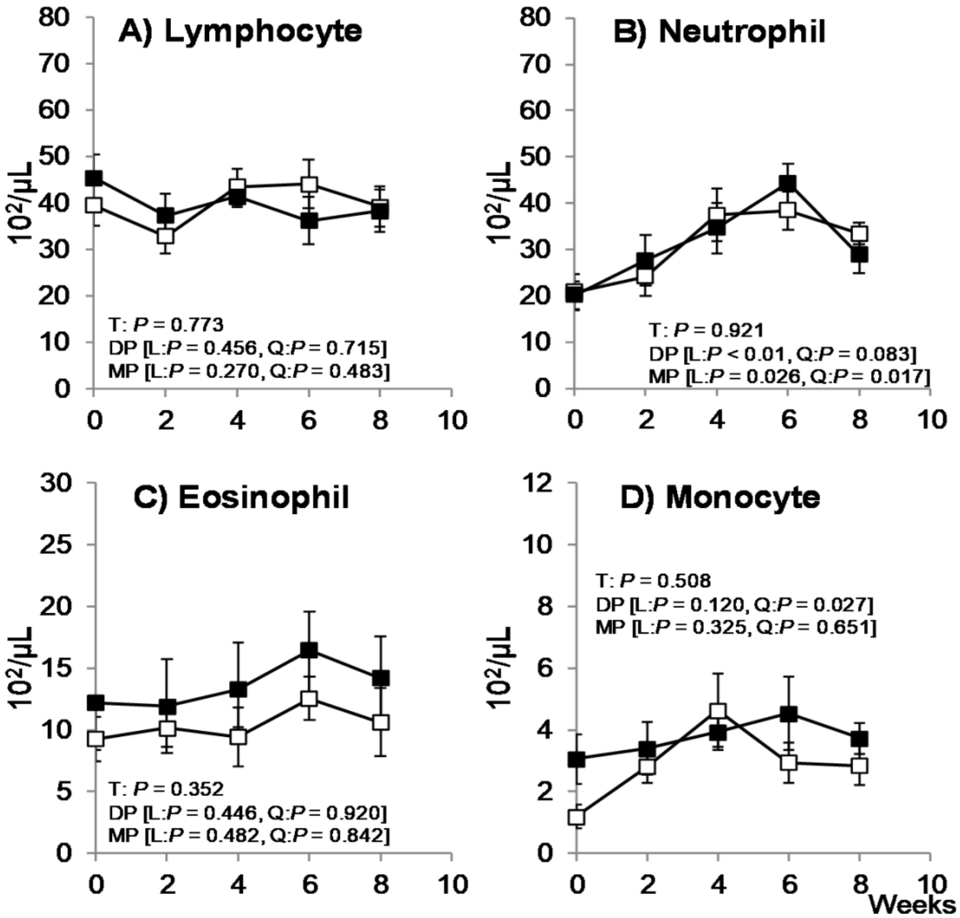

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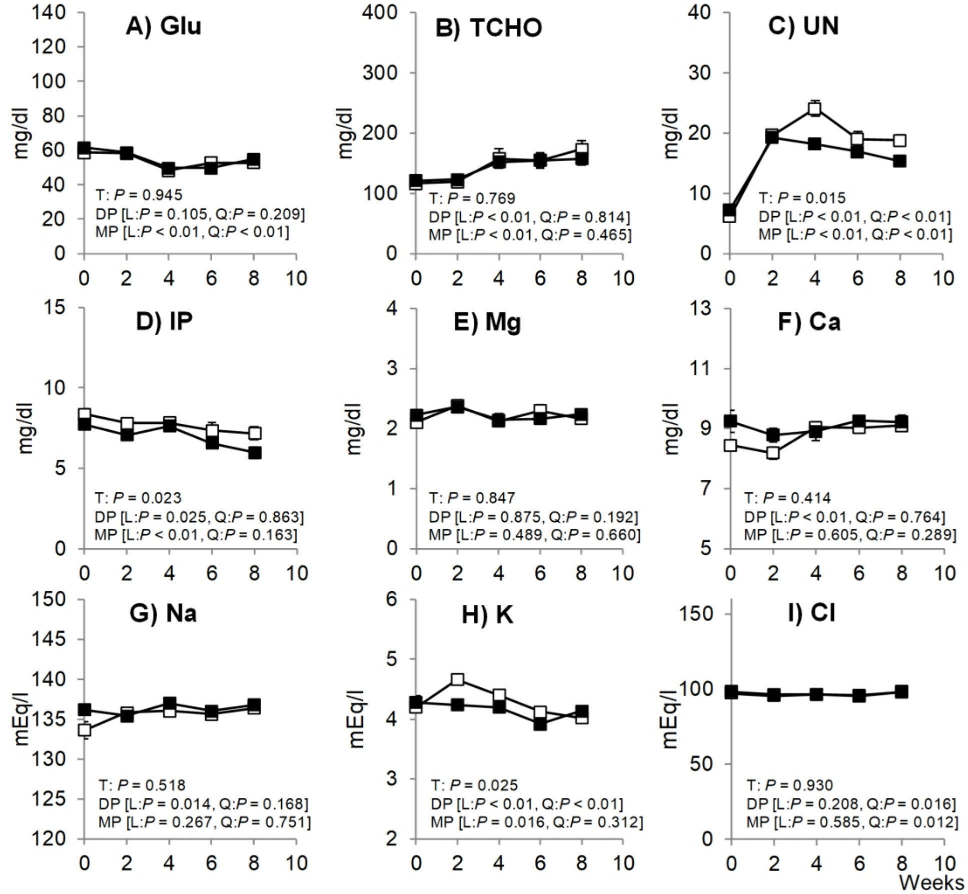

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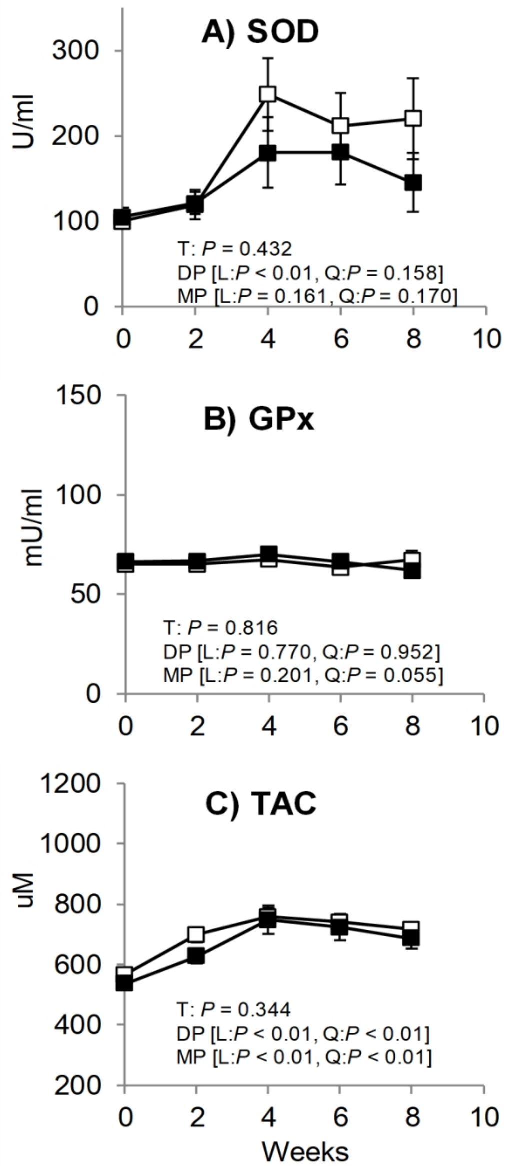

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