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Effects of feeding level on nutrient digestibility and enteric methane production in growing goats (*Capra hircus hircus*) and Sika deer (*Cervus nippon hortulorum*)

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Objective: Two experiments were conducted to determine the effects of feeding level on nutrient digestibility and enteric methane (CH_4) emissions in growing goats and Sika deer.
Methods: Three growing male goats (initial body weight [BW] of 22.4±0.9 kg) and three growing male deer (initial BW of 20.2±4.8 kg) were each allotted to a respiration-metabolism chamber for an adaptation period of 7 d and a data collection period of 3 d. An experimental diet was offered to each animal at one of three feeding levels (1.5%, 2.0%, and 2.5% of BW) in a 3×3 Latin square design. The chambers were used for measuring enteric CH_4 emission.
Results: Nutrient digestibility decreased linearly in goats as feeding level increased, whereas Sika deer digestibility was not affected by feeding level. The enteric production of CH_4 expressed as g/kg dry matter intake (DMI), g/kg organic matter intake, and % of gross energy intake decreased linearly with increased feeding level in goats; however, that of Sika deer was not affected by feeding level. Six equations were estimated for predicting the enteric CH_4 emission from goats and Sika deer. For goat, equation 1 was found to be of the highest accuracy: CH_4 (g/d) = 6.2 (±14.1)+10.2 (±7.01)×DMI (kg/d)+0.0048 (±0.0275)×dry matter digestibility (DMD, g/kg)–0.0070 (±0.0187)×neutral detergent fiber digestibility (NDFD; g/kg). For Sika deer, equation 4 was found to be of the highest accuracy: CH_4 (g/d) = –13.0 (±30.8)+29.4 (±3.93)×DMI (kg/d)+0.046 (0.094)×DMD (g/kg)–0.0363 (±0.0636)×NDFD (g/kg).
Conclusion: Increasing the feeding level increased CH_4 production in both goats and Sika deer, and predictive models of enteric CH_4 production by goats and Sika deer were estimated.

Keywords: *Capra hircus hircus*; *Cervus nippon hortulorum*; Methane; Greenhouse Gas; Feeding Level

INTRODUCTION

Enteric methane (CH_4) production in ruminants is recognized as one of the major sources of greenhouse gas emissions associated with agriculture [1]. Although a large number of goats and Sika deer inhabit East Asia [2], few studies have estimated enteric CH_4 production of goats and Sika deer. Enteric CH_4 production by ruminants is generally influenced by various dietary factors such as the forage-to-concentrate ratio [3], carbohydrate type [2,4], forage processing [5], fat addition [6], and ionophore addition [7]. Fundamentally, because enteric methane production is a part of the energy utilization process of ruminants, the amount of feed intake has been suggested as the major factor driving enteric CH_4 production. Changes of feed intake level affect passage rate and rumen fermentation characteristics and, thereby, can alter nutrient digestibility and CH_4 production of the animal [8]. Therefore, the aims of the present study were i) to determine the effects of feeding level on nutrient digestibility and enteric CH_4 production and ii) to estimate the for the respective enteric CH_4 production prediction equations for growing goats and Sika deer.

MATERIALS AND METHODS

Experiments were conducted to determine the nutrient digestibility and enteric CH_4 and CO_2 emissions in goats (*Capra hircus hircus*) and Sika deer (*Cervus nippon hortulorum*) in accordance with the Institutional Animal Care and Use Committee of Konkuk University.

Animals, diets, and experimental design

Three growing male goats (initial body weight [BW] of 22.4±0.9 kg) and three growing male deer (initial BW of 20.2±4.8 kg) were used in this study. Each animal was housed individually in a respiration-metabolism chamber as described by Li et al [9] for a diet adaptation period of 7 d and a data collection period of 3 days [10,11]. The same experimental diet was prepared and used for both goat and deer experiments (Table 1), and animals were randomly assigned to one of three feeding levels (1.5%, 2.0%, or 2.5% of BW), which were applied in a 3×3 Latin square design. Experimental diets were fed daily at 1100 h, and water and mineral block (contained 3,250 K IU/kg vitamin A, 500 K IU/kg vitamin D, 2,500 IU/kg vitamin K, 200 mg/kg vitamin B_1, 200 mg/kg vitamin B_2, 250 μg/kg vitamin B_{12}, 3,500 mg/kg niacin, 9,000 mg/kg choline chloride, 48,000 mg/kg methionine, 10,200 mg/kg glycine, 100 mg/kg β-carotene, 254 g/kg calcium, 38 g/kg phosphorus, 4,000 mg/kg manganese, 9,050 mg/kg zinc, 4,500 mg/kg iron, 200 mg/kg iodine, 45 mg/kg cobalt, 11,000 mg/kg magnesium, 2,300 mg/kg copper, 92 mg/kg selenium) were freely accessible. Sampling unit was feces which collected daily (n = 3 d/animal). Total fecal samples were collected at 1000 h and immediately dried for subsequent chemical analysis. Digestibility coefficients were calculated by the following formula: nutrient digestibility (%) = [nutrient intake (g) – nutrient in the fecal (g)]/nutrient intake (g)×100. Experimental unit was animal (n = 3 animals/treatment).

Table 1. Ingredients and nutrient composition of experiment diets

Items	Experimental diet
Ingredients (%, DM basis)	
Ground corn	25.5
Soybean meal	24.5
Tall fescue, hay	50.5
Nutrient composition	
DM (%)	89.3
OM (% DM)	92.1
CP (% DM)	16.0
NDF (% DM)	44.0
GE (MJ/kg DM)	15.9

DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fiber; GE, gross energy.

Chemical analysis

Feed ingredients and fecal samples were analyzed in duplicate for dry matter (DM), organic matter (OM), crude protein (CP), and ether extract as described by AOAC [12]. Neutral detergent fiber (NDF) was analyzed using heat stable α-amylase (Sigma A3306; Sigma Chemical Co., St. Louis, MO, USA) according to the method described by Van Soest et al [13]. Gross energy (GE) was analyzed using a bomb calorimeter (C5000; IKA, Staufen, Germany).

Gas production measurement

A respiration-metabolism chamber system was used to measure enteric CH_4 and CO_2 production [9], and a gas flow meter (GFM57, Aalborg Instruments & Controls Inc., Orangeburg, NY, USA) and a sample pump (Columbus Instruments, Columbus, OH, USA) were used to collect inlet and outlet gas samples. Collected gas samples were passed through a desiccant ($CaSO_4$) before the samples flew into the gas analyzer. A non-dispersive infrared gas analyzer (VA-3000; Horiba Stec Co., Kyoto, Japan) was used to analyze the concentrations of CH_4 and CO_2.

Statistical analysis

All variables were analyzed using SAS PROC MIXED (Version 9.2; SAS Institute Inc., Cary, NC, USA). The statistical model included treatment as a fixed effect and animal and period as random effects. The orthogonal polynomial contrast was tested using the CONTRAST statement to examine the linear and quadratic effects of feeding level on the response variables. Significant differences were declared at $p<0.05$, and trends were defined at $0.05 \le p<0.10$. SAS PROC REG (Version 9.2; SAS Institute Inc., USA) was used to estimate simple and multiple linear equations. Equations were evaluated based on the root mean square error (RMSE), adjusted-R^2, and the p-value.

RESULTS

Dry matter intake (DMI) of both goats and Sika deer increased linearly ($p<0.05$) as feeding level increased (Table 2). The DM, OM, CP, and neutral detergent fiber digestibility (NDFD) of goats decreased linearly ($p<0.05$) as the feeding level increased. However, the DM, OM, CP, and NDFD of Sika deer did not differ significantly among feeding level treatment groups.

In goats, enteric emission of CH_4 expressed as g/d and g/kg $BW^{0.75}$ increased linearly ($p<0.05$) with increasing feeding level (Table 3); however, when expressed as g/kg DMI, g/kg organic matter intake (OMI), and % of gross energy intake (GEI), it decreased linearly ($p<0.05$) with increasing of feeding level. No difference in enteric CH_4 production when expressed as g/kg digested dry matter intake (DDMI) and g/kg digested organic matter intake (DOMI) was observed among feeding

Table 2. Effect of feeding levels on nutrient digestibility in goats (*Capra hircus hircus*) and Sika deer (*Cervus nippon hortulorum*)

Items	Feeding level (% of BW)			SEM	p-value	
	1.5	2.0	2.5		Linear	Quadratic
Dry matter intake (g)						
Goats	330	454	559	11.5	< 0.001	0.665
Sika deer	316	385	508	61.1	0.025	0.361
Digestibility (%)						
Goats						
DM	77.3	70.9	67.2	1.31	0.015	0.423
OM	79.0	72.9	69.4	1.02	0.013	0.394
CP	83.2	77.9	76.3	1.37	< 0.001	0.011
NDF	67.7	58.1	54.0	2.25	0.002	0.200
Sika deer						
DM	66.9	63.5	66.7	1.59	0.891	0.152
OM	69.4	66.4	69.0	1.48	0.819	0.185
CP	77.6	76.3	77.0	2.17	0.814	0.704
NDF	53.0	47.8	53.2	2.38	0.983	0.159

BW, body weight; SEM, standard error of the mean; DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fiber.

levels. In Sika deer, enteric emission of CH_4 expressed as g/d and g/kg $BW^{0.75}$ increased linearly (p<0.05) with increasing feeding level, while it was not affected by feeding level when expressed as g/kg DMI, g/kg OMI, or % of GEI. In goats, CO_2 production expressed as g/d and g/kg $BW^{0.75}$ increased linearly (p<0.05) with increasing feeding level, and in Sika deer, enteric CO_2 production expressed as g/kg was not affected by feeding level. However, CO_2 production expressed as g/kg $BW^{0.75}$ increased linearly (p<0.01) with increasing feeding level.

For goats, the model that used the DMI, DM digestibility (DMD), and NDFD as independent variables (equation 1), was most accurate (Table 4; R^2 = 0.69, RMSE = 1.05, and p = 0.096), whereas for Sika deer, the model that used the DMI, DMD, and NDFD as independent variables, was most accurate (R^2 = 0.95, RMSE = 0.98, and p = 0.001).

DISCUSSION

The amount of feed intake is fundamentally important for animals because it directly affects animal productivity. In general, feeding level and nutrient digestibility in ruminants are negatively correlated [14,15] because a change in feeding level can alter the passage rate and fermentation pattern of the reticulo-rumen [16]. In the current experiment, the nutrient

Table 3. Effect of feeding levels on enteric methane and carbon dioxide in goats (*Capra hircus hircus*) and Sika deer (*Cervus nippon hortulorum*)

Items	Feeding level (% of BW)			SEM	p-value	
	1.5	2.0	2.5		Linear	Quadratic
Goats						
CH_4 production						
CH_4 (g/d)	8.5	10.3	11.2	0.570	0.008	0.460
CH_4 (g/kg $BW^{0.75}$)	0.83	0.99	1.09	0.050	0.010	0.642
CH_4 (% of GEI)	9.0	8.0	7.0	0.400	0.046	0.914
CH_4 (g/kg DMI)	25.7	22.7	20.1	1.13	0.047	0.912
CH_4 (g/kg DDMI)	33.4	32.0	30.0	1.90	0.283	0.872
CH_4 (g/kg OMI)	27.9	24.6	21.9	1.23	0.047	0.913
CH_4 (g/kg DOMI)	35.4	33.8	31.5	1.85	0.169	0.859
CO_2 production						
CO_2 (g/d)	351	435	462	6.90	0.001	0.020
CO_2 (g/kg $BW^{0.75}$)	34.6	41.9	45.0	1.07	0.002	0.180
Sika deer						
CH_4 production						
CH_4 (g/d)	8.1	10.2	13.3	1.810	0.004	0.448
CH_4 (g/kg $BW^{0.75}$)	0.81	1.09	1.38	0.081	0.001	0.769
CH_4 (% of GEI)	9.0	9.2	9.2	0.380	0.480	0.625
CH_4 (g/kg DMI)	25.4	26.1	26.1	1.08	0.472	0.616
CH_4 (g/kg DDMI)	38.2	41.1	39.1	1.93	0.665	0.278
CH_4 (g/kg OMI)	27.6	28.4	28.3	1.18	0.472	0.621
CH_4 (g/kg DOMI)	39.9	42.7	41.1	1.98	0.651	0.391
CO_2 production						
CO_2 (g/d)	410	410	489	68.4	0.141	0.278
CO_2 (g/kg $BW^{0.75}$)	41.7	44.4	50.7	2.17	0.006	0.228

BW, body weight; SEM, standard error of the mean; CH_4, methane; GEI, gross energy intake; DMI, dry matter intake; DDMI, digested dry matter intake; OMI, organic matter intake; DOMI, digested organic matter intake; CO_2, carbon dioxide.

Table 4. Equations for enteric methane emission from goats (*Capra hircus hircus*) and Sika deer (*Cervus nippon hortulorum*)

Items	Equations	Statistical parameters		
		R^2	RMSE	p-value
Goats				
1	CH_4 (g/d) = 6.2 (±14.1)+10.2 (±7.01)×DMI (kg/d)+0.0048 (±0.0275)×DMD (g/kg)–0.0070 (±0.0187)×NDFD (g/kg)	0.69	1.05	0.096
2	CH_4 (g/d) = 8.1 (±14.1)–10.5 (±6.43)×DMI (kg/d)–0.0039 (±0.0135)×DMD (g/kg)	0.68	0.97	0.032
3	CH_4 (g/d) = 4.6 (±1.45)+12.1 (±3.16)×DMI (kg/d)	0.68	0.90	0.006
Sika deer				
4	CH_4 (g/d) = –13.0 (±30.8)+29.4 (± 3.93) × DMI (kg/d)+0.046 (± 0.094)×DMD (g/kg)–0.0363 (0.0636)×NDFD (g/kg)	0.95	0.98	0.001
5	CH_4 (g/d) = 4.06 (±7.49)+27.8 (±2.7)×DMI (kg/d)–0.0073 (±0.0118)×DMD (g/kg)	0.95	0.92	<0.001
6	CH_4 (g/d) = –0.54 (±1.04)+27.4 (±2.47)×DMI (kg/d)	0.95	0.88	<0.001

RMSE, root mean square error; CH_4, methane; DMI, dry matter intake; DMD, dry matter digestibility; NDFD, neutral detergent fiber digestibility.

digestibility of goats decreased linearly (p<0.05) as feeding level increased. This result was consistent with the findings of previous studies using other ruminants [14,15,17]. However, interestingly, nutrient digestibility of Sika deer was not affected by increasing the feeding level. Although a few studies have determined both nutrient digestibility and the DMI of white-tailed and roe deer [18,19], to our knowledge, the effect of feeding level on digestibility has not been previously determined for Sika deer. Nutrient digestibility varies for different species of ruminants [20,21]. Galbraith et al [22] reported that OM, NDF, and ADF digestibility of white-tailed deer tend to be lower than those of Wapiti. [2,23]. According to the morphophysiological classification of Hofmann [24], Sika deer are classified as concentrate eaters. Therefore, it seems that the nutrient digestibility of Sika deer is different from that of goats and cattle because rumen capacity and retention time of Sika deer are lower than those of both intermediate-type and grass/roughage eaters. In addition, since the experimental diet of Sika deer in this study was comprised of 50% roughage sources, it may have been difficult for the deer to digest a large amount of fiber contents due to being concentrate eaters rather than to the feeding levels.

In both goats and Sika deer, enteric emissions of CH_4 expressed as g/d and g/kg $BW^{0.75}$ increased linearly (p<0.05) with increasing feeding level, and in goats, enteric production of CH_4 expressed as g/kg DMI, g/kg OMI, and % of GEI decreased linearly (p<0.05) with increasing of feeding level. These results agreed with those of studies that have found that CH_4 production (g/kg DMI) decreased with increased feeding level in cows [25,26] and sheep [27]. Because the residence time of rumen contents decreases with increased feed intake, consuming larger amounts of feed can result in shorter exposure time to rumen microbes [28,29]. However, enteric production of CH_4 expressed as g/kg DMI, g/kg OMI, and % of GEI were not affected by increasing feeding levels in Sika deer, and, as mentioned above, the morphophysiological differences of the gastro-intestinal tracts of Sika deer can affect rumen capacity and retention time [24]. Consequently, in comparison with both intermediate-type and grass/roughage eaters, it seems that the overall effect of feeding level on enteric CH_4 production can be diminished in Sika deer. In both goats and Sika deer, enteric CH_4 production expressed as g/kg DDMI and g/kg DOMI did not differ statistically. Although digestibility is difficult to measure and requires complex equipment, Na et al [3] reported that digested nutrient intake is a critical factor that explains CH_4 production in goats and Sika deer. Therefore, digested nutrient intake can be used as a CH_4 prediction factor when the experimental condition allows. Mineral salt supplementation can influence the enteric methane emission and methanogenic archaea population in rumen [30]. In the current study, mineral block intake did not measure, however, it appears that the effect of mineral block was slight because the animals can freely access to the mineral block.

The models that used nutrient digestibility as a factor exhibited the greatest accuracy in this as well as other experiments [3,31]. However, equations 3 and 4, which used the DMI as the only factor, can also be used to predict enteric CH_4 because the model without digestibility is more practical. To our knowledge, only one other study has predicted CH_4 production in Sika deer [3]. Therefore, the models evaluated in the current study contribute to our understanding of enteric CH_4 emissions in Sika deer.

CONCLUSION

Nutrient digestibility of goats decreased linearly as the feeding level increased, whereas the digestibility of Sika deer was not affected by feeding level. The enteric production of CH_4 expressed as g/kg DMI, g/kg OMI, and % of GEI decreased linearly with increasing feeding levels in goats. However, enteric production of CH_4 expressed as g/kg DMI, g/kg OMI, and % of GEI of Sika deer were not affected by feeding level.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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