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Effects of different ration scenarios on fecal-greenhouse gas emissions from awassi ewes

Farklı rasyon senaryolarının İvesi koyunlarında dışkı kaynaklı sera gazları emisyonuna etkileri

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ABSTRACT

C4 plants are known as plants with high photosynthesis ability, which can be well adapted to the sunny regions, and corn/maize is a good example. This study investigated the effect of diets with different ratios of C4:C3 plant materials on CH_4, CO_2, and N_2O emissions from manure produced by Awassi ewes. The trial compared the production of greenhouse gases from manures produced by sheep fed diets with low C4:C3 ratios (Group A–0.95:1) to those fed high C4:C3 plant ratios (Groups B–1:1; C–1.5:1; D–2:1). The trial was a randomized design with four groups and each group contained four sheep (total 16). The main feedstuffs were alfalfa and maize based combinations with similar nutrient contents. The body weight gain (BWG) and the feed conversion ratio (FCR) of the sheep were affected by C4:C3 ratio. They increased in the higher C4 plant-consuming groups, despite similar feed consumption. Manure components were not affected by the feed ratio, except for N, pH, and Neutral detergent fiber (NDF). Total diet digestibility of N increased cubically when maize silage was included (Groups B and D). The use of C3 plants as forage or grain sources led to increased manure and manure gas production (P = 0.04 and linear effect: 0.03). Regardless of the C4:C3 ratio, alfalfa increased N_2O emissions from manures because more manure was produced by groups A and C. The temperature, humidity and mold of the manures were the main causes of the rise in the amount of manure based CO_2 in the alfalfa and maize based groups.

Key Words: C3-C4 plants, Gas production, Manure, Methane, Nitrous Oxide

ÖZ

C4 bitkileri güneşli bölgelere iyi adapte olabilen ve yüksek fotosentez yeteneğine sahip bitkiler olarak bilinir ve mısır buna iyi bir örnektir. Bu çalışma farklı oranda C4:C3 bitkilerinden oluşan rasyonların İvesi koyunlarında dışkı gaz emisyonlarına etkilerini incelemek için yapılmıştır. Düşük düzeyde C4:C3 (Grup A–0.95:1) oranına sahip rasyonlardan yüksek C4:C3 oranlı (B–1:1; C–1.5:1; D–2:1) rasyonlara kadar değişen gruplarda TMR alan koyunların dışkılarından yayılan sera gazları karşılaştırıldı. Deneme her grupta 4 ferdi tekerrür olan ve 4 gruptan oluşan (toplam:16) tesadüf parselleri desenine göre düzenlendi. Rasyonlarda ana faktörler yonca ve mısır olmuştur. Canlı ağırlık değişimi ve yem dönüşüm oranı farklı C4:C3 oranlarından etkilenmiştir. Bu değerler benzer yem tüketimlerine rağmen daha yüksek miktarda C4 bitkisi tüketen gruplarda daha iyi durumda olmuştur. Dışkı içeriği, N, pH, ve NDF hariç etkilenmemiştir. Grup B ve CD'de toplam N sindirim düzeyi rasyona mısır silajı dâhil edilince artmıştır. C3 bitkilerinin kaba ve konsantre yemler olarak tercih edilmesi hem dışkı miktarını hem de dışkıdan yayılan gaz düzeyini artırmıştır(P = 0.04 ve lineer etki = 0.03). C4:C3 oranından bağımsız olarak yonca kuru otu Grup A ve C'de hem dışkı miktarını hem de dışkıdan yayılan N_2O emisyonunu artırmıştır. Ayrıca yine C4:C3 oranından bağımsız olarak sıcaklık, nem ve küf dışkıdan CO_2 salınmasını önemli düzeyde artırmıştır.

Anahtar Kelimeler: C3-C4 bitkileri, Sindirilebilirlik, Gaz üretimi, Dışkı, Metan, Nitroz Oksit

Introduction

The livestock population has increased in Turkey over the past 5 years (TUIK, 2016). This increase has been accompanied by a large rise in feces output (approximately 120 million tons) per year in Turkey. Manure directly contributes to N_2O emissions by stimulating nitrification and denitrification (Mosier et al., 1998). Although the anaerobic digestion of livestock slurry produces beneficial products, such as biogas, the production of these products is not yet economically viable in Turkey.

Ruminants excrete 75 % of the N they ingest and excess dietary N is mainly excreted in urine, but feces N excretion remains relatively constant (Castillo et al., 2000). The key factor is the content of urea in the urine. Normally, animals excrete manures via urine and dung, and their separation is difficult under normal farm conditions. Strategies to improve N cycling in the rumen environment can also lead to lower N_2O emissions. Maize silages are known to have a lower methane potential compared to grass silage (Lettat et al., 2013). They can also potentially reduce CH_4 and N_2O in manures.

High temperatures influence both yields and nutritive value, mainly through differences in the photosynthetic pathways of C3 plants (for example barley, cotton, alfalfa, wheat in the current study). C4 plants photosynthesize two times more than C3 plants. Maize is an important C4 plant and has a higher growth rate and soluble sugar contents than other forages because of its greater photosynthetic activity. The yearly average dry matter yield per hectare is more than double that of alfalfa. Furthermore, maize may be more resistant to global droughts, which is important in Sanliurfa Province, which is located in southeast Turkey and has a fairly long summer season.

Adding plant oil or improving forage quality by feeding animals with forages that contain less fiber and higher soluble carbohydrate contents, such as C4 plants like maize and maize silage, can reduce feces production and GHG emissions caused by manure (feces and urine). Cotton production is very important in southeast Turkey, which means that there is a considerable amount of cotton oil that could be used in the animal feed sector. However there is a need for an *in vivo* investigation to determine whether these oils can be used to inhibit rumen methanogens, which would reduce manure emissions.

The aim of this project was to measure greenhouse gases emissions from manures in uncovered manure stores and to determine the effect of different forages and cotton oil additions on some GHG emissions from sheep manures under normal farming conditions.

Materials and Methods

Research on animals was conducted according to institutional committee on animal use (ethical document number: KSU-2013/08-3). The experiments took place between 2 and 10 May, 2014 at the Sanliurfa province in the Southeast Anatolian Region of Turkey, which lies on 37°9’32.9364 latitude and 38°47’48.8724 longitude. The mean air temperature was 25°C, and the warmest temperature was 29°C. The average humidity ranged from 52% to 67.5%.

Animals and experimental groups

Sun-dried alfalfa hay was ground in a mill with 3–5-cm sieves. The other source of forage was maize silage that had a particle size of 3–5 cm. The diets were designed to be iso caloric and iso nitrogenous over 2 weeks. They contained the same macronutrient proportions and energy: protein balance (Table 1).

All ratios were adjusted so that the diets met the NRC (2007) requirements for sheep (Levis et al., 2007). Awassi sheep that had an average live body weight of 49.5±4.32 kg were allocated randomly into four groups based on body weight and they were 2–3 years old and 1-2 months pregnant. A total of 16 adult fat-tailed Awassi ewes Awassi females) were divided into the four groups, which meant that there were four replicates. There was one ewe per 1.5 × 1.5 m pen. The animals were housed in individual cages (one ewe per1.5 × 1.5 m cage) under a protective roof and had free access to fresh water throughout the study. They were fed separately and *ad libitum* with a total mixed ration (TMR) containing mixtures of alfalfa hay or maize silage. The trial measured the production of greenhouse gases from manures produced by sheep fed diets with a low C4:C3 ratio (A–0.95:1) and compared them to sheep fed with high C4:C3 plant ratios (B–1:1; C–1.5:1; D–2:1). Maize has a C4 photosynthetic pathway, whereas barley feeds, wheat bran, cotton oil, and cotton seed meal are derived from C3 plants. Cotton seed oil was used as the plant oil source because the region was a major cotton production center.

The sheep were fed with diets containing different ratios of C4 plant derived feeds, such as maize grain and maize silage (B–1:1 and D–2:1; Table 1). The variables measured were total manure production, manure content or its gas accumulation and methane, carbon dioxide and nitrous oxide production in manure gas, dry matter intake (DMI), and the change in body weight (kg). The experiments, which included a one-week adaptation period, lasted for two weeks.

All the sheep were weighed individually at the beginning, at the start of adaptation period, at the end of adaptation period, and during the data collection period. Feed intakes were measured daily over the adaptation period. Feed intakes and feed refusals were collected before the morning feeding and weighed. The measured intake was the difference between the total amount of feed offered and refused.

Table 1.Dietary ingredients and chemical composition

of the total mixed rations

Çizelge 1. TMR rasyonunun bileşimi ve kimyasal kompozisyonu

C4:C3	*A.0.95:1*	*B.1:1*	*C.1.5:1*	*D.2:1*
Barley *Arpa*	1.0	1.8	1.0	1.0
Corn *Mısır*	48.6	41.6	60.8	59.5
Wheat bran *Buğday kepeği*	1.3	35.9	1.0	17.7
Cotton seed meal *Pamuk tohumu küspesi*	11.7	11.0	9.0	14.4
Alfalfa hay *Yonca kuru otu*	35.7	0.0	28.1	0.0
Corn silage *Mısır silajı*	0.0	7.0	0.0	6.6
Cotton oil *Pamuk yağı*	1.6	2.0	0.0	0.0
Lime stone *Kireç taşı*	0.5	0.6	0.0	0.7
Vitamin[1]	0.1	0.1	0.1	0.1
Total	100.0	100.0	100.0	100.0
DM (%)	89	85	89	85
CP (%)	13.9	13.2	13.2	13.3
ME (Mcal kg^1)	2.5	2.5	2.5	2.5
Ca (%)	0.5	0.3	0.4	0.3
P (%)	0.4	0.7	0.4	0.5
CF (%)	12.0	6.2	10.7	5.1
ADF (%)	14.8	7.7	13.2	6.6
NDF (%)	22.2	21.9	20.4	16.6
Ether extract (%)	4.8	5.8	2.9	3.3
Ash (%)	4.3	3.8	3.9	3.2
C4 ratio (%)	48.6	48.6	60.8	66.10
C3 ratio (%)	51.3	48.7	39.1	33.14

Calculation based on NRC (2007); [1]: Each kilogram of vitamin–mineral premix provides vitamin A, 800 000 IU; vitamin D_3, 100 000 IU; vitamin E, 3000 mg; Mn, 5000 mg; Fe, 5000 mg; Cu, 1000 mg; Co, 150 mg; I, 800 mg; and Se, 150 mg; DM: dry matter, CP: crude protein, ME: metabolic energy, P: phosphorus, CF: crude fiber, ADF: acid detergent fiber, NDF: neutral detergent fiber.

Manure Sample Collection and Gas Sampling

The urine was not separated from the feces. Feces and urine were collected during the data collection period and were weighed together twice a week. A daily sample was collected, mixed thoroughly, and kept for dry matter (DM), total ash, Acid detergent fiber (ADF), NDF, and total Kjeldahl N analysis. The nitrogen content was used as an indicator of N_2O emissions. During the collection period, total feces excretion was measured by collecting the feces from a mat laid on the on the bottom of the sheep pen. The manure samples from each ewe were collected daily during the 3-day collection periods and then weighed ($kgday^1$). The pH was also recorded.

Special bottles (250 ml) were used to measure total gas production and they were connected by their glass neck to the bottles used to collect the gas. Representative samples of manure (100 or 150 g) were put in these bottles and incubated in oven at 45°C for one hour. Plastic sealed covers were placed on a side pipe with a piece of parafilm to prevent gaseous leakage during the incubation. The manures were kept in sealed bottles and the gas was sampled three times a day with syringes that had a sealed cover.

Atmospheric barometers were attached to these bottles to measure the production of emitted total gas from manure per hour (g h^1). However, after gas sampling, oxygen was allowed to enter bottles by opening the top cover and then the sampling was repeated. Total gas accumulation was estimated using the pressure values recorded by the barometers and then gas accumulation was estimated one hour later using the following formula (Petrucci et al., 2010).

$$m = PV/RT \qquad \textit{Equation (1)}$$

where m is total gas (g), P is atmospheric pressure (paschal), V is volume (cm^3), R is the natural gas constant value, and T is temperature (K). The temperature and humidity data were obtained from a meteorological center every 60 min over 24 hours. The gases were also measured from manure samples obtained from the total manure collection to establish the effect of air temperature and decay on manure release of CH_4, N_2O, and CO_2.

The CH_4, N_2O, and CO_2 contents of these samples were measured by gas chromatography (SRI Instruments - European Greenhouse Gas Chromatograph (GC) System Germany). Samples were introduced to the injection port via plastic syringes. 3 m Hayesep D packed column was used for CO_2 and CH_4 analysis and 3m Hayesep D column was used for N_2O analysis. Operating conditions for the GC were as follows: injector temperature 95 °C, column temperature 85 °C, and detector temperature 320 - 350 °C for GC.

After drying at room temperature, the other compositions of the manure samples were determined according to standard AOAC procedures (AOAC, 2016). The samples were dried in an oven at 105°C for dry matter and then ashed for total ash analysis. NDF and ADF were analyzed by an ", Macedon, NY, USA) according to Van Soestet al. (1991). After the one-week collection period, the manure samples were kept at room temperature for 7 days and gas sampling was done again to observe the effects of temperature and fungi on manures.

Apparent In Vivo Digestibility

In vivo dry matter digestibility and nitrogen balance data were collected from the feces produced by the sheep fed on the experimental diets. All the sheep were used to measure the apparent *in vivo* digestibility. The animals were kept in their pens. Before the *in vivo* studies, all the animals received their respective daily experimental ratios and had free access to water. Each experimental period lasted for 14 days, which comprised of a 7-days adaptation to the diets period and 7-days feces collection period where the total feces was collected as previously described. Gas sampling was done on feces collected with urine, and then urine was filtered. As the studies were done in female sheep, it was difficult to separate feces and urine. Because the feces of the sheep were in a pelletized structure, the urine was very small and the effect of urine was very insignificant.

The experiment was a randomized design. Data were analyzed by one-way analyses of variance and significant differences between means were determined by Duncan's multiple range tests (Statistical Package for the Social Sciences, SPSS, Version: 22.0; 2013). When the F-test was significant, single degree of freedom orthogonal contrasts were examined to determine the linear, quadratic, or cubic effects of the C4:C3 ratios. This test was also used to see if there was an interaction that was not due to the C4:C3 ratio. There were no linear, quadratic and cubic effects detected for C4:C3 ratios. Therefore, only the fungi results are reported in Table 5.

Results and Discussion

The effects of the different diets on the measured performance parameters are shown in Table 2. The results show that the C4:C3 ratio had significantly affected body weight and feed conversion ratio in all groups. These results show that the body weight change and the feed conversion ratio were affected linearly, quadratically, and cubically by the maize grain and maize silage ratios ($P<0.01$).

The addition of oil generally reduced the change in body weight gain in groups A and B and the feed conversion ratio had strong linear and quadratic effects ($P<0.01$). This was particularly apparent in silage-based groups B and D (Table 2). However the sources of the forages, oil addition, and the interaction between oil and forage sources were not significantly different in terms of feed intake ($P>0.05$).

Table 2. Some performance values in sheep fed with different C4: C3 ratios
Çizelge 2.Farklı C4:C3 oranlı rasyonlarla beslenen koyunlarda bazı performans değerleri

							Contrasts		
C4:C3	A.0.95:1	B.1:1	C.1.5:1	D.2:1	SEM	P	L	Q	C
Initial weight (kg) *Başlangıç ağırlığı*	49.6	48.8	47.5	50.0	3.0	0.93			
Feed intake (kg day^{-1}) *Yem tüketimi*	2.0	1.7	1.7	1.8	0.1	0.36	0.24	0.20	0.37
BWC (kg) *Canlı ağırlık değişimi*	-0.4^{a}	-0.0^{a}	-0.3^{a}	0.5^{b}	0.1	< 0.01	< 0.01	0.02	< 0.01
FCR *Yemden yararlanma*	-0.2^{a}	0.0^{a}	-0.14^{a}	0.2^{b}	0.08	< 0.01	< 0.01	0.02	< 0.01

BWC: Body weight change, FCR: Feed conversion ratio, ^{a,b,c} **means in the same row with different superscripts differ significantly, SEM: Standard error of the mean; L: Linear; Q: Quadratic; C: Cubic effects.**

There were no significant differences in DM, ash, and ADF contentsof manure between any of the groups. There were significant changes in N, pH, and NDF contents in the manures (Table 3). Manure pH increased linearly and quadratically as the C4 plant content in the feed rose. The NDF did not change linearly as the C4:C3 proportion rose but it did change quadratically (Q= 0.02) or cubically (C= 0.05) with the C4:C3 ratio.

Table 3. Chemical compositions of manure and In vivo digestibility (%) of the dry matter and N in the different group diets
Çizelge 3.Dışkının kimyasal bileşimi ve gruplarda in vivo kuru madde ile N sindirilebilirliği (%)

							Contrasts		
C4:C3	A.0.95:1	B.1:1	C.1.5:1	D.2:1	SEM	P	L	Q	C
DM (%)	46.8	52.4	51.8	51.7	4.8	0.72	0.37	0.26	0.20
N (%)	1.3^{b}	1.01^{ab}	0.6^{a}	1.1^{ab}	0.1	0.05	0.33	0.04	0.06
Ash (%)	7.5	9.5	12.9	7.9	1.5	0.39	0.51	0.16	0.17
pH	6.5^{a}	8.3^{ab}	7.9^{b}	8.1^{b}	0.4	< 0.01	0.03	0.03	0.04
ADF (%)	50.2	51.66	53.4	42.3	2.7	0.16	0.28	0.10	0.17
NDF (%)	58.0^{ab}	63.3^{b}	59.7^{ab}	49.4^{a}	2.1	0.05	0.09	0.02	0.05
In vivo digestibilities *In vivo* sindirilebilirlik									
OM (%)	70.3	68.8	66.7	69.7	3.3	0.86	0.75	0.74	0.86
N (%)	68.7^{a}	68.0^{a}	82.6^{b}	78.2^{b}	2.0	< 0.01	0.02	0.04	< 0.01

DM: dry matter, OM: organic matter, SEM: Standard error of the mean; L: Linear; Q: Quadratic; C: Cubic effects

Adding cotton oil to the different C4:C3 ratio diets did not affect OM digestibility (Table 3) during the data collection phase. However, total diet digestibility of N increased linearly, quadratically, and cubically (P< 0.01) as theC4:C3 ratio rose after the inclusion of maize silage (Groups B and D).

Finally, daily manure output and total gas accumulation were affected by the C4:C3 ratios (Table 4) because orthogonal comparisons were significant for daily feces output (P<0.05) or showed a strong trend for daily gas consumption against the C4:C3 ratio. Significant C4:C3 ratio linear effects were observed (P=0.03) for manure output and there was a slightly insignificant effect on total gas accumulation (P = 0.06). Feces output was higher in sheep fed alfalfa hay in groups A and C according to the cubic effect results, although no interaction analysis was performed between the alfalfa and maize groups.

The feed ratios had no significant effects on CH_4, CO_2,and N_2O emissions per ml gas syringed from the manures (P>0.05). However, there was a small linear downward trend (P=0.07) in the production of hourly CO_2 (mg hour manure^{-1}) as the C4:C3 ratio increased (Table 4). Total GHG emissions, including CO_2 (slightly tendency) and N_2O (P=0.03), from the daily feces and urine samples were higher in the alfalfa groups (A and C) than in the maize silage groups.

Table 4. Manure outputs and GHG gas distribution by the different treatments and GHG emissions from the total manure samples

Çizelge 4.Dışkı miktarı ve gruplarda toplam dışkıdan yayılan sera gazı emisyon dağılımları

							Contrasts		
C4:C3	A.0.95:1	B.1:1	C.1.5:1	D.2:1	SEM	P	L	Q	C
Manure output (g day^{-1}) *Dışkı miktarı g gün^{-1}*	1102^{b}	879^{ab}	1014^{ab}	768^{a}	93.0	0.04	0.03	0.14	0.08
Total gas (g day m^{-1}) *Günlük dışkıdan çıkan toplam gaz*	402^{b}	169^{a}	317^{b}	130^{a}	68.9	0.06	0.06	0.15	0.09
CH_4 (ppm ml^{-1})	1.6	1.8	1.6	2.0	0.2	0.58	0.40	0.65	0.58
CO_2 (ppm ml^{-1})	539	467	494	420	51.2	0.25	0.08	0.24	0.25
N_2O (ppm ml^{-1})	11.9	3.4	19.1	11.7	6.1	0.41	0.63	0.86	0.41
CH_4 (mg h^{-1}M)	0.0007	0.0006	0.0006	0.0009	0.0001	0.70	0.59	0.35	0.52
CO_2 (mg h^{-1} M)	0.52	0.49	0.42	0.40	0.03	0.30	0.06	0.12	0.11
N_2O, (mg h^{-1} M)	0.004	0.006	0.02	0.009	0.003	0.42	0.30	0.37	0.42
CH_4 (ppm T.M^{-1})	636^{ab}	247^{a}	828^{b}	262^{a}	154	0.09	0.12	0.20	0.11
CO_2 (ppm T.M^{-1})	217029^{ab}	80498^{a}	265883^{b}	54323^{a}	49677	0.06	0.03	0.09	0.04
N_2O(ppm T.M^{-1})	4794^{bc}	573^{a}	6079^{c}	1529^{ab}	1394	0.03	0.38	0.61	0.03

^{a,b,c} means in the same row with different superscripts differ significantly. M: manure, T.M: daily total manure, h: hour; SEM: Standard error of the mean, L: Linear, Q: Quadratic, C: Cubic effects.

Seasonal variations in gas emissions were also observed and the results revealed that air humidity, temperature, and decay also played an important role because they led to high fungi concentration in the manure (Table 5 and Figure 1). Carbon dioxide emissions measured at the end of digestion process (decaying) ranged from 5000 to 5350 ppm ml^{1}, which were higher than the first fresh manures (P<0.01).

Table 5. The effects of fungi on the slurries and the emissions of specific GHG gases from Awassi sheep

Çizelge 5.İvesi koyunlarının dışkılarında küflenmenin bazı spesifik gaz emisyonlarına etkileri

	Alfalfa based *Yonca bazlı*		Maize based *Mısır bazlı*			P		
	Fresh *Taze*	Moldy *Küflü*	Fresh *Taze*	Moldy *Küflü*	SEM	Forages *Kaba Yem*	Moldy *Küflenme*	Forages*Moldy *Kaba Y*Küflenme*
CH_4 (*ppm* ml^{-1})	1.6	1.9	1.8	1.7	0.19	0.84	0.69	0.46
CO_2 (*ppm* ml^{-1})	519^{a}	5357^{b}	439^{a}	5062^{b}	90.6	0.16	< 0.01	0.42
N_2O (*ppm* ml^{-1})	15.5	14.0	8.5	4.2	4.2	0.16	0.62	0.81

Data not shown for the C4:C3 ratio and fat addition because the results were insignificant.

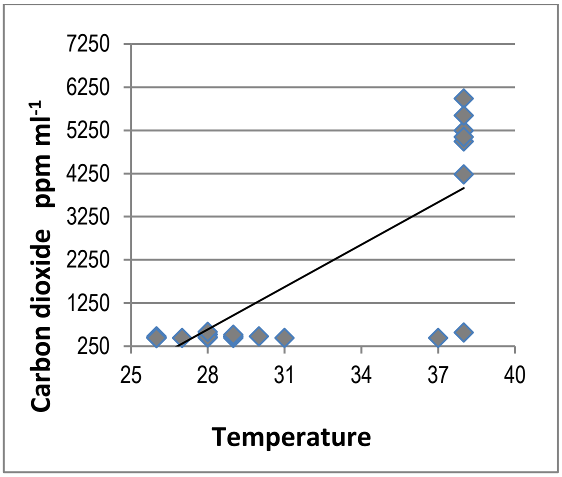

Figure 1. CO_2 emissions from manure at different temperatures

Şekil 1.Farklı sıcaklıklarda dışkıdan CO_2emisyonu

Manure output increased as the alfalfa concentration increased. Weiss et al. (2009) suggested that feces output increased as digestibility, dietary starch concentrations, and metabolic protein decreased. When maize silage and starch were included in the ration, the manure output decreased significantly. Dried alfalfa is known to reduce feed digestibility because it increases fiber content, especially NDF, compared to maize silages. This result was confirmed by the reduction in manure NDF content in silage-based groups (B and D). The NDF didn't change linearly, but it changed quadratically or cubically as the C4:C3 ratio rose. This change seems to be related to the oil-free diets in groups C and D.

In this study, all nutrient digestibility were not measured in the 16 sheep consuming maize silage, and alfalfa based diets, but the results did show that the excessive excretion of manure in the alfalfa based groups was caused by a decrease in diet digestibility. Furthermore, the feed intakes were very similar in all groups and therefore, the negative effect of alfalfa on digestibility was clearer, which suggests that silage feeding is an important strategy for reducing the amount of feces produced.

Substituting maize (C4 plant) in the diets up to 2:1 on a DM basis did not affect specific manure gas emissions (CH_4, CO_2, and N_2O), but linearly ($P < 0.01$) improved body weight, feed conversion efficiency, and decreased manure production. The change in body weight and the feed conversion ratio were affected linearly ($P<0.01$), quadratically ($P=0.03$), and cubically ($P<0.01$), which showed that this change was due to the alterations in the maize component and the C4:C3 ratio.

Daily total gas accumulation values calculated for the manure decreased due to the higher C4 ratio. Total GHG emissions, including CO_2 (slight tendency $P=0.06$) and N_2O ($P<0.05$) from daily feces and urine, were higher in the alfalfa groups (A and C) than for maize silage. This was mainly due to a corresponding increase in manure output from these groups. In this study, it was not clear whether the CH_4, CO_2,and N_2O were from feces or urine. However, the objectives of the study were to determine specific GHG emissions from feces and urine together. Weiss et al. (2009) observed that the amount of NH_3-N produced per gram of manure decreased as the alfalfa content in the diet increased because excreted N shifted from urine to manure. This is important because the C4 plant, whole-crop maize (*Zea mays L.*) silage, is an important dietary component of intensively managed ruminants and provides a reliable source of roughage, has high energy content, and can be consumed in large quantities in arid regions. However, it is well known that microbial degradation can lead to the production of gases that then end up in the stall air (Czepiel et al., 1996).

According to Caswell and Reed (1976), C4 plants are resistant to bacterial degradation *in vitro* because these plants concentrate their protein in highly-vascularized bundle sheath cells. Ehleringer and Monson (1993) suggested that C4 grasses have a lower dry matter digestibility (DMD) than their C3 counterparts. In this study, the C4 diets were significantly more digestible than the C3 diets in terms of nitrogen (mean *in vivo* N digestibility of %80.4), which is probably due to maize energy efficiency. Sponheimer et al. (2003) suggested that apparent nitrogen digestibility was higher for some species, such as goats, llamas, and rabbits, if they are fed on C3 grass rather than C4 grass. However, this experiment did not investigate these species and grasses. Our results are for maize, which can be evaluated as grain or silage rather than as a grass.

The individual values for rumen pH were not measured and daily mean fecal pH values obtained ranged from 6 to 8, which are considered normal for sheep fed on alfalfa or maize diets. Furthermore, none of the animals in this study were at risk from ruminal acidosis. It was expected that the rapid fermentation of readily available carbohydrates in maize silages may reduce ruminal and manure pH. However, this study showed that a ration combination containing maize grain and maize silage led to manure with a higher pH and N. Hassanat et al. (2017) observed that a decline in manure N excretion and a shift in N excretion from urine to feces when corn silages were used in the diet reduced manure N volatilization.

The results of this study suggest that maize silages can reduce GHG emission because they decrease total gas producing fermentative processes. Furthermore, lower manure production in silage fed sheep can lead to lower CH_4, CO_2, and N_2O production. It has been shown that increasing the maize silage component compared to alfalfa could contribute to lower manure production and lower GHG production in extreme climatic conditions. According to Kissinger et al. (2007), feed ration choices may affect the quantity of nutrients in the manure. The generation of N_2O depends on the total nitrogen content of the manures as well as the temperature, pH, and O_2in the feces.

Table 3 shows that manures obtained in this experiment had a variable N content; and that the greatest differences in manure pH were observed between the 0.95:1 group and the other groups. The manures of sheep fed silage-based diets emitted less total gas, but the result was insignificant (P=0.07). This was probably due to the reduction in manure pH as the C4:C3 ratio increased. Hristov et al. (2013) suggested that the inhibition of nitrification was related to pH and our results indicate a significant shift in N from N_2O to NH_3as the maize silage in diets increased. Ammonia gas is undesirable on farms, but it is not a greenhouse gas. Although statistically significant differences were also observed for pH; there was also an interaction between forage and oil addition, and total gas production. This interaction was probably due to the quadratic effect because the additional fat in the cotton oil might have affected rumen fermentation when it was added to starch-rich based diets (Chung et al., 2011). There is considerable evidence that clearly shows that oils are an important carbon source. The increase in CO_2 emissions that we observed in the oil-fed groups may be due to a significant increase in the carbon load (Table 4). There was a linear increase tendency in CO_2 emissions per hour (P=0.06) in the lower C4:C3 groups and this result may also be related to a significant carbon loading from the cotton oil in groups A and B. However, CO_2 emissions were higher in groups A and C when the total manure or fecal matters were measured, and this is entirely related to the amount of manure in these groups.

The generation of CH_4 is closely connected with a lack of O_2 in the feed substrate (Hellebrand et al., 2001). In this study, the CH_4 emission was not measured in a completely closed system. Due to the necessity of providing normal conditions, the bottles were always open, except when the manures were in the bottles during each sampling period. Wulf et al. (2002) explained that after a field application of manure, GHG emissions were dominated by N_2O and NH_3, whereas CH_4 was of minor importance. Ramin and Huhtanen (2013) predicted that there would be a slight decrease in CH_4 production when the crude protein (CP) concentration in the diet increased, but concluded that the impact of dietary CP on CH_4 production is quantitatively small. Møller et al. (2014) evaluated the effect of fat level on dairy cow diets, and the manure composition and methane (CH_4) potential from manure. The fat was supplied along with rapeseed in the diet. They found that the fat level had a significant influence on manure composition and CH_4 yield. We found that the cotton oil level had no significant influence on manure composition and CH_4 yield. This was probably due to an oxygenated environment. In our study, after gas sampling, oxygen was allowed to enter when the bottles were opened for N_2O measurement.

Relationship between air temperature and GHG emissions

Carbon dioxide releases measured at the end of digestion process (decaying) ranged from 5000 to 5350 ppm ml^{-1}, which were higher than for fresh manures. Manure CO_2 concentration did not fall below 5000 ppm ml^{-1}, which was its equilibrium value after it had been left to digest for two weeks (Figure 1). Carbon dioxide production in the manures probably increased after defecation because carbon emissions are dominated by CH_4, which was probably due to an oxygenated environment.

Temperature and humidity are the main factors that lead to a rise in fungi and carbon dioxide. After the one-week collection period, the manure samples were kept in a room for 7 days and gas sampling was undertaken to measure the effect of temperature and fungal growth on manures. The results revealed that gas emission varied over time and that air temperature and decay also played important roles because they increased the fungi concentration in the manure (Table 5).

During the sampling period, the average ambient temperature changed from 26 to 39°C as the manure decayed, but there were no differences in N_2O and CH_4 production. Therefore, the temperature and fungi concentration in the manure stimulated gas emissions, especially carbon dioxide production (ppm ml^{-1}), and the total waiting time was not related to the forage sources and cotton oil addition. However, the changes in CH_4 and N_2O were not significantly different (Figure 1).

Under storage conditions, considerably more CH_4 emissions may occur because during anaerobic fermentation, organic matter is degraded to CH_4. The experimental conditions were not fully anaerobic and considerably more O_2 was available to the manure than would be expected under storage conditions. This study showed that methane and carbon dioxide emissions from manures may be independent of the rumen environment and direct inhibition by methane producing bacteria. Johnson et al. (2000) suggested that only up to %1 of the CH_4emissions from the excreta of grazing cattle are due to anaerobic conditions. Therefore in ruminant production systems, the CH_4 emissions from animal excreta are often neglected.

Carbon dioxide emissions from feces depended on experimental storage conditions and were highest from moldy manures in the later stages of the experiment. Although there have only been a few studies on CH_4 emissions from outdoor manures, our results suggest that manures are not important sources of methane under outdoor conditions and they do not pose a risk to the environment.

Replacing corn silage with alfalfa hay as the forage component in sheep rations can lead to significant reductions in CO_2 emissions from livestock production. According to Hawkins et al. (2015), this change leads to the capture and storage of C on the farm. Perennial plants exploit more soil organic matter in arid regions, such as the Sanliurfa region. This means that alfalfa hay can be largely replaced by maize silages and that this will lead to a reduction in manure output. Furthermore, maize will become more important in future drought-prone areas.

Conclusion

The main objective of this study was not to promote maize grain and maize silages, but “to promote the perception about maize as an important C4 plant against global warming in the future”. Mitigation of GHG emissions can be achieved because manure output falls when sheep consume maize silage. In conclusion, CH_4 production from manure occurs mainly during storage, and cotton oil addition does not reduce manure N_2O emissions.

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References

AOAC - Association of Official Analytical Chemistry. 2016. Official methods of analysis. 20th ed. AOAC International, Washington, DC, USA

Castillo, A.R., Kebreab, E., Beever, D.E., and France J. 2000. Review of efficiency of nitrogen utilization in lactating dairy cows and its relationship with environmental pollution. *Journal Animal and Feed Science* 9:1-32.

Caswell, H., and Reed, F.C. 1976. Plant–herbivore interactions: the indigestibility of C4 bundle sheath cells by grasshoppers. *Oecologia* 26: 151–156.

Chung, Y.E., He, M.L., McGinn, S.M., McAllister, T.A., and Beauchemin, B.A. 2011. Linseed suppresses enteric methane emissions from cattle fed barley silage but not from those fed grass hay. *Animal Feed Science and Technology* 166: 167321-329.

Czepiel, P., Douglas, E.,Harriss, R., and Crill, P. 1996. Measurements of N_2O from composted organic wastes. *Environmental Science and Technology* 30: 2519–2525.

Ehleringer, J.R., and Monson, R.K. 1993. Evolutionary and ecological aspects of photosynthetic pathway variation. *Annual Review of Ecology and Systematics* 24: 411–439.

Hassanat, F.,Gervais, R., and Benchaar, C. 2017.Methane production, ruminal fermentation characteristics, nutrient digestibility, nitrogen excretion, and milk production of dairy cows fed conventional or brown midrib corn silage. *Journal of Dairy Science* 4:2625–2636.

Hawkins, J., Weersink, A., Wagner-Riddle, C., and Fox, G. 2015.Optimizing ration formulation as a strategy for greenhouse gas mitigation in intensive dairy production systems. *Agricultural Systems* 137: 1–11.

Hellebrand, H.J., and Kalk, W.D. 2001.Emission of methane, nitrous oxide, and ammonia from dung windrows. *Nutrient Cycling in Agroecosystems* 60:83–87.

Hristov, A.N., Oh, J., Lee, C., Meinen, R., Montes, F., Ott, T., Firkins, J., Rotz, A., Dell, C., Adesogan, A., Yang, W., Tricarico, J., Kebreab, E., Waghorn, G., Dijkstra, J., and Oosting, S. 2013. Mitigation of greenhouse gas emissions in livestock production. P.25-26. In: A review of technical options for non-CO_2 emission. FAO, Rome, Italy. Gerber, P., Henderson, B., Makkar, H.

Johnson, D.E., Johnson, K.A., Ward, G.M., and Braine, M.E. 2000. Ruminants and other animals p.112-133. In: *Atmospheric Methane*: Its role the atmospheric environment. Springer-Verlag Berlin Germany. Khalil, M.A.K.

Kissinger, W.E., Koelsch, R.K., Erickson, G.E., and Klopfenstein, T.J. 2007.Charecteristics of slurry harvested from beef cattle feedlots. *Applied Engineering in Agriculture* 23(3):357-365.

Lettat, A., Hassanat, F., and Benchaar, C. 2013. Corn silage in dairy cow diets to reduce ruminal methanogenesis: effects on the rumen metabolically active microbial communities. *Journal of Dairy Science* 96(8):5237-5248.

Levis, A.J.,Jonker, J.S.,Jameison, D.L.,Arieti, R.S., and Tsai, P. 2007. Nutrient requirements of sheep tables. p. 244. In: NRC. Nutrient Requirements of small ruminants. National Academy Press. Washington DC, USA. Paula, T. Whitacre.

Møller, H.B.,Moset, V.,Brask, M.,Weisbjerg, M.R., and Lund, P. 2014. Slurries composition and slurry derived methane yield from dairy cows: influence of diet with focus on fat supplement and roughage type. *Atmospheric Environment* 94: 36–43.

Mosier, A.,Kroeze, C.,Nevison, C.,Oenema, O.,Seitzinger, S., and Cleemput, O.V. 1998.Closing the global N_2O budget: nitrous oxide emissions through the agricultural nitrogen cycle. *Nutrient Cycling in Agroecosystems* 52: 225–248.

Petrucci, R.H., Herring, G., Madura, J., and Bissonnette, C. 2010. Thermo chemistry. Chapter 7.p.282. In: Petrucci, R.H. (eds) General Chemistry. Principles and modern applications with mastering chemistry. 10th ed. Canada. Published by Pearson. Petrucci, R.H.

Ramin, M., and Huhtanen, P. 2013. Development of equations for predicting methane emissions from ruminants. *Journal of Dairy Science* 96: 2476–2493.

Sponheimer, M., Robinson, T., Roeder, B., Hammer, J., Ayliffe, L., Passey, B., Cerling, T., Dearing, D., and Ehleringer, J. 2003. Digestion and passage rates of grass hays by llamas, alpacas, goats, rabbits, and horses. *Small Ruminant Research* 48: 149–154.

TUIK, 2016.Turkish Statistical Institute. Animal production statistics. Hayvansal üretim istatistikleri. T.C. Kalkınma Bakanlığı. *Özel ihtisas komisyon raporu* (In Turkish). Ankara. Turkey

Van Soest, P.J., Robertsoni, J.B., and Lewis, B.A. 1991.Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* 74: 583-3597.

Weiss, W.P., Willet, L.B., St Pierre, N.R., Borger, D.C., Mckelvey, T.R., and Wyatt, D.J. 2009. Varying forage type, metabolizable protein concentration, and carbohydrate source affects slurry excretion, slurry ammonia, and nitrogen metabolism of dairy cows. *Journal of Dairy Science* 92: 5607–5619.

Wulf, S., Maeting, M., and Clemens, J. 2002. Effect of application technique on the emission of trace gases (NH_3, N_2O, CH_4) after spreading co fermented slurry on arable and grassland. Part 2. GHG emissions. *Journal of Environmental Quality* 31: 1795-1801.