Effects of different levels of lespedeza and supplementation with monensin, coconut oil, or soybean oil on ruminal methane emission by mature Boer goat wethers after different lengths of feeding

Ryszard Puchala, Shirron LeShure, Terry A. Gipson, Kesete Tesfa, Michael D. Flythe and Arthur L. Goetsch

American Institute for Goat Research, Langston University, Langston, OK, USA; Forage Animal Production Research Unit, ARS, USDA, Lexington, KY, USA

ABSTRACT
Mature Boer goat wethers were supplemented with 0.5% BW rolled corn and consumed pelleted alfalfa (CON), pelleted Sericea lespedeza (HSL; 6.4% condensed tannins), a 1:1 mixture of alfalfa and lespedeza (MSL), or alfalfa with monensin (ION; 22 mg/kg), coconut oil (CCO; 4%), or soybean oil (SBO; 4%). Total DM intake in the 20-wk study (3.86%, 3.75%, 3.52%, 3.69%, and 3.64% BW) and total tract OM digestibility determined every 5 wk (72.8%, 69.5%, 70.3%, 72.0%, and 71.1%) were not affected by treatment, although there were differences in nitrogen digestion (77.5%, 70.7%, 67.0%, 77.0%, 75.7%, and 73.6% for CON, MSL, HSL, ION, CCO, and SBO, respectively; SEM = 1.76). Ruminal methane emission was not influenced by period and was lowest among treatments for CON expressed as percentages of gross (10.3%, 6.8%, 6.3%, 7.2%, 6.5%, and 6.5%; SEM = 0.35) and digestible energy (14.8%, 10.2%, 9.3%, 10.6%, 9.8%, and 10.1% for CON, MSL, HSL, ION, CCO, and SBO, respectively; SEM = 0.62). In conclusion, both levels of lespedeza elicited similar depressions in ruminal methane emission, with a magnitude of change similar to that of an ionophore and coconut and soybean oils, and effects did not vary with week of the study.

1. Introduction
A commonly cited proportion of the total greenhouse gas radiative force attributable to methane is 20% (IPCC 2001). Of this 20%, based on a review of the literature, Bodas et al. (2012) concluded that domesticated ruminants account for 15%–33%. Therefore, ruminant livestock make a substantial contribution to anthropogenic greenhouse gas emission and, thus, climate change. A number of means to decrease methane emission by domesticated ruminant livestock have been studied. Various additives and natural feed ingredients have received attention, one of the earliest being ionophores (Chalupa 1980). Moreover, in some cases consumer pressure has caused shifts from antibiotics towards ‘natural’ alternatives. These include essential oils, oils or fats high in medium-chain or long-chain polyunsaturated fatty acids (FA), saponins, organosulfur compounds, tannins (Wallace et al. 2002; Makkar 2003; Goel and Makkar 2012), and other plant secondary metabolites (Bodas et al. 2012). With the banning of antibiotic use in livestock production in some countries, research with such substances will likely increase in the future, and it is possible that some modes of action may be shared with more studied ionophores.

In recent experiments with sources of lespedeza high in condensed tannins (CT) (Animut et al. 2008a, 2008b; Puchala et al. 2012a, 2012b), the magnitude of decrease in ruminal methane emission (29%–52% relative to digestible energy intake) was greater or in the high range of findings of CT studies reviewed by Goel and Makkar (2012). However, it is unclear if, over time, there is microbial adaptation to CT resulting in a lessening or disappearance of the effect on ruminal methane emission. There have been different ways suggested by which adaptation to plant secondary metabolites such as CT might occur, including bacterial modification or degradation of CT, changes in saliva composition for increased CT binding and reduced ruminal activity, development of a protective layer around bacterial cells and other cell membrane modifications lessening CT effects (Makkar 2003; Bodas et al. 2012), dissociation of CT-substrate complexes, metal ion sequestration, and inactivation of high-affinity binders (Smith et al. 2005).

In general, ionophores cause shifts from Gram-positive to negative bacteria in the rumen that are associated with decreases in the acetate-to-propionate ratio. In the review of McAllister et al. (1996), this was described by greater inhibition of bacteria producing hydrogen and formate than ones yielding succinate and propionate. Decreased methanogenesis is an indirect effect relating to decreased hydrogen availability for methanogens because of the greater degree of reduction of propionate vs. acetate. Monensin also can lessen numbers of protozoa and methanogenic bacteria in symbiotic association (Tomkins et al. 2015). Protozoa produce hydrogen, and there is less hydrogen available for methanogenesis when they are inhibited. Some research has indicated that adaptation to ionophores occurs so that with advancing time the effect on ruminal methane production disappears (Rumpler et al. 1986; Sauer...
et al. 1998; Guan et al. 2006), and McAllister et al. (1996) cited additional experiments supporting adaptation. Conversely, other studies have shown continued impact without adaptation (Davies et al. 1982; Rogers et al. 1997; Odongo et al. 2007). Specific modifications of ruminal microbial conditions by ionophores that may be responsible for adaptation when it appears to have occurred have not been delineated (Odongo et al. 2007). However, Johnson and Johnson (1995) suggested that adaptation could be more likely with diets high vs. low in available energy, in accordance with a longer effect of monensin on methane emission by beef cattle consuming a low- vs. high concentrate diet noted by Guan et al. (2006). Hence, a forage-based diet could be conducive to ionophore effects on ruminal methane emission that would not lessen with advancing time via microbial adaptation.

Inclusion of various sources of fats and oils in ruminant diets can lessen ruminal methane emission, although effects vary considerably because of the many different sources (McAllister et al. 1996). The nature of the diet is very important as well, such as levels of fibre influencing capacity for adsorption, rather than interaction with microbial cells, and calcium available for formation of inert soaps (Patra 2013). General modes of action of fat sources on ruminal methane emission are known, as described by McAllister et al. (1996), Machmüller (2006), and Patra (2013). Long-chain polyunsaturated FA may have a minor indirect effect by providing a hydrogen sink and decreasing availability to methanogens. Both medium-chain FA and long-chain polyunsaturated FA appear to have direct effects on methane emission by decreasing the number and activity of methanogenic bacteria, although specific actions have not been elucidated. Conversely, McAllister et al. (1996) attributed effects of long-chain FA on methane emission to toxicity to methanogenic and gram-positive and protozoa. Likewise, there is conflicting data concerning the involvement of protozoa in the methane-depressing effect of coconut oil (Machmüller 2006; Patra 2013). As addressed for CT and ionophores, adaptation over time for diminishing effects of medium-chain and long-chain polyunsaturated FA on ruminal methane emission are possible and have not received much attention (Patra 2013).

A primary objective of the experiment was to evaluate effects of different levels of a forage source containing Sericea lespedeza (Lespedeza cuneata) that is high in CT on ruminal methane emission by meat goats relative to potential changes with inclusion of an ionophore and oils high in medium-chain and long-chain polyunsaturated FA (i.e. coconut and soybean, respectively). A second goal was to determine if the magnitude of any such effects would diminish with different lengths of feeding relating to potential adaptation processes.

2. Materials and methods

2.1. Animals, periods, and housing

The experiment was approved by the Langston University Animal Care and Use Committee. The study was 140 days in length, with four 5-wk periods. Thirty-six Boer goat wethers were used, 18 of which were approximately 2.5 yr of age at the beginning of the experiment and 18 that were 3.5 yr old. Three wethers of each age were assigned randomly to the six treatments. Two wethers of each treatment, one or two of the two ages, were assigned to three measurement sets of 12 animals that started the experiment sequentially at 2-wk intervals. During the first 6 wk of the periods, wethers in treatment groups resided in six 6.1 × 5.6 m pens in an enclosed building that had a 6.1 × 1.35 m area with a concrete floor and a 6.1 × 4.25 m unpaved floor area. The six pens with Calan gate feeders (American Calan Inc., Northwood, NH, USA) were aligned in a row adjacent to one another. With diets potentially differing in palatability and similar environmental conditions among pens, the same diet was fed in a pen to avoid problems with wethers attempting to gain access to feeders other than their own. In wk 7 wethers were situated in 0.7 × 1.2 m metabolism crates, with ‘training’ head boxes in the first day for adaptation to later conditions of measurements with the calorimetry system. During the subsequent 6 days feces and urine were collected and animals were cycled in groups of four into a room with metabolism cages fitted with headboxes of a respiration calorimetry system for 2-day periods.

2.2. Diets

Treatments entailed feeding of two different forages with four concentrate supplements. One forage was dehydrated alfalfa (Medicago sativa; AL) pellets (Stillwater Milling, Stillwater, OK, USA; AL) and the other a pelleted source of hay high in Sericea lespedeza (SL) but also containing some warm season grasses. The lespedeza hay was purchased from a farm in central Arkansas. Approximately 1 month before harvest it appeared that the level of lespedeza would be high, but based on the analyzed CT concentration compared with that in previous studies (Animut et al. 2008a, 2008b; Puchala et al. 2012a, 2012b), apparently the level of grass had increased. However, Muir et al. (2017) reported total CT levels of 6.1%–10.0% in Sericea lespedeza harvested at five locations in the southeastern USA.

The Control treatment (CON) was AL supplemented with rolled corn at approximately 0.5% BW (DM). A medium SL treatment (MSL) entailed a 1:1 (as fed) mixture of AL and SL and a high SL treatment (HSL) consisted of feeding SL alone, both with rolled corn supplementation. Other treatments were feeding AL with monensin (Rumensin 90°; Elanco, Greenfield, IN, USA) added to rolled corn for a feeding rate of approximately 0.67 mg/kg BW and 22 mg/kg DM intake (ION) or coconut oil (CCO) (Butcher Boy, 76° Coconut Oil # 550; Columbus Vegetable Oils, Des Plaines, IL, USA) or soybean oil (SBO) (International Ingredient Corp., St. Louis, MO, USA) to be approximately 0.15% BW and 4% of the total diet. The coconut oil was non-hydrogenated, refined, bleached, and deodorized, and soybean oil was fully refined, bleached, and deodorized as well.

The feeding rates of CCO and SBO supplements were slightly greater than that of CON, MSL, and HSL so that corn intake would be similar among treatments, with oil levels of approximately 23.1% of DM. The same was true for the ION supplement, although the level of Rumensin 90° in the supplement was much lower (0.073% DM). The forages were offered at approximately 120% of consumption on previous
days at 08:00 h after refusals of pellets were collected and concentration was fed and consumed. The quantity of pellets offered also was adjusted in accordance with the amount consumed. Moreover, wethers had access to blocks of trace mineralized salt (96.5%–99.5% NaCl, 4000 mg/kg Zn, 1600 mg/kg Fe, 1200 mg/kg Mn, 260–390 mg/kg Cu, 100 mg/kg I, and 40 mg/kg Co) in Calan gate feeders and small block pieces in met abolism cage feeders.

2.3. Measures

Body weight (BW) was determined before the morning meal at the beginning of periods and the start and end of wk 5 and calorimetry measures. Average daily gain (ADG) was determined by regressing BW at the start of the experiment and end of periods against time. Wethers resided in metabolism crates in wk 7 for 1 day of adjustment followed by 6 days when feces and urine were collected. Urine was acidified with 30 ml of 30% (vol/vol) H2SO4 placed in collection vessels to maintain pH below 3.0. Composite samples of feces and urine were formed by collecting 15% daily aliquots. Partial DM concentration in feces was determined by drying in a forced-air oven at 55° C for 48 h. Forages and concentrates were sampled to form composites, and feed and fecal samples were analyzed for dry matter (DM), ash (AOAC 2006), nitrogen (N; Leco TruMac CN, St. Joseph, MI, USA), neutral detergent fibre (NDF) with use of heat stable amylase (Van Soest et al. 1991) and containing residual ash, acid detergent fibre (ADF), acid detergent lignin (ADL; filter bag technique of ANKOM Technology Corp., Fairport, NY, USA), ether extract (EE) with an ANKOM100 unit (ANKOM Technology Corp.; AOCS Official Procedure Am 5-04), and GE using a bomb calorimeter (Parr 6300; Parr Instrument Co., Inc., Moline, IL, USA). Samples were also analyzed for CT by the procedure of Dalzell and Kerven (1998), without addition of Fe3+, with inclusion of ascorbic acid, and using CT extracted from Sericea lespedeza as the standard. Urine was analyzed for DM (lyophilization) and gross energy (GE). Feed intake is presented during the entire experiment and wk 5, and digestibilities were also applied to intake throughout the experiment.

There was a separate room with four metabolism cages fitted with head boxes of an indirect, open-circuit respiration calorimetry system (Sable Systems International). Prior to measures, the analyzers were calibrated with gases of known concentrations. Energy lost as methane was total methane emitted in l/day × 39.5388 kJ/l (Brouwer 1965), and metabolizable energy (ME) was the difference between digestible energy (DE) and the sum of energy in urine and methane.

Ruminal fluid was sampled by stomach tube on the last day of wk 4 of period 4 at 4 h after feeding. The pH was measured with a digital metre and then 4 ml were placed into a tube with 1 ml of a 250 g/l metaphosphoric acid solution and frozen at −20°C for later volatile fatty acid (VFA) analysis. Likewise, 3 ml were placed into a tube with 2 ml of 3 M HCl and frozen at −20°C for ammonia analysis. Analyses of VFA and ammonia N were by procedures of Lu et al. (1990) and Broderick and Kang (1980), respectively. Blood samples were collected at this time and also in earlier periods, centrifuged at 3,000 × g for 20 min at 10°C to harvest plasma, stored frozen at −20°C, and later thawed and analyzed for urea N (Chaney and Marbach 1962).

2.4. Statistical analyses

Variables measured in each period were analyzed using a mixed effects model with SAS (Littell et al. 1996; SAS 2011). Fixed effects were treatment, period, and treatment × period, with the repeated measure of period and random effect of animal within treatment. The model for variables with one value, such as ADG, had treatment as a fixed effect. Because of numerical differences among treatments in initial BW, this variable was used as a covariate for intake and digestion variables.

3. Results

3.1. Feedstuff and diet composition

As expected, CCO and SBO supplements were much higher in EE than supplements of CON and ION (Table 1). Alfalfa was slightly higher in ash than lespedeza, which was accompanied by a lower level of GE. Lespedeza pellets were considerably lower in CP than alfalfa, presumably due in part to more non-legume forage in lespedeza. Likewise, alfalfa was lower in NDF, ADF, and ADL than lespedeza. With the level of supplemental concentrate, the total dietary CT level was approximately 2.7% and 5.5% for MSL and HSL, respectively. Differences among treatments in the composition of the diet consumed (Table 2) were in accordance with the composition of forages and supplemental concentrates.

3.2. BW and ADG

As noted earlier, initial BW numerically differed among treatments (Table 3). However, initial BW included as a covariate did not affect ADG (P = 0.837), with means from the two analyses the same or differing by 1 g. The ADG was lower for HSL than for other treatments (P < 0.05) except CCO and higher for ION vs. CCO (P < 0.05). Values for CON, MSL, and SBO were numerically (P > 0.05) intermediate to those for ION and CCO.

Table 1. Chemical composition of supplement and forage.

| Item       | CON (%) DM | ION (%) DM | CCO (%) DM | SBO (%) DM | Alfalfa (%) DM | Lespedeza (%) DM |
|------------|------------|------------|------------|------------|----------------|-----------------|
| OM (%)     | 97.4       | 97.4       | 97.5       | 97.5       | 87.4           | 93.9            |
| CP (%)     | 10.0       | 10.0       | 9.4        | 9.2        | 21.4           | 13.3            |
| NDF (%)    | 12.7       | 12.7       | 11.4       | 11.4       | 45.1           | 52.4            |
| ADF (%)    | 4.9        | 5.0        | 4.3        | 4.4        | 37.3           | 43.0            |
| ADL (%)    | 1.0        | 1.0        | 0.9        | 0.8        | 13.4           | 17.2            |
| Fat (%)    | 5.6        | 5.4        | 14.2       | 14.4       | 2.4            | 1.4             |
| Gross energy (MJ/kg DM) | 18.8 | 18.8 | 20.8 | 20.8 | 17.3 | 18.8 |
| Condensed tannins (%) DM | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 6.4 |

*CON = Control, used for the Control treatment and treatments with feeding of lespedeza; ION = ionophore; CCO = coconut oil; SBO = soybean oil.
3.3. Feed intake during entire experiment

There were some differences among treatments in concentrate DM intake in g/day during the entire study (Table 4) in part because of adjustments of amounts offered in accordance with change in BW from the last period and projected change in the next. Intake of concentrate DM relative to total intake was 14%–15% for CON, MSL, HSL, and ION and slightly greater \((P < 0.05)\) as expected for CCO and SBO. Forage and total DM intakes in g/day were affected by interactions between treatment and period \((P = 0.011 \text{ and } 0.030, \text{ respectively})\), but in % BW interactions were nonsignificant \((P \geq 0.078)\). Neither forage nor total DM intake in % BW was affected by dietary treatment \((P = 0.283)\). Forage and total DM intake in % BW were both less in period 1 than in periods 2, 3, and 4 \((P < 0.05)\). Intake of digestible DM was similar among treatments \((P > 0.05)\) and lowest among periods in period 1 \((P < 0.05)\).

As for DM intake in g/day, there were treatment × period interactions in intakes of OM, N, NDF, and GE \((P < 0.05; \text{Table } 4)\). One factor contributing to these interactions was much smaller differences among periods for HSL and CCO compared with other treatments for which intake was lowest among periods in period 1. Also, intake was fairly similar among CON, MSL, ION, and SBO treatments in periods 3 and 4 but lowest for CON in period 1 and highest in period 2. Lastly, intake tended to be lowest among treatments for CON in period 1 and highest in period 2.

The only treatment effect on intake of a digested fraction was for N \((P < 0.001)\), although the \(P\) value for NDF approached significance \((P = 0.061; \text{Table } 4)\). Digested N intake was lowest among treatments for HSL \((P < 0.05)\) and lower for MSL than for other treatments \((P < 0.05)\) except SBO \((P = 0.065)\). Digested NDF intake was numerically lowest for CCO and SBO treatments.

3.4. Digestibility and energy measures

Apparent total tract digestibilities of DM, OM, NDF, and GE were not influenced by treatment \((P > 0.05; \text{Table } 5)\). Digestibility of N was lower for HSL than for all treatments \((P < 0.05)\) except MSL, and the value for MSL was lower than for CON and ION \((P < 0.05)\). Digestibility of fat was greater for CCO than for CON, MSL, and ION \((P < 0.05)\), with intermediate \((P > 0.05)\) values for HSL and SBO. However, it should be noted that total DM intake was lower in wk 5 when digestibilities were determined than period averages. Differences were greatest for CON and ION, lowest for HSL, and intermediate for HSL, CCO, and SBO. Factors responsible for these findings are unclear, although adaptation for more than a day could have been beneficial. But, a portion of such differences, notably the smallest difference for HSL, relates to use of initial BW as a covariate.

There were differences among periods in digestibilities \((P < 0.05)\) but not significant treatment × period interactions \((P > 0.05)\). Digestibility of fat was greater for CCO than for CON, MSL, and ION \((P < 0.05)\), with intermediate \((P > 0.05)\) values for HSL and SBO. However, it should be noted that total DM intake was lower in wk 5 when digestibilities were determined than period averages. Differences were greatest for CON and ION, lowest for HSL, and intermediate for HSL, CCO, and SBO. Factors responsible for these findings are unclear, although adaptation for more than a day could have been beneficial. But, a portion of such differences, notably the smallest difference for HSL, relates to use of initial BW as a covariate.

There were differences among periods in digestibilities \((P < 0.05)\) but not significant treatment × period interactions \((P > 0.05)\). Digestibility of fat was greater for CCO than for CON, MSL, and ION \((P < 0.05)\), with intermediate \((P > 0.05)\) values for HSL and SBO. However, it should be noted that total DM intake was lower in wk 5 when digestibilities were determined than period averages. Differences were greatest for CON and ION, lowest for HSL, and intermediate for HSL, CCO, and SBO. Factors responsible for these findings are unclear, although adaptation for more than a day could have been beneficial. But, a portion of such differences, notably the smallest difference for HSL, relates to use of initial BW as a covariate.

There were differences among periods in digestibilities \((P < 0.05)\) but not significant treatment × period interactions \((P > 0.05)\). Digestibility of fat was greater for CCO than for CON, MSL, and ION \((P < 0.05)\), with intermediate \((P > 0.05)\) values for HSL and SBO. However, it should be noted that total DM intake was lower in wk 5 when digestibilities were determined than period averages. Differences were greatest for CON and ION, lowest for HSL, and intermediate for HSL, CCO, and SBO. Factors responsible for these findings are unclear, although adaptation for more than a day could have been beneficial. But, a portion of such differences, notably the smallest difference for HSL, relates to use of initial BW as a covariate.

3.5. Ruminal fluid measures and serum urea N

Ruminal pH in period 4 was not affected by treatment \((P > 0.05; \text{Table } 6)\). Period 4 ruminal VFA concentrations in mmol/l were quite variable, with treatment differences only in isobutyrate and valerate. Numerically, total VFA concentration ranked HSL
Table 4. Feed intake during the entire trial and digested fractions based on digestibility determined in wk 5 of periods.

| Item                      | Effect P values1 | Treatment2 | Period |
|---------------------------|------------------|------------|--------|
| Item                      | Trt Prd Trt*Prd Cov Prd | CON  MSL HSL ION CCO SBO | SEM 1 2 3 4 SEM |
| DM intake (g/day)         | 0.017 0.108 0.704 <0.001 | 336ab 314a 306a 363abc 395c 382bc | 20.2 323 366 364 344 14.7 |
| Forage                    | 0.022 <0.001 0.011 <0.001 | 1602 1668 1695 1662 1731 1470 | 125.2 |
| Total                     | 0.051 <0.001 0.030 <0.001 | 1865 1936 1999 2066 2092 1807 | 141.5 |
| Digested (g/day)          | 0.108 <0.001 0.271 <0.001 | 1719 1548 1415 1658 1501 1435 | 85.8 1363a 1608b 1564b 1649b 52.1 |
| Concentrate (% total)     | <0.001 0.098 0.615 0.691 | 13.8a 13.8a 15.4a 15.1a 18.2b 17.7b | 0.68 16.2 16.1 15.6 14.8 0.46 |
| DM intake                 | 0.055 <0.001 0.119 0.001 | 3.86 3.75 3.52 3.69 3.64 3.54 | 0.157 3.37a 3.80b 3.69b 3.79b 0.092 |
| Total (% BW)              | 0.025 0.314 0.668 0.029 | 0.53ab 0.52a 0.54ab 0.56abc 0.66c 0.62bc | 0.033 0.55 0.61 0.57 0.56 0.023 |
| Forage (% BW)             | 0.283 <0.001 0.078 0.002 | 3.33 3.23 2.98 3.12 2.98 2.92 | 0.142 2.82ab 3.20b 3.12b 3.24b 0.081 |
| OM intake (g/day)         | 0.165 <0.001 0.035 <0.001 | 1656 1774 1884 1846 1865 1613 | 127.4 |
| Total                     | 0.163 <0.001 0.305 <0.001 | 1577 1460 1351 1526 1385 1329 | 76.1 1280a 1514b 1445b 1512b 46.5 |
| Digested (g/day)          | <0.001 <0.001 0.011 <0.001 | 59.2 50.5 41.7 63.6 64.8 55.4 | 4.26 |
| N intake (g/day)          | 0.048 <0.001 0.017 <0.001 | 756 848 922 800 821 701 | 60.1 |
| Total                     | <0.001 <0.001 0.001 | 1098 1009 972 972 828 866 | 866 |
| Digested NDF intake (g/day) | <0.001 <0.001 0.082 <0.001 | 60.2ab 42.3b 28.8a 58.0de 50.9de 49.3bc | 2.59 41.6a 48.8a 50.3b 52.3b 1.56 |
| GE (MJ/day)               | 0.061 <0.001 0.444 0.008 | 529 508 502 501 410 395 | 36.8 412a 482b 452ab 551c 23.6 |
| Total                     | <0.001 <0.001 0.095 <0.001 | 69.8b 55.8ab 43.6a 66.6c 98.4d 97.5f | 3.30 63.3a 74.7b 76.1b 73.2b 1.83 |
| Digested GE (MJ/day)      | <0.001 <0.001 0.549 <0.001 | 46.2ab 34.6ab 30.2a 41.7bc 76.8d 68.4d | 3.89 41.7a 53.9b 52.1b 51.0b 2.04 |
| Total                     | 0.291 <0.001 0.036 <0.001 | 32.63 35.15 37.50 36.30 37.44 32.4a | 2.52 |
| Digested                  | 0.363 <0.001 0.366 <0.001 | 29.84 27.87 26.22 28.80 26.97 25.53 | 1.518 24.59 29.06 27.42 29.08 0.931 |

1Trt = treatment; Prd = period; Cov = covariate (initial BW).
2CON = Control; MSL = moderate level of lespedeza; HSL = high level of lespedeza; ION = ionophore; CCO = coconut oil; SBO = soybean oil.

Main effect means in a row without a common superscript letter differ (P < 0.05).
Table 5. Feed intake and digestion in wk 5 of periods.

| Item          | Effect P values | Treatment | Period | SEM |
|---------------|-----------------|-----------|--------|-----|
| **DM**       |                 |           |        |     |
| Intake (g/day) | 0.803 <0.001   | 0.690 0.001 | 1817 1966 | 1915 1821 | 1731 1775 | 129.3 1662 a 1743a 1808a 2140b 71.6 |
| Digestion (%) | 0.675 0.008    | 0.540 0.467 | 70.5 67.6 69.5 69.5 68.6 66.2 | 1.95 69.6 b 69.4b 65.5 70.0b 1.17 |
| Digested (g/day) | 0.797 <0.001 | 0.617 0.006 | 1277 1326 1350 1273 1199 1176 | 100.2 1150a 1209b 1198a 1510b 58.2 |
| **OM**       |                 |           |        |     |
| Intake (g/day) | 0.495 <0.001   | 0.696 0.001 | 1622 1804 | 1808 1625 | 1804 1553 | 1593 1172 | 1150a 1588b 1639a 1932b 64.3 |
| Digestion (%) | 0.618 <0.001   | 0.650 0.592 | 72.8 69.5 70.3 72.0 71.1 68.8 | 1.80 72.3b 72.4b 67.2a 71.2b 1.09 |
| Digested (g/day) | 0.611 <0.001 | 0.681 0.003 | 1177 1251 1289 1174 1103 | 90.2 1084a 1147b 1112a 1387b 52.2 |
| **N**        |                 |           |        |     |
| Intake (g/day) | 0.041 <0.001   | 0.597 0.004 | 56.1b 50.8b 39.5a 56.5b 51.6b | 53.4b 3.78 46.1a 47.5a 50.6a 61.0b 2.16 |
| Digestion (%) | 0.001 0.013    | 0.453 0.727 | 77.5c 70.2ab 67.0a 77.0c | 75.2bc 73.6bc 1.76 74.0b 72.6a 71.9a 75.8b 1.04 |
| Digested (g/day) | 0.005 <0.001 | 0.460 0.008 | 43.4b 35.8b 27.1a | 43.8b 39.5b 39.4b 2.97 34.2a 34.5a 37.1a 46.9b 1.81 |
| **NDF**      |                 |           |        |     |
| Intake (g/day) | 0.057 <0.001   | 0.769 0.004 | 708 840 878 | 711 645 | 673 60.3 670a 690b 725c 886b 32.9 |
| Digestion (%) | 0.441 0.001    | 0.450 0.208 | 53.7 50.6 53.0 | 52.3 48.1 | 46.6 2.87 51.3bc 50.1ab 45.6a 55.9b 1.87 |
| Digested (g/day) | 0.086 <0.001 | 0.553 0.095 | 380 425 480 | 383 322 | 316 42.3 341a 351a 341a 504b 25.3 |
| **Ether extract** | <0.001 <0.001 | 0.467 <0.001 | 55.0b 50.0ab | 42.2a | 52.6b 88.7c 87.7c 3.73 57.2a 62.4b 62.6b 68.6c 2.00 |
| Intake (g/day) | 0.026 0.008    | 0.189 0.876 | 66.2a 62.8a 68.5ab | 62.3a 76.3b 70.1ab | 3.02 64.3a 71.5b 67.1a 67.9ab 1.75 |
| Digestion (%) | <0.001 <0.001   | 0.652 0.001 | 36.3a 31.0a 29.3 | 32.8a 69.0b 61.9b | 3.79 37.6a 45.2ab 43.2a 47.6c 2.03 |
| **Gross energy** | <0.001 <0.001 | 0.661 0.587 | 69.9 67.0 68.5 | 69.0 68.8 | 65.8 1.99 69.0b 69.8b 64.1a 68.9b 1.19 |
| Intake (MJ/day) | 0.632 <0.001   | 0.690 0.004 | 22.26 23.87 25.03 | 22.14 | 21.71 21.09 1.762 20.84a 22.03a 21.14a 26.72b 1.030 |
| Digestion (%) | <0.001 <0.001   | 0.319 0.002 | 9.72c 9.4ab 8.02a | 8.62b 10.5a | 10.2a 0.081 0.97 0.93 0.89 0.84 0.047 |
| **Methane (MJ/day)** | <0.001 <0.001 | 0.326 0.033 | 3.06b 2.31b 2.12ab | 2.21b 1.96a | 2.04ab 1.108 2.41b 2.37b 2.30b 2.05b 0.068 |
| Intake (MJ/day) | <0.001 <0.001 | 0.501 0.003 | 10.3b 6.8a 6.3a | 7.2a 6.5a | 6.5a 0.35 8.3c 7.7bc 7.4a 5.6a 0.28 |
| **Metabolizable** | <0.001 <0.001 | 0.468 0.036 | 14.8b 10.2a 9.3a | 10.6a 9.8a | 10.1a 0.62 11.9b 11.2b 11.6b 8.4a 0.51 |
| **kJ/kg BW** | 0.439 <0.001 | 0.693 0.007 | 18.23 20.62 22.41 | 18.95 18.88 | 17.87 17.07 17.46a 18.74a 17.95a 23.83b 1.001 |
| **kcal/kg BW** | 0.146 0.001 | 0.667 0.401 | 820 955 1069 | 870 893 | 831 69.2 889b 896a 806b 1035a 43.3 |

1Trt = treatment; Prd = period; Cov = covariate (initial BW).
2CON = Control; MSL = moderate level of lespedeza; HSL = high level of lespedeza; ION = ionophore; CCO = coconut oil; SBO = soybean oil.
3Main effect means in a row without a common superscript letter differ (P < 0.05).

Table 5. Feed intake and digestion in wk 5 of periods.
< CCO < CON, MSL, and SBO. There were differences in molar percentages of acetate and valerate (P < 0.05), but magnitudes were small. The ruminal ammonia N concentration in period 4 was lowest among treatments for HSL (P < 0.05). This was also true for serum urea N concentration measured in each period, also with a lower level for CCO than CON, ION, and SBO (P < 0.05). Serum urea N concentration was less in periods 1 and 2 than in periods 3 and 4 (P < 0.05).

4. Discussion

4.1. BW and ADG

The wethers began the experiment in moderate body condition, with a body condition score (BCS) of approximately 3 on a scale of 1 to 5. With the assumption that a 20% change in BW of that at a BCS of 3 corresponds to a 1 unit change in BCS (i.e. to a BCS of 2 or 4; Ngwa et al. 2007), observed increases of 13%, 11%, 6%, 14%, 10%, and 11% of initial BW represent increases of 0.67, 0.56, 0.30, 0.72, 0.48, and 0.55 BCS units for CON, MSL, HSL, ION, CCO, and SBO, respectively. Pelleting presumably contributed to increasing BW by facilitating relatively high feed intake and efficiency of energy utilization (AFRC 1998). The estimate of digested N intake for HSL was less than one-half of that for CON, but it is doubtful that protein status limited ADG by HSL wethers because as a percentage of DM intake digested N for HSL on a CP basis was 9%. Rather, energy absorption may have been responsible, as average GE and DE intakes during the entire experiment were 12% less for HSL than for CON.

4.2. Feed intake

The interaction between treatment and period in total intake of DM and other constituents in g or MJ/day during the entire experiment suggests that a characteristic of the HSL diet, such as the highest CT concentration, and the medium-chain and long-chain polyunsaturated FA of CCO and SBO diets, respectively, had negative effects in periods 2, 3, and 4 but not in period 1. Regarding lespedeza, this is in slight contrast to previous studies of Animut et al. (2008a, 2008b) and Puchala et al. (2012a, 2012b) in which CT from lespedeza did not affect intake. Relatedly, data of the present study are not available to attribute such effects solely to CT, with possible involvement of other forage characteristics such as higher levels of NDF, ADF, and ADL in lespedeza. With application of digestibilities determined at the end of periods to average intake during entire periods, there were no effects of treatment on digested DM, OM, or energy and no treatment by period interactions. Somewhat comparable results were noted by Kouakou et al. (1994) with mature beef cattle, in that corn and soybean oil when supplemented together at similar levels decreased intake of legume and cool-season grass hay sources. Likewise, Galloway et al. (1993) observed little effect on intake and digestion in Holstein steer calves consuming a low-quality grass hay supplemented with different levels of monensin and lasalocid.

4.3. Digestibility and energy measures

4.3.1. Digestibilities and urinary energy

The factor most likely responsible for relatively low N digestibility, ruminal ammonia N concentration, and level of urea N in serum for HSL is binding of protein in the rumen by CT of lespedeza (Robbins et al. 1987; Hoste et al. 2016). This is also supported by lower N digestibility for the MSL vs. CON treatment and the greater magnitude of difference between CON and MSL (6.8 percentage units) than between MSL and HSL (3.7 percentage units). The findings suggest that either there was not complete dissociation of proteins initially bound to CT in the rumen when acidic conditions of the abomasum were encountered or rebinding of CT to proteins in the intestines occurred as pH rose, as also suggested by Puchala et al. (2012a, 2012b).
However, the magnitude of differences among diets with and without lespedeza was not great. For example, based on 88% true protein digestibility and 2.67% DM of metabolic fecal CP (Moore et al. 2004), expected apparent total tract N digestibility was 74.1%, 71.4%, and 67.0% for CON, MSL, and HSL, respectively, with the measured value for CON slightly less than predicted and observed values for diets with lespedeza close to ones expected.

Based on levels of EE in diets consumed during weeks when digestibility was determined, 43.4% and 40.0% of EE was from coconut and soybean oils for CCO and SBO treatments, respectively. Assuming that digestibility of EE for the CON treatment was the same as that from ingredients other than oils added to CCO and SBO diets, digestibility of EE of coconut and soybean oils was 92.9% and 77.2%, respectively. The value for coconut oil is slightly greater than 83%, 85%, and 91% estimated from data of Bhatt et al. (2011) for lambs consuming a diet of *Ailanthus* leaves and concentrate with 2.5%, 5.0%, and 7.5% oil, respectively, both offered free-choice. The value for soybean oil is lower than 90% determined by Ferreira et al. (2016) for lambs consuming a diet with 4% oil. Although, a similar value of 77% was determined by van Cleef et al. (2016) for lambs consuming a 60% concentrate diet including soybean oil at 6%.

The magnitude of decrease in urinary energy for HSL was slightly greater than expected based on the effect on total tract N digestibility, and no change for the MSL treatment was not anticipated either. However, for the latter result, GE intake was numerically greater for MSL than for CON.

### 4.3.2. Methane

Table 7 summarizes effects of CT of lespedeza on ruminal methane emission by goats in some previous experiments and the present one. In earlier studies, forage CT markedly decreased ruminal methane emission. The effect increased with increasing level of CT, but per unit of CT change was greatest at low dietary levels. Also, effects were immediate with maximal impact the first day of feeding. Perhaps the relatively low level of CT in the source of lespedeza used in the present experiment contributed to the lack of difference in ruminal methane emission between MSL and HSL treatments. Despite the less than anticipated dietary CT levels for these treatments, magnitudes of change in ruminal methane emission were fairly similar to those of Animut et al. (2008a) with a diet of 33% Kobe lespedeza (*Lespedeza striata*) and 67% grass containing 5.0% CT and Puchala et al. (2012b) with feeding fresh lespedeza every 4 days with an overall average dietary CT level of 4.0%. Conversely, a similar magnitude of change was noted by Puchala et al. (2012a) but with a lespedeza hay diet much higher in CT at 15.3%. Nonetheless, results of the present experiment suggest that the magnitude of effect of feeding lespedeza with a relatively low level of CT on ruminal methane emission is similar to that of the ionophore monensin and coconut and soybean oils as rich sources of medium-chain and long-chain polyunsaturated FA, respectively, included at their respective levels.

There have been some comparisons of effects of CT sources, ionophores, and different oils on methane production, but none with the number or array of dietary components addressed in the present experiment. These results differ somewhat from those of Liu et al. (2011) with young sheep consuming a 48% concentrate diet supplemented with 1% or 3% chestnut tannins alone or with coconut oil at 2.5% of the diet. Methane emission was not affected by the low level of chestnut tannins but was decreased by the higher level. Coconut oil alone did not significantly affect methane emission, but numerically there was a greater depression when both chestnut tannins and coconut oil were included in the diet (i.e. 22.3 vs. 24.9 g/kg DM intake; SEM = 0.61). In somewhat accordance with results of the present experiment, Perna Junior et al. (2017) reported similar decreases in methane emission of 10.7% and 8.0% when monensin and a tannin-rich extract from *Acacia mearnsii* were included in a 50% concentrate diet of dry Holstein cows at 11 mg/kg and 0.6% DM intake, respectively. Although an in vitro study, results of Johnson et al. (2009) were fairly comparable to those of the present experiment, in that coconut oil at 5% and monensin at 22 mg/kg DM intake elicited similar decreases in methane production.

As opposed to studies overviewed in Table 7 that consisted all or primarily of forage, diets of the present experiment included rolled corn though at a relatively low level that should not have deleteriously affected fibre digestion (Galyean and Goetsch 1993; Dolebo et al. 2017). But, even small additions of cereal grain high in starch to forage diets can impact the number and types of ruminal protozoa present and their actions and activities (Mould et al. 1983; Uden 1984). In this regard, results of all studies in Table 7 were strongly supportive of impact on ruminal methane emission via direct effects of lespedeza CT on the number and/or activity of methanogenic bacteria. Only results of Animut et al. (2008b) suggested that negative effects of CT on activity of non-methanogenic bacteria, particularly fibrolytics, to lessen hydrogen availability to methanogens and indirectly lessen methane production. Findings of studies of Animut et al. (2008a, 2008b) were strongly indicative and some of Puchala et al. (2012b) and the Puchala et al. (2012a) experiment with fresh forage were somewhat supportive of decreased methane emission via direct effects of CT on the number and/or activity of protozoa, thus with indirect effect by impacting hydrogen availability to symbiotic methanogens in physical association with methanogens (Piñeiro-Vázquez et al. 2015; Tapio et al. 2017). Hence, it is conceivable that effects of supplemental concentrate in the present experiment on the number and/or activity of protozoa influenced impact of lespedeza CT on ruminal methane emission, and the same is possible for effects of monensin, coconut oil, and soybean oil as well. In accordance, future research should address potential interactions between dietary grain and CT levels as well as those of other modifiers of methane emission.

As noted before, besides comparing different potential modifiers of methane emission, an important goal of the study was to address potential adaptation in ruminal methane emission to effects of CT and other substances evaluated. In accordance, in some studies addressed in Table 7 with days of adaptation ranging from 15 to 34, inclusion of qualifying statements in manuscripts regarding the potential for less or no effects on ruminal methane emission with longer experiments was deemed warranted. However, the lack of interaction...
between treatment and period in the present study indicates that no such adaptation occurred and that effects after a few weeks of feeding any of these fermentation modifiers would be similar even a few months later.

5. Conclusion

A 1:1 mixture of pelleted alfalfa and a source of lespedeza, with a low to moderate level of CT, had a similar effect on ruminal methane emission as lespedeza as the sole forage (31% and 37% decreases relative to DE intake, respectively), both with rolled corn supplemented at approximately 0.5% BW and 14%–15% of the diet. Inclusion in basal alfalfa diets of monensin at approximately 22 mg/kg DM intake and coconut and soybean oils at 4% elicited similar decreases in methane emission (28, 34, and 32%, respectively). There was no evidence of adaptation to any of the modifiers, with methane emission (28, 34, and 32%, respectively). There was no evidence of adaptation to any of the modifiers, with methane emission determined in wk 5, 10, 15, and 20.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

**Funding**

The project was supported by the USDA National Institute of Food and Agriculture (NIFA), Project OKLUAGOETSCH2014, Accession Number 1004179.

**References**

AFRC. 1998. The nutrition of goats. New York (NY): CAB International.

Animut G, Puchala R, Goetsch AL, Patra AK, Sahu T, Varel VH, Wells J. 2008. Methane emission by goats consuming different sources of condensed tannins. Anim Feed Sci Technol. 144:228–241.

AOAC. 2006. Official methods of analysis, 18th ed. Gaithersburg (MD): AOAC International.

Bhatt RS, Soren NM, Tripathi MK, Karim SA. 2011. Effects of different levels of coconut oil supplementation on performance, digestibility, rumen fermentation and carcass traits of malpura lambs. Anim Feed Sci Technol. 164:29–37.

Bodas R, Prieto N, Garcia-González R, Andrés S, Giráldez FJ, López S. 2012. Manipulation of rumen fermentation and methane production with plant secondary metabolites. Anim Feed Sci Technol. 176:78–93.

Bruderick GA, Kang JH. 1980. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and In vitro media. J Dairy Sci. 63:64–75.

Brouwer E. 1965. Report of sub-committee on constants and factors. In: Blaxter KL, editor. Energy metabolism. Proc. 3rd Symp European Assoc Anim Prod Publ No. 11. London (UK): Academic Press; p. 441–443.

Chalupa W. 1980. Chemical control of rumen microbial metabolism. In: Ruckebusch Y, Thivend P, editor. Digestive physiology and metabolism of ruminants. Westport (CT): AVI Publishing Co. Inc.; p. 325–443.

Chaney AL, Marbach EP. 1962. Modified reagents for determination of urea and ammonia. Clin Chem. 8:130–136.

Dalzell SA, Kerven GL. 1998. A rapid method for the measurement of Leucaena spp proanthocyanidins by the proanthocyanidin (butanol/HCl) assay. J Sci Food Agric. 78:405–416.

Davies A, Nwaonu MN, Stanier G, Boyle FT. 1982. Properties of a novel series of inhibitors of rumen methanogenesis; in vitro and in vivo experiments including growth trials on 2,4-bis (trichloromethyl)-benzo [1, 3]dioxin-6-carboxylic acid. Br J Nutr. 47:565–576.

Dolebo AT, Puchala R, Gipson TA, Dawson LJ, Sahu T, Goetsch AL. 2017. Effects of supplemental concentrate level and forage source on intake and digestion by growing and yearling Boer goat wethers and evaluation of a method of predicting negative feedstuff associative effects. J Appl Anim Res. 45:470–479.

Ferreira EM, Pires AV, Susin I, Biehl MV, Gentili RS, Parente M, Polizel DM, Ribeiro CVD, de Almeida E. 2016. Nutrient digestibility and ruminal fatty acid metabolism in lambs supplemented with soybean oil partially replaced by fish oil blend. Anim Feed Sci Technol. 216:30–39.
Galloway DL Sr, Goetsch AL, Patil AR, Forster LA Jr, Johnson ZB. 1993. Feed intake and digestion by Holstein steer calves consuming low-quality grass supplemented with lasalocid or monensin. Can J Anim Sci. 73:869–879.

Galyean ML, Goetsch AL. 1993. Utilization of forage fiber by ruminants. In: Jung HG, Buxton DR, Hatfield RD, Ralph J, editors. Forage cell wall structure and digestibility. Madison (WI): Am. Soc. Agron., Crop Sci. Soc., Am., Soil Sci. Soc. Am.; p. 33–72.

Goel G, Makkar HPS. 2012. Methane mitigation from ruminants using tannins and saponins. Trop Anim Health Prod. 44:729–739.

Guan G, Wittenberg KM, Ominski KH, Krause DO. 2006. Efficacy of ionophores in cattle diets for mitigation of enteric methane. J Anim Sci. 84:1896–1906.

Hoste H, Torres-Acosta JFJ, Quijada J, Chan-Perez I, Kommuru Goel G, Makkar HPS. 2012. Methane mitigation from ruminants using monensin or monensin as feed additives on ruminal fermentation efficiency in cattle. Livest Sci. 203:21–29.

Pinto-Vázquez AT, Canul-Solís JR, Alayón-Gamboa JA, Chan-Canal AJ, Ayala-Burgos AJ, Aguilar-Pérez CF, Solorio-Sánchez FJ, Ku-Vera JC. 2015. Potential of condensed tannins for the reduction of emissions of enteric methane and their effect on ruminant productivity. Arch Med Vet. 47:263–272.

Puchala R, Animut G, Patra AK, Dettweiler GD, Wells JE, Varel VH, Sahlu T, Goetsch AL. 2012a. Effects of different fresh-cut forages and their hays on feed intake, digestibility, heat production, and ruminal methane emission by Boer × Spanish goats. J Anim Sci. 90:2754–2762.

Puchala R, Animut G, Patra AK, Dettweiler GD, Wells JE, Varel VH, Sahlu T, Goetsch AL. 2012b. Methane emissions by goats consuming sericea lespedeza at different feeding frequencies. Anim Feed Sci Technol. 175:76–84.

Puchala R, Tovar-Luna I, Goetsch AL, Sahlu T, Carstens GE, Freytl HC. 2007. The relationship between heart rate and energy expenditure in alpine, angora, Boer and Spanish goat wethers consuming different quality diets at level of intake near maintenance or fasting. Small Rumin Res. 70:183–193.

Puchala R, Tovar-Luna I, Sahlu T, Freytl HC, Goetsch AL. 2009. Technical note: The relationship between heart rate and energy expenditure in growing crossbred Boer and Spanish wethers. J Anim Sci. 87:1714–1721.

Robbins CT, Hanley TA, Hagerman AE, Baker DL, Schwartz CC, Mautz WW. 1987. Role of tannins in defending plants against ruminants: reduction in protein availability. Ecology. 68:98–107.

Rogers M, Jouany JP, Thivend P, Fontenot JP. 1997. The effects of short-term and long-term monensin supplementation, and its subsequent withdrawal on digestion in sheep. Anim Feed Sci Technol. 65:113–127.

Rumphler WV, Johnson DE, Bates DB. 1986. The effect of high dietary cation concentration on methane output and steers fed with and without ionophores. J Anim Sci. 62:1737–1741.

SAS. 2011. SAS/STAT® 9.3 user’s guide. Cary (NC): SAS Inst. Inc.

Sauer FD, Fellner V, Kinsman R, Kramer JKG, Jackson HA, Lee AJ, Chen S. 1998. Methane output and lactation response in Holstein cattle with monensin or unsaturated fat added to the diet. J Anim Sci. 76:906–914.

Smith AH, Zoetendal E, Mackie RI. 2005. Bacterial mechanisms to overcome inhibitory effects of dietary tannins. Microbiol Ecol. 50:197–205.

Tapiol I, Snelling TJ, Strozzi F, Wallace RJ. 2017. The ruminal microbiome associated with methane emissions from ruminant livestock. J Anim Sci Biotechnol. 8:7. (11 pages).

Tomkins NW, Denman SE, Plagnin R, Wanapat M, McSweeney CS, Elliott R. 2015. Manipulating rumen fermentation and methane emissions using an essential oil and monensin in beef cattle fed a tropical grass hay. Anim Feed Sci Technol. 200:25–34.

Uden P. 1984. Digestibility and digesta retention in dairy cows receiving hay or silage at varying concentrate levels. Anim Feed Sci Technol. 11:279–291.

van Cleef FDOS, Eziquel JMB, D’Aurea AP, Almeida MTC, Perez HL, van Cleef EHC. 2016. Feeding behavior, nutrient digestibility, feedlot performance, carcass traits, and meat characteristics of crossbred lambs fed high levels of yellow grease or soybean oil. Small Rumin Res. 137:151–156.

Van Soest PJ, Robertson JB, Lewis BA. 1991. Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. J Dairy Sci. 74:3583–3597.

Wallace RJ, McEwan NR, Mchintosh FM, Teveredegne B, Newbold CJ. 2002. Natural products as manipulators of rumen fermentation. Austral J Anim Sci. 15:1458–1468.