793 Effects of of 2-hydroxy 4-(methylthio) butanoic acid iso-propyl ester (HMBi) and DL-Met on in vitro fermentation characters of high-yielding dairy cow diets. B. B. Nobari*[1], A. Taghizadeh[1], M. Khorvash[2], S. Alijani[1], J. Shodja[1], and F. Parnian, and K. Dizaj[1], *[1]Department of Animal Sciences, Faculty of Agriculture, University of Tabriz, Tabriz, Eastern Azarbaijan, Iran, [2]Department of Animal Science, College of Agriculture, Isfahan, Isfahan, Iran.*

An in vitro gas production and degradability study has been conducted to investigate the effects of 4 treatments including: no supplement (cnt), 0.065% HMBi/ DM diet (HMBi-1), 0.13% HMBi/ DM diet (HMBi-2) and 0.088% DL-Met/ DM diet (DL-Met) on typical dairy cow diets. Two diets with 17.7% (HCP, high crude protein) and 15.7% (LCP, low crude protein) CP have been formulated by different ingredients for early lactating Holstein dairy cows (DIM 55 ± 7, BW 650 and Milk yield 55 ± 6.4). Results revealed that digestibility of DM were significantly increased by HMBi and DL-Met addition while digestibilities of ADF, NDF and HEMI was decreased for the DL-Met treated diets compared with the diet containing the equivalent amount of Met supplied as HMBi-2. Asymptote gas production (A) has been affected by HBMi and DL-met supplementation ($P < 0.01$) but fractional gas production rate (c) parameter of gas production has not affected by treatments. Regarding fermentation parameters, pH has not been affected by supplements or CP levels. Also, there are linear and quadratic effects of HMBi incremental levels on ammonia-N, whereas its concentration decreased with addition of HMBi. Supplementation of HMBi and low crude protein diets can reduce excess ammonia-N load in rumen and increase N utilization in dairy industry which will modify animal health as well as environment friendly farming.

Table 1. Nutrient digestibility, gas production and fermentation parameters of two diets in batch culture including two concentrations of HMBi or DL-Met

	HMBi (%)					Contrasts				*P*-value		
Item	0	0.065	0.13	dl-met	SEM	L	Q	HMBi2 vs. dl-met	Cnt vs All	M	CP	M × CP
GP parameters												
A (ml)												
HCP	214.6	224.6	212.9	223.0	0.96	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.001
LCP	217.1	221.6	199.6	187.6								
c (%/h)												
HCP	0.061	0.063	0.060	0.063	0.003	0.989	0.594	0.251	0.510	0.616	<0.001	0.721
LCP	0.068	0.075	0.075	0.075								
Lag (h)												
HCP	0.30	0.25	0.56	0.63	0.16	0.117	0.457	0.201	0.407	0.368	0.916	0.266
LCP	0.51	0.46	0.20	0.61								
Fermentation parameter												
pH												
HCP	5.39	5.38	5.40	5.37	0.02	0.164	0.426	0.164	0.564	0.404	0.323	0.400
LCP	5.36	5.37	5.42	5.38								
Ammonia-N (mg/dL)												
HCP	5.57	5.31	4.91	5.30	0.40	0.016	0.312	0.002	0.031	0.012	0.607	0.850
LCP	5.37	5.25	4.93	5.37								

Key Words: degradability, fermentation, HMBi

794 Study of the effect of flavonoid substances on methanogenesis on in vitro fermentation of rumen liquor coming from different experimental diets in beef cattle. A. R. Seradj*[1], J. Balcells[1], H. J. Morazan[1], D. V. Mata[1], J. Crespo[2], and M. Fondevila[3], *[1]Dept. Animal Production, University of Lleida, Lleida, Spain, [2]Interquim, S.A. (Ferrer HealthTech), Barcelona, Spain, [3]Dept. Animal production and nutrition, University of Zaragoza, Zaragoza, Spain.*

Two in vitro incubation trials in a randomized complete block design were conducted to evaluate the effect of Neohesperidine (NH) and Bioflavex (BF) at 5 mg/L compared with an unsupplemented control (CTR) on total gas and methane production. Two groups of steers given a total mixed ration (TMR) and concentrate plus straw were used as donors of rumen liquid, which was filtered and immediately used as inoculum. Bottles of 120 mL were filled in anaerobic conditions with 80 mL of incubation solution including 20% strained rumen fluid, and kept at 39 °C for 72 h in triplicate. The pressure measurements were carried out at different incubation times and converted to volume. From 12h, a gas sample (0.1 mL) was taken manually using a gas tight syringe and immediately analyzed for methane concentration using gas chromatography (GC). One bottle from each treatment was opened under anaerobic conditions and sampled for pH and volatile fatty acids (VFA) using GC at 0, 12 and 72 h. A nonlinear model for rate determination with lag time [$y = a (1 - e^{-b (t-c)})$] was applied to determine the potential cumulative gas production, fractional rate and discrete lag time. No effect of BF and NH was recorded ($P > 0.05$) on gas production despite the diet given to donor animals; however, NH produced more gas ($P < 0.05$) than BF and CTR Both BF and NH reduced ($P < 0.01$) the production and proportion of methane respect to CTR in rumen liquid from both origins. Compared with NH, BF showed lower methane production (170.2 vs. 188.6 mL; SEM 3.37; $P < 0.01$) and methane percentage (13.52 vs. 13.88% SEM 0.22; $P > 0.05$). Total VFA concentration decreased with BF ($P < 0.01$) and NH ($P > 0.05$), and both BF and NH decreased ($P < 0.01$) the acetate to propionate ratio as compared with control. The 2 sources of flavonoids did not affect gas production pattern. However, VFA concentration increased and acetate to propionate molar proportion decreased, and consequently methane production was reduced.

Key Words: Bioflavex, methanogenesis, Neohesperidine

795 Gastrointestinal bacterial and methanogenic archaea diversity in response to feeding condensed tannins-containing pine bark diet to goats using 16S rDNA amplicon pyrosequencing. B. R. Min*[1], S. Solaiman[1], R. Shange[1], and J. S. Eun[2], *[1]Tuskegee University, Tuskegee, AL, [2]Utah State University, Logan.*

Eighteen Kiko-cross goats (33.4 ± 0.98 kg; n = 6) were used to measure gastrointestinal (GI) bacteria and methanogenic archaea (MA) diversity when fed condensed tannins (CT)-containing pine bark (PB). The GI fecal collection was performed during 7 d in 2 different periods. Three dietary treatments were tested: control diet (0% PB and 30% wheat straw (WS; 0.17% CT DM); 15% PB and 15% WS (1.6% CT DM), and 30% PB and 0% WS (3.2% CT DM). Populations of the GI bacteria and MA were measured using a 16S rDNA bacterial tag-encoded FLX amplicon pyrosequencing technique to characterize and elucidate changes in GI bacteria and MA diversity among the diets. All the statistical analyses were conducted using the MIXED procedures of SAS, and orthogonal polynomial contrasts were performed to determine linear and quadratic effects of feeding PB in the diets. Total 416 bacteria (60 unknown spe-

cies) and 26 MA genera were detected in goat samples. *Proteobacteria* was the most dominant phyla with mean relative abundance values ranging from 39.7% (30% PB) to 46.5% (control) and 47.1% (15% PB). The remaining phyla accounted for fewer than 25% of the relative abundance observed. Of these groups, *Gammatoproteobacteria* ($P < 0.05$), *Flavobacteria* ($P < 0.01$), *Proteobacteria* ($P < 0.05$), and Bacteroides ($P < 0.05$) were linearly decreased ($P < 0.05$) with increasing dietary PB concentration. However, Clostridia ($P < 0.01$) and *Firmicutes* ($P < 0.05$) were linearly increased with increasing PB concentration. Predominant GI genera among methanogens were *Methanobrevibacter* (75, 72, and 49%), *Akkermansia* (17, 23, and 41%), *Methanosphaera* (3.3, 2.3, and 3.4%), and *Methanobacteriaceae* (1.2, 0.6, and 0.7%) population in control, 15, and 30% PB, respectively, and they were linearly decreased or increased with increasing PB concentration ($P < 0.05$). Other 22 GI fecal MA genera population (<1%) varied among treatments. These results indicated that feeding PB selectively reduced bacteria and main MA populations in the GI tract of meat goats.

Key Words: bacteria, methanogenic archaea, pine bark

797 Effect of polymer-coated urea and sodium bentonite on digestibility, nitrogen retention and rumen fermentation in sheep fed high levels of corn stalk. A. R. Chegeni[1,2], Y. L. Li*[1], C. G. Jiang[1], Q. Y. Diao[1], *[1]Feed Research Institute, Chinese Academy of Agricultural Sciences, Beijing, China, [2]Lorestan Agricultural and Natural Resources Research Center, Khorramabad, Lorestan, Iran.*

The experiment of this study was conducted to evaluate the effect of polymer-coated urea (Optigen II, Alltech Inc., Nicholasville, KY) and sodium bentonite (SB) on nutrient digestibility, nitrogen retention and rumen fermentation in sheep fed high level of corn stalk. Four ruminally cannulated thin-tailed Han × Dorper crossbreed wethers were used in a 4 × 4 Latin square design experiment with 4 isonitrogenous and isocaloric diets composed with 60% corn stalk (DM basis) and 40% concentrate (DM basis). The 4 treatments were control (CON), PCU (replacing soybean meal with 1.8% Optigen on control diet), SB (supplementing 2% SB on CON diet), and PCUSB (supplementing 2% SB on PCU diet). The objective of this study was to evaluate if Optigen could be used as a substitution for soybean meal and if SB could improve N utilization. There were no differences in DMI for different treatments. The digestibilities of OM ($P = 0.02$) and nitrogen ($P < 0.01$) for PCU were greater than those in other treatments. The fecal N were numerically lower ($P = 0.09$) for treatments with Optigen (PCU and PCUSB), and nitrogen retentions were numerically ($P = 0.09$) greater. The pH and total VFA were not influenced by different treatments, whereas the proportions of propionate were greater ($P < 0.01$) for treatments with Optigen than those without Optigen. The concentrations of NH_3-N in sheep fed Optigen were greater ($P < 0.05$) at the beginning after feeding, but decreased to the same level with other treatments at the time of 7h. The results showed that using Optigen as N source to replace soybean meal in sheep fed high levels of corn stalk improved parts nutrient digestibilities, likewise increased nitrogen retention and the proportion of propionate. However, adding SB to Optigen had no further beneficial effects on N utilization. It is concluded that Optigen could be used as a substitution for soybean meal in sheep fed high level of corn stalk and had no negative effect on nutrient digestibility, nitrogen retention and rumen fermentation.

Key Words: polymer-coated urea, sheep, sodium bentonite

798 Essential oils modify rumen bacterial compositions in vitro as revealed by microarray analysis. A. K. Patra[1,2] and Z. Yu*[1], *[1]The Ohio State University, Columbus, [2]West Bengal University of Animal and Fishery Sciences, Kolkata, West Bengal, India.*

This experiment was conducted to examine the effect on rumen bacterial composition of 3 different types of essential oils (EOs): origanum oil (ORO), garlic oil (GAO) and peppermint oil (PEO), using a rumen microarray that was developed recently in our laboratory. Each of the EOs was used at 0.50 g/L of the fermentation medium. The number of bacterial operational taxonomic units (OUTs) in the phylum *Firmicutes* was lowered, especially in the class Clostridia by ORO and GAO, but increased by PEO compared with control. The number of OTUs in the genus *Butyrivibrio* was lowered by all the EOs. However, OTUs in the phylum *Bacteroidetes* were increased by ORO and PEO, but not affected by GAO. Increases of OTUs in *Bacteroidetes* mainly resulted from increases of Prevotella OTUs. Overall, 113 individual OTUs showed significant differences ($P \leq 0.10$) among the EOs. Among the predominant OTUs, some OTUs assigned to *Syntrophococcus sucromutans*, *Lachnospiraceae incertae sedis*, and unclassified *Ruminococcaceae* were decreased, while others classified to *Succiniclasticum ruminis*, *Prevotella*, and unclassified *Bacteroidales*, *Lachnospiraceae*, and *Prevotellaceae* were increased markedly by ORO. Garlic oil increased some OTUs related to *S. ruminis*, *Prevotella*, *Clostridium*, *Mogibacterium*, and unclassified *Ruminococcaceae*, while decreasing some OTUs of *Lachnobacterium bovis* and *Bacillus* substantially. For PEO, some OTUs belong to *S. sucromutans*, *S. ruminis*, and unclassified *Ruminococcaceae* and *Lachnospiraceae* were decreased, but other OTUs mainly related to *Roseburia*, *Prevotella*, *Pseudobutyrivibrio*, and unclassified *Ruminococcaceae*, *Lachnospiraceae*, *Clostridiales* and *Bacteroidales* were increased notably. However, principal component analysis indicated that PEO resulted in a distinct ruminal bacterial community, but not GAO or ORO. In conclusion, this study demonstrated that EOs can affect the population dynamics of several bacteria, especially those in the families *Prevotellaceae*, *Lachnospiraceae* and *Ruminococcaceae* depending upon the EO types, resulting in modification on rumen fermentation.

Key Words: essential oil, rumen bacterial composition, microarray

799 Chemical composition and digestion kinetic of urea-molasses treated wheat straw ensiled with exogenous enzyme in ruminally cannulated buffalo bulls. M. Nisa, M. Sarwar, O.A. Khan*, A. Rehman, and M. A. Shazad, *Institute of Animal Nutrition and Feed Technology, University of Agriculture Faisalabad, Faisalabad, Punjab, Pakistan.*

Two experiments were conducted to evaluate the chemical composition and digestion kinetics of urea molasses treated wheat straw (UMWS) ensiled with varying level of exogenous fibrolytic enzyme. Thirty-six laboratory silos, 9 each in completely randomized design of UMWS were treated with 4% urea and 6% molasses with 0 (E0), 1 (E1), 2 (E2) and 3 g (E3) enzyme /kg UMWS on DM for 21 d. Dry matter, OM, NDF, ADF, crude protein, true protein and pH were analyzed. For digestion kinetics, 4 ruminally cannulated Nili Ravi buffalo bulls were used in 4 × 4 Latin square design. The 4 test diets were placed in the rumen of bulls alternatively during 4 periods of 5 d each. Fractional digestion rate was calculated by regressing natural log of potentially digestible fraction remaining at different time intervals over time. The data were analyzed using GLM procedures of SAS. Dry matter, NDF, ADF, crude protein and true protein of UMWS ensiled without or with enzyme remained unaltered. The pH of UMWS without or with enzyme ranged from 8.42 to 8.47 and remained unchanged across all treatments. The lag time, digestion rate, in situ digestibility and extent of digestion of DM, NDF and ADF did not change across all treatments. In conclusion, ensiling UMWS with enzyme did not influence its chemical composition and digestion kinetics.

Key Words: digestion kinetic, exogenous enzyme

800 Effects of nitrate, saponins, sulfate, and their combinations on rumen methanogenesis, fermentation and microbial communities in vitro. A. K. Patra*[1,2] and Z. Yu[1], [1]*The Ohio State University, Columbus,* [2]*West Bengal University of Animal and Fishery Sciences, Kolkata, West Bengal, India.*

This study investigated the anti-methanogenic effects of quillaja saponins (QS), nitrate, and sulfate, and their effects on rumen fermentation and ruminal microbial communities in vitro. Nitrate (5 m*M*), sulfate (5 m*M*), and QS (0.6 g/L) were tested individually and in all possible combinations. All the treatments (8 in total) except sulfate decreased methane production compared with control. Sulfate and its combinations with nitrate or QS did not show cumulative depression in methane production. In contrast, combinations of nitrate with QS or/and sulfate additively lowered methane production, with the lowest (45.7% reduction) methanogenesis caused by the combination of QS, nitrate and sulfate. The combination of these 3 compounds did not affect dry matter or fiber digestion. Concentrations of total volatile fatty acids and molar percentage of acetate were not affected by any of the treatments. Inclusion of QS in the medium, except when nitrate was also added, increased ($P < 0.05$) the molar percentages of propionate. Conversely, molar percentages of butyrate were lowered ($P < 0.05$) by the combination of QS with the other 2 inhibitors. The acetate to propionate ratios were lower ($P < 0.05$) than control when QS and sulfate were added together. Neither any of the compounds nor their combinations altered the abundances of total bacteria or *Ruminococcus albus*. Adding QS alone or together with nitrate or/and sulfate stimulated the growth of *Fibrobacter succinogenes* and *Ruminococcus flavefaciens*, whereas nitrate and sulfate did not. Rumen archaeal populations did not differ among the treatments except between nitrate and the combination of all 3 compounds. Addition of QS, either individually or in combinations, inhibited ($P < 0.05$) the growth of protozoa. The archaeal and bacterial diversity differed ($P < 0.05$) among the treatments. This study demonstrated that feeding QS, nitrate and sulfate together at low concentrates might effectively decrease methanogenesis in an additive manner, while not adversely affecting, even improving, rumen fermentation characteristics.

Key Words: saponin-nitrate-sulfate combination, methanogenesis, rumen fermentation