

Rumen ecology of Red Sokoto goats fed diets containing varying levels of soybean milk residue as a replacement for soybean meal

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Received 21st September 2024; Accepted 13th October 2024

ABSTRACT: In this study, 25 weaned bucks weighing 8.40kg were used to evaluate rumen kinetics (rumen pH, bacteria, fungi, protozoa, NH₃-N production and volatile fatty acids production) of Red Sokoto bucks at 30, 60 and 90 days of feeding diets containing varying levels of soybean milk residue as a replacement for soybean meal. The diets were designated T1, T2, T3, T4, and T5. The Red Sokoto goats were fed diets containing varying levels of soybean milk residue with *Ficus lyrata* as a basal diet at 30, 60 and 90 days of the experiment. Complete Randomized Design (CRD) was used in this experiment. Rumen pH did not change significantly throughout the experiment but fluctuated around 6.03 and 6.56. NH₃-N production in the rumen reduced at 60 days but picked up at 90 days. Volatile fatty acids also maintained a similar production profile throughout the experiment. Protozoa, fungi and bacteria counts reduced at 60 days but increased at 90 days. Protozoa counts were highest for bucks receiving diets containing 100% soybean milk residue throughout the experiment. Fungi count was highest in T3 (28.03) for buck receiving diets containing 50% soybean milk residue at 30 days and T2 (21.46) for buck receiving diets containing 25% soybean milk residue at 90 days. The least bacteria count was recorded in T4 (9.61) for buck receiving diets containing 75% soybean milk residue at 30 days. The highest bacteria count was recorded in T5 (41.16) for buck receiving diets containing 100% soybean milk residue at 90 days. Soybean milk residue supplementation did not cause any disruption in the rumen ecology.

Keywords: pH, microbes, NH₃-N, rumen, soybean milk residue, volatile fatty acids.

INTRODUCTION

There is an alarming shortage of meat in the daily diet of an average Nigerian due to the high cost of feeding during animal production which resulted in the high cost of meat products. Feed cost accounts for between 50% and 75% of the total cost of production (Orayaga, 2016). Cheaper alternative sources of feedstuff for livestock that will reduce feeding costs and boost production for the utmost product supply are important to improve animal protein intake and sustain the livestock sector (Ahamefule *et al.*, 2001; Esonu *et al.*, 2003). Hence, the solution to high feed

costs in animal production may lie in the use of farm or agro-industrial by-products such as soybean milk residue to replace the already expensive soybean meal. Soybean milk residue is the residue (sludge) remaining after extracting juice from ground soybeans. It is rich in protein and fibre but usually dumped on refuse sites after soymilk extraction (Li *et al.*, 2013). It is readily available and cheap in towns and villages where cheese is widely produced and eaten (Iyeghe-Erakpotobor, 2010). A conscious effort to properly channel this seemingly good quality feedstuff for

feeding ruminants like goats will enhance additional production value. Ruminants have evolved to possess a diverse symbiotic microbiota in their rumen, mainly consisting of bacteria, archaea, ciliated protozoa, fungi, and viruses (Firkins and Yu, 2015). These microbiotas are associated with ruminant production and health traits such as feed efficiency (Li and Guan, 2017), methane (CH₄) yield (Wallace *et al.*, 2015), milk composition (Jami *et al.*, 2014), and ruminal acidosis (McCann *et al.*, 2016). Digestion of plant fibre are potential target for manipulation to improve ruminant productivity as well as to reduce CH₄ emissions (Li *et al.*, 2019). Maintaining rumen pH is very important for the persistence and stability of the gut microbiota (Franzolin and Dehority, 2010). Rumen NH₃-N is the major source of rumen nitrogen and the end product of ruminal proteolysis (Ikyume, 2021). Rumen volatile fatty acids (VFA) have long been recognized as important intermediate compounds in the energy utilization of ruminants (Hamada *et al.*, 1985). Rumen microbial fermentation transforms feed components into useful VFAs (Ikyume, 2021). This study is, therefore, aimed at evaluating the stability of the rumen pH, NH₃-N and VFAs production, bacteria, fungi and protozoa of Red Sokoto bucks when fed soybean milk residue as a replacement for soybean meal.

MATERIALS AND METHODS

This research was carried out in the Teaching and Research Farm, and Biochemistry Laboratory of Federal Polytechnic, Bali B ward, Bali Local Government Area (LGA) of Taraba State, Nigeria. Bali covers a total land area of about 5,500 km and extends between latitude 8° 35' 00" North of the equator and 10° 46' 00" East of the Greenwich Meridian (Taraba State Government, 2020). Whole maize grains are sourced and crushed without dehulling. Maize offals were sourced from millers. Soybean milk residues were bought from sellers of soybean milk. The residues were dried properly and crushed. Soybean meal was bought from AFCOT Oil Mill, Ngurore, Adamawa State. The SBM is produced after milling out vegetable oil from full-fat soybeans. Twenty-five (25) Red Sokoto bucks were assigned to the treatment diets using a completely randomized design. Each treatment was assigned 5 bucks with each buck representing a replicate. 70 ml of rumen samples were collected from each buck at 30, 60 and 90 days six hours post feeding using a stomach suction tube according to the procedure by Babayemi and Bamikole (2006). Immediately after sample collection, rumen pH was determined using a pH meter (Universal pH Test Kit- Digital pH Meter®). The samples were then filtered using a muslin with four layers. Each sample filtrate was divided into three equal portions. One portion was placed in a test tube to which 1 ml sulphuric acid has been added to stop microbial

fermentation and then centrifuged for about 10 minutes. The supernatant was collected and used to analyze NH₃-N using the Kjeldahl distillation and titration process according to AOAC (2005) method.

$$\text{NH}_3 - \text{N} = \frac{\text{Titre value} \times 0.1 \times 17.1 \times 100}{10}$$

A second portion of the filtered sample was used for microbial count. The protozoan population was determined by direct observation using a magnified (x10) microscope. Bacteria and fungi colonies were determined using nutrient agar and potato dextrose agar respectively. The agar plate was incubated for 24 hours at room temperature after which all colonies formed were counted using a digitally illuminated colony counter. The third portion of the filtered sample was used to determine the total Volatile Fatty Acid (VFA) by titration. Rumen liquor was titrated with 0.1N NaOH solution in the presence of an indicator to express the VFA content of the rumen. This method is a modification of the conventional potentiometric titration method. NaOH was matched with VFA content in the samples (Siedlecka *et al.*, 2008). All data collected in this study were subjected to Analysis of variance (ANOVA) appropriate using Minitab16 (2004) statistical software. Significant differences between treatment means were separated by Fisher's least square difference using the same statistical package.

RESULTS AND DISCUSSION

The diets were designated as T1, T2, T3, T4, and T5 as seen in Table 1. Tables 2, 3 and 4 show the rumen ecology of Red Sokoto goats fed diets containing varying levels of soybean milk residue with *Ficus lyrata* as a basal diet at 30, 60 and 90 days of the experiment.

The results obtained show that there was a significant difference ($p < 0.05$) in the rumen pH values at 30 days of feeding and no significant difference ($p > 0.05$) at 60 and 90 days of feeding soybean milk based diets. The rumen pH is within the 6-6.4 (Franzolin and Dehority, 2010), 6.33-6.50 (Ikyume *et al.*, 2017) and 6.8-7.5 (Falk *et al.*, 2016) range acceptable for rumen functioning. Stability in the rumen pH may be due to the rate of absorption of short-chain fatty acids (Aeschbacher *et al.*, 2010). An increase in rumen pH above 7.0 may make the rumen intolerable for fibrolytic bacteria (Roger *et al.*, 1990) and reduce rumen microbial proliferation (van Soest, 1994).

Soybean milk inclusion did not affect ($p > 0.05$) the rumen ammonia nitrogen (NH₃-N) at 30 and 60 days of feeding except ($p < 0.05$) at 90 days which is similar to the findings of Terry *et al.* (2018) and Ikyume (2021) when they fed humate diets to heifers and West African dwarf goats, respectively. However, Ikyume (2021) observed a significant difference in NH₃-N at 90 days of feeding humate diets.

Table 1. Composition of experimental diet.

Ingredients	Experimental diet				
	T1	T2	T3	T4	T5
Maize	5.00	5.00	5.00	5.00	5.00
Sorghum chaff	10.00	10.00	10.00	10.00	10.00
Maize offal	57.74	57.74	57.74	57.74	57.74
SBMR	0.00	6.06	12.13	18.20	24.26
SBM	24.26	18.20	12.13	6.06	0.00
Bone ash	2.00	2.00	2.00	2.00	2.00
Common salt	1.00	1.00	1.00	1.00	1.00
Total	100	100	100	100	100
Calculated analysis %					
Crude Protein	18.00	16.31	14.62	12.92	11.23
Crude Fibre	7.11	7.30	7.49	7.69	7.84
Ether Extract	3.26	3.39	3.53	3.66	3.79
Ash	4.63	4.46	4.29	4.12	3.95
NFE	67.00	68.60	70.07	71.67	73.19
ME (Kcal/kg)	2477.50	2401.37	2325.12	2248.84	2172.71

SBMR= soybean milk residue, SBN=soybean meal, NFE= nitrogen free extract, ME= metabolizable energy. T1= 0% Soybean milk residue, T2= 25% Soybean milk residue, T3= 50% Soybean milk residue, T4= 75% Soybean milk residue, T5= 100% Soybean milk residue.

Table 2. Rumen ecology (at 30 days) of Red Sokoto goats fed diets containing varying levels of soybean milk residue with *Ficus 3yrate* as basal diet.

Parameters	Treatments					SEM	P- value
	T1	T2	T3	T4	T5		
pH	6.14 ^b	6.30 ^{ab}	6.36 ^{ab}	6.28 ^b	6.56 ^a	0.07	0.0036
NH ₃ -N (g100 ⁻¹)	21.89	19.54	21.78	21.14	18.31	0.90	0.0447
TVFA (mmol/100mol)	7.13	7.07	7.06	6.90	6.88	0.08	0.1756
Acetate (mmol/100mol)	3.37	3.27	3.35	3.29	3.24	0.05	0.3119
Propionate (mmol/100mol)	2.25	2.17	2.19	2.18	2.15	0.03	0.3567
Butyrate (mmol/100mol)	0.33	0.30	0.32	0.30	0.29	0.02	0.2887
A:P	1.50 ^d	1.51 ^{bc}	1.53 ^a	1.51 ^b	1.50 ^{cd}	0.00	0.0001
Protozoa (x10 ³ cell/ml)	34.37 ^b	37.46 ^{ab}	38.06 ^{ab}	39.45 ^a	40.39 ^a	1.12	0.0119
Fungi (cfu.ml)	16.28 ^d	19.29 ^c	28.03 ^a	20.54 ^c	24.47 ^b	0.40	0.0001
Bacteria (cfu.ml)	10.44 ^{cd}	20.09 ^a	12.46 ^c	9.61 ^d	15.17 ^b	0.50	0.0001

^{abcd}Means with different superscript within the same row differ significantly (P<0.05). NH₃-N= ammonia nitrogen, TVFA= total volatile fatty acids, A:P= acetate-propionate ratio.

The concentration of ammonia nitrogen in the rumen depends on the diet, reaches its peak at 2-4 hours post-feeding (Wang *et al.*, 2009) and could be affected by deamination (Wallace, 2004). According to Wanapat *et al.* (2009), an increase in NH₃-N absorption by rumen epithelium could reduce its concentration in the rumen. Rumen NH₃-N is the major source of rumen nitrogen and the end product of ruminal proteolysis (Ikyume, 2021).

Rumen volatile fatty acids (VFA) are intermediate

compounds associated with energy utilization by ruminants (Ikyume *et al.*, 2017). Non-significant effects (p>0.05) observed for total volatile acid at 30 and 60 days of feeding soybean milk diets are in line with the observations of Terry *et al.* (2018) and Ikyume (2021) and the significant effects (p<0.05) in acetate, butyrate and propionate throughout the feeding trial and the total volatile acid values obtained at 90 days of feeding soybean milk diets are consistent with the report of Ogunbosoye *et al.*

Table 3. Rumen ecology (at 60 days) of Red Sokoto goats fed diets containing varying levels of soybean milk residue with *Ficus lyrata* as basal diet.

Parameters	Treatments					SEM	P- value
	T1	T2	T3	T4	T5		
pH	6.19	6.10	6.22	6.18	6.44	0.12	0.4145
NH ₃ -N (g100 ⁻¹)	19.92	14.59	19.25	18.97	15.8	1.40	0.0534
TVFA (mmol/100mol)	7.59	7.36	7.63	7.85	7.44	0.12	0.0641
Acetate (mmol/100mol)	3.30 ^b	3.62 ^{ab}	3.62 ^{ab}	4.00 ^a	3.40 ^b	0.10	0.0015
Propionate (mmol/100mol)	2.21 ^b	2.49 ^{ab}	2.40 ^{ab}	2.66 ^a	2.35 ^b	0.07	0.0028
Butyrate (mmol/100mol)	0.29 ^b	0.34 ^{ab}	0.33 ^{ab}	0.39 ^a	0.30 ^b	0.02	0.0055
A:P	1.49 ^{ab}	1.45 ^b	1.51 ^a	1.50 ^a	1.44 ^b	0.01	0.0017
Protozoa (x10 ³ cell/ml)	28.51	29.52	30.85	31.98	33.30	1.41	0.1653
Fungi (x10 ³ cell/ml)	12.96 ^c	11.83 ^c	15.56 ^{ab}	16.74 ^a	13.62 ^{bc}	0.54	0.0001
Bacteria (x10 ³ cell/ml)	22.38	16.64	12.87	11.08	17.22	0.88	0.0001

^{abc}Means with different superscript within the same row differ significantly (P<0.05). NH₃-N= ammonia nitrogen, TVFA= total volatile fatty acids, A:P= acetate-propionate ratio.

Table 4. Rumen ecology (at 90 days) of Red Sokoto goats fed diets containing varying levels of soybean milk residue with *Ficus lyrata* as basal diet.

Parameters	Treatments					SEM	P- value
	T1	T2	T3	T4	T5		
pH	6.03	6.27	6.26	6.22	6.43	0.15	0.4878
NH ₃ -N (g100 ⁻¹)	21.44 ^a	19.61 ^{ab}	20.77 ^a	16.49 ^c	17.07 ^{bc}	0.69	0.0001
TVFA (mmol/100mol)	7.34 ^a	6.39 ^c	6.60 ^{bc}	7.30 ^a	7.05 ^{ab}	0.12	0.0001
Acetate (mmol/100mol)	3.45 ^{ab}	3.13 ^c	3.17 ^{bc}	3.51 ^a	3.32 ^{abc}	0.07	0.0033
Propionate (mmol/100mol)	2.30 ^a	2.08 ^b	2.10 ^b	2.33 ^a	2.22 ^{ab}	0.04	0.0019
Butyrate (mmol/100mol)	0.31	0.32	0.29	0.32	0.31	0.01	0.2373
A:P	1.49	1.50	1.51	1.51	1.50	0.01	0.0559
Protozoa (x10 ³ cell/ml)	32.84	41.08	33.54	36.09	42.74	3.03	0.1106
Fungi (x10 ³ cell/ml)	17.04 ^b	21.46 ^a	16.64 ^b	10.70 ^d	13.66 ^c	0.14	0.0001
Bacteria (x10 ³ cell/ml)	36.18 ^c	39.55 ^b	32.42 ^e	33.58 ^d	41.16 ^a	0.27	0.0001

^{abcde}Means with different superscript within the same row differ significantly (P<0.05). NH₃-N= ammonia nitrogen, TVFA= total volatile fatty acids, A:P= acetate-propionate ratio.

(2022). The reduction in acetate, butyrate and propionate at 90 days of feeding agrees with the observation of El-Zaiat *et al.* (2018) under *in vivo* conditions. Most VFAs are absorbed across the reticulorumen wall, directly into the bloodstream, and are used by the ruminant as substrates for energy production and biosynthesis (Diao *et al.*, 2019). VFA proportions in the rumen depend largely on the diet fed to the animals (Ikyume, 2021). The acetate-propionate ratios were significant (p<0.05) at 30 and 60 days of feeding but there was no significant difference (p>0.05) among treatments at 90 days of feeding soybean milk diets. The values for the acetate-propionate ratios were similar to those reported by France and Dijkstra (2005) and Ikyume (2021).

Rumen protozoa counts varied among treatment diets at 30 days of feeding but did not vary at 60 and 90 days of

feeding soybean milk diets. The rumen protozoa counts were high at 30 days, reduced at 60 days and increased at 90 days. This may be due to stable rumen pH in those days. The availability of protozoa helps degrade and digest feed carbohydrates, especially starch, sugars and protein (McCann *et al.*, 2016). Fungi counts were significant (p<0.05) throughout the feeding trial. This is not consistent with the findings of Galip *et al.* (2010) and Ikyume (2021) when they fed humate to rams and WAD goats, respectively. The presence of rumen fungi is important because they hydrolyse some ester linkages between lignin and hemicellulose or cellulose, and help break down digesta particles (Li *et al.*, 2019). Bacteria count was not significant (p>0.05) at 30 days but had an effect (p<0.05) at 60 and 90 days. The reduction of bacteria at 60 days may be due to feed efficiency (Li and Guan, 2017)

methane (CH₄) yield (Wallace *et al.*, 2015) or ruminal acidosis (McCann *et al.*, 2016). Rumen bacteria preferentially digest structural carbohydrates, non-structural carbohydrates, and protein, respectively (Li and Guan, 2017).

Conclusion

Soybean milk residue supplementation did not cause any disruption in the rumen ecology and when supplemented up to 100% to replace soybean meal in the diets, ensured a very stable rumen ecology for goats.

Recommendation

Complete replacement of soybean meal by soybean milk residue in goat diets can support a healthy rumen and ultimate feed efficiency.

CONFLICT OF INTEREST

The authors declare that they have no conflict interest.

REFERENCES

- Aeschbacher, M., Sander, M., & Schwarzenbach, R. P. (2010). Novel electrochemical approach to assess the redox properties of humic substances. *Environmental science & technology*, 44(1), 87-93.
- Ahamefule, F. O., Ibeawuchi, J. A., Ibemere, J. C., & Okpara, M. (2001). Digestibility and nitrogen balance studies in West African Dwarf sheep fed fortified cassava peel meal-poultry waste diets. *Journal of Applied Chemistry and Agricultural Research*, 7(1), 69-74.
- AOAC (2005). Official Methods of Analysis. Association of Official Analytical Chemists. 16th Edition. William Try Press. Richard Virginia USA. pp17-34.
- Babayemi, O. J., & Bamikole, M. A. (2006). Effect of *Tephrosia candida* leaf and its mixtures with guinea grass on *in vitro* fermentation changes as feed for ruminants in Nigeria. *Pakistan Journal of Nutrition*, 486(5), 14-18.
- Diao, Q., Zhang, R., & Fu, T. (2019). Review of strategies to promote rumen development in calves. *Animals*, 9(8), 490.
- El-Zaiat, H. M., Morsy, A. S., El-Wakeel, E. A., Anwer, M. M., & Sallam, S. M. (2018). Impact of humic acid as an organic additive on ruminal fermentation constituents, blood parameters and milk production in goats and their kids growth rate. *Journal of Animal & Feed Sciences*, 27(2), 105-113.
- Esonu, B. O., Theukwumere, F. C., Iwuji, T. C., Akanu, N., & Nwugo, O. H. (2003). Evaluation of *Microdesmis puberula* leaf meal as feed ingredient in broiler starter diets. *Nigerian Journal of Animal Production*, 30(1), 3-8.
- Falk, M., Munger, A., & Dohme-Meier, F. (2016). A comparison of reticular and ruminal pH monitored continuously with 2 measurement systems at different weeks of early lactation. *Journal of Dairy Science*, 99(3), 1951-1955.
- Firkins, J. L., & Yu, Z. (2015). Ruminant nutrition symposium: how to use data on the rumen microbiome to improve our understanding of ruminant nutrition. *Journal of Animal Science*, 93(4), 1450-1470.
- France, J., & Dijkstra, J. (2005). Volatile fatty acid production. In Quantitative aspects of ruminant digestion and metabolism. Dijkstra, J., Forbes, J. M., & France, J. (eds.). Second Edition, CABI Publishing, Wallingford, UK. Pp. 157-176.
- Franzolin, R., & Dehority, B. A. (2010). The role of pH on the survival of rumen protozoa in steers. *Revista Brasileira de Zootecnia*, 39(10), 576-581.
- Galip, N., Polat, U., & Biricik, H. (2010). Effects of supplemental humic acid on ruminal fermentation and blood variables in rams. *Italian Journal of Animal Science*, 9(4), e74.
- Ikyume, T. T. (2021). Performance and welfare parameters of semi-intensively managed West African Dwarf goats fed diets containing varying levels of sodium humate. A PhD Thesis submitted to the Department of Animal Production and Health, Federal University of Agriculture, Abeokuta, Nigeria. pp38-39.
- Ikyume, T. T., Sowande, O. S., Dele, P. A., Yusuf, A. O., Monday, S., Egunjobi, O. K., & Fatoba, O. (2017). Effect of varying levels of garlic (*Allium sativum*) powder on growth, apparent digestibility, rumen ecology, blood profile and cost analysis of feeding West African Dwarf goats. *Malaysian Journal of Animal Science*, 20(2), 61-74.
- Iyeghe-Erakpotobor, G. T. (2010). Performance of grower rabbits fed concentrate and stylosanthes (verano) combinations under tropical conditions. *Animal Science Journal*, 77(1), 71-78.
- Jami, E., White, B. A., & Mizrahi, I. (2014). Potential role of the bovine rumen microbiome in modulating milk composition and feed efficiency. *PloS one*, 9(1), e85423.
- Li, F., & Guan, L. L. (2017). Metatranscriptomic profiling reveals linkages between the active rumen microbiome and feed efficiency in beef cattle. *Applied and Environmental Microbiology*, 83(9), e00061-17.
- Li, F., Li, C., Chen, Y., Liu, J., Zhang, C., Irving, B., Fitzsimmons, C., Plastow, G., & Guan, L. L. (2019). Host genetics influence the rumen microbiota and heritable rumen microbial features associate with feed efficiency in cattle. *Microbiome*, 7, article number 92.
- Li, S., Zhu, D., Li, K., Yang, Y., Lei, Z., & Zhang, Z. (2013). Soybean curd residue: Composition, utilization, and related limiting factors. *International Scholarly Research Notices*, 2013(1), 423590.
- McCann, J. C., Luan, S., Cardoso, F. C., Derakhshani, H., Khafipour, E., & Looor, J. J. (2016). Induction of subacute ruminal acidosis affects the ruminal microbiome and epithelium. *Frontiers in Microbiology*, 7, article number 701.
- Minitab (2014). Minitab statistical software version 16. Retrieved from <http://www.amazon.com>
- Ogunbosoye, D. O., Taye-Olurotimi, A., & Abayomi, A. (2022). Influence of ensiled guinea grass-cassava peels enriched with soybean waste on performance of WAD goats. *Turkish Journal of Agriculture-Food Science and Technology*, 10(12), 2397-2404.
- Orayaga, K. T. (2016). Effects of composite mango (*Mangifera indica*) fruit reject meal on growth performance, digestibility and economics of production of rabbits. *Nigerian Journal of Animal Science*. 18(1), 65-75.
- Roger, V. R., Fonty, G., Komisarczuk-Bondy, S., & Gouet, P. (1990). Effects of physiochemical factors on the adhesion to cellulose avicel of the rumen bacteria *Ruminococcus*

- flavefaciens* and *Fibrobacter succinogenes*. *Applied Environmental Microbiology*, 56, 3081-3087.
- Siedlecka, E. M., Kumirska, J., Ossowski, T., Glamowki, P., Golebiowski, M., Gajdus, J., Kacznski, Z., & Stepnowski, P. (2008). Determination of volatile fatty acids in environmental aqueous samples. *Polish Journal of Environmental Studies*, 17(3), 351-356.
- Taraba State Government (2020). Climate bulletin of Taraba State. Ministry of Environment, Jalingo, Taraba State.
- Terry, S. A., Ribeiro, G. O., Gruninger, R. J., Hunerberg, M., Sheng, P., Alex, V. C., Burllet, J., Beauchemin, K. A., & McAllister, T. A. (2018). Effect of humic substances on rumen fermentation, nutrient digestibility, methane emissions, and rumen microbiota in beef heifers. *Journal of Animal Sciences*, 96(1), 3863-3877.
- Van Soest, P. J (1994). Nutritional ecology of the ruminant. 2nd Edition. Cornell University Press. Ithaca, NY, USA.
- Wallace, R. J. (2004). Antimicrobial properties of plant secondary metabolites. *Proceedings of the Nutrition Society*, 63(4), 621-629.
- Wallace, R. J., Rooke, J. A., McKain, N., Duthie, C. A., Hyslop, J. J., Ross, D. W., Waterhouse, A., Watson, M., & Roehe, R. (2015). The rumen microbial metagenome associated with high methane production in cattle. *BMC Genomics*, 16, article number 839.
- Wanapat, M., Polyrach, S., Boonnop, K., Mapato, C., & Cherdthong, A. (2009). Effect of treating rice straw with urea and calcium hydroxide upon intake, digestibility, rumen fermentation and milk yield of dairy cows. *Livestock Science*, 125, 238-243.
- Wang, C. J., Wang, S. P., & Zhou, H. (2009). Influences of flavomycin, ropadiar, and saponin on nutrient digestibility, rumen fermentation, and methane emission from sheep. *Animal Feed Science and Technology*, 148(2-4), 157-166.