

Effects of Spent Craft Brewers' Yeast on Fermentation and Methane Production by Rumen Microorganisms

Virginia L. Pszczolkowski¹, Robert W. Bryant², Brittany E. Harlow³, Glen E. Aiken³,
Langdon J. Martin¹, Michael D. Flythe^{3,4}

¹Department of Chemistry, Warren Wilson College, Asheville, NC, USA

²Asheville Flavor Innovations, LLC, Asheville, NC, USA

³Forage-Animal Production Research Unit, Agricultural Research Service, USDA, Lexington, KY, USA

⁴Department of Animal and Food Sciences, University of Kentucky, Lexington, KY, USA

Email: michael.flythe@ars.usda.gov

Received 15 June 2016; accepted 14 August 2016; published 17 August 2016

Copyright © 2016 by authors and Scientific Research Publishing Inc.

This work is licensed under the Creative Commons Attribution-NonCommercial International License (CC BY-NC).

<http://creativecommons.org/licenses/by-nc/4.0/>



Open Access

Abstract

Saccharomyces cerevisiae is a key component of beer brewing and a major by-product. The leftover, spent brewers' yeast from large breweries has been used as a protein supplement in cattle; however the possible advantages of spent yeast from smaller craft breweries, containing much higher levels of bioactive hop acids, have not been evaluated. Hops secondary metabolites from the hops (*Humulus lupulus* L.) used to make beer are concentrated in the yeast during brewing, and have antimicrobial activity against Gram-positive bacteria. Uncultivated suspensions of bovine rumen microorganisms produced less methane during fructose fermentation when exposed to inactivated, and freeze-dried spent craft brewers' yeast than a bakers' yeast control. The experiment was repeated with caprine rumen microorganisms and ground grass hay as the substrate. Likewise, in the presence of craft brewers' yeast less methane was produced (2.7% vs. 6.9% CH₄). Both experiments also revealed a decrease in acetic acid production, but not propionic acid production, when craft brewers' yeast was included. These results indicated that spent yeast could represent a co-product for craft breweries, and a feed supplement for ruminants that has a favorable impact on methane production.

Keywords

Antimicrobials, Brewing, Co-Product, Feed Supplement, Feed Efficiency, Hops Acids, *Humulus Lupulus*, Plant Secondary Metabolite, Phytochemicals

1. Introduction

The multiplication of yeast during fermentation was one observation that led Pasteur to propose their centrality in that process [1]. Yeast growth during beer fermentation is considerable, such that large amounts remain beyond what is needed for seeding the next fermentation. This excess spent brewers' yeast has long been fed to cattle [2], and there are now many commercial feed supplements that include *Saccharomyces cerevisiae*. The reported benefits for ruminants range from increased micronutrients to modulation of rumen fermentation [3]. One of the simplest benefits of yeast for ruminants might be that yeast is rich in protein. However, the differences between commercial feed additives and spent yeast from brewing, and how those different components could interact with the complexities of ruminant digestion, should be considered.

Ruminants are distinguished by their complex digestive tracts, particularly the pre-gastric rumen, which acts as a fermentation chamber. Like all mammals, ruminants lack the enzymes necessary to digest fiber (*i.e.*, cellulose, hemicellulose) and instead rely on an internal ecosystem of anaerobic microorganisms to ferment these otherwise indigestible feed components [4]. Most microorganisms in the rumen and lower digestive tract use fermentation to fuel their cellular functions, and produce Short-Chain Fatty Acids (SCFAs) as a byproduct. The SCFAs, namely acetate, propionate, and butyrate, are subsequently absorbed through the rumen wall and metabolized by the host [5].

Methanogenesis is an important electron sink in the rumen ecosystem, but methanogens preclude full host use of carbohydrates by converting metabolites into methane (CH₄), which, unlike SCFAs, is not usable as a host energy source. Between 2% - 15% of feed energy is lost through methanogenesis, depending on the ruminant species and diet [6]. Ionophores and other antibiotics have long been a strategy for combating these losses in feed efficiency. Monensin is an ionophore that functions by dissipating the protonmotive force and ion transport, and inhibits H₂- and formate-producing bacteria, which ultimately leads to decreased methane production [7] [8]. Monensin also decreases ammonia production from feed protein, and taken together the improvement in feed efficiency saves U.S. cattle producers an estimated 1.4 million tons of feed per year.

Concern has risen over excessive antibiotic use in livestock production [9] [10]. Plant-derived alternatives to antibiotics have proven useful in inhibiting both ammonia production and methanogenesis [11]. Various saponins, tannins, essential oils, organosulfur compounds, and flavonoids have all been shown to have efficacy in this regard [12] [13]. Hops (*Humulus lupulus*) α - and β -acids are another suite of plant secondary metabolites shown to have antimicrobial properties against Gram-positive bacteria, including rumen bacteria [14] [15]. Up to 25% of the dry weight of hops consists of these acids [16]. The hydrophobic structures of the hops acids induce membrane perturbation, due to interactions via prenyl and aryl side chains [17], and thereby equilibrate trans-membrane ion gradients, such as pH and potassium [15]. Additionally, hops have been shown to decrease concentrations of acetate (used by some methanogens as a metabolic precursor to methane) and increase concentrations of propionate (a competitive pathway for hydrogen use) in the rumen [18]-[21].

The hops acids are found in the flower of the female hops plant, which is used in beer production to inhibit Gram-positive lactic acid bacteria that cause beer spoilage. During the brewing process, the α - and β -acids adsorb to the cell walls of the brewers' yeast such that their concentrations in yeast cells exceed that in the beer by greater than 100-fold [22]. This results in a significant proportion of the acids ending up in the spent yeast rather than in the finished beer. Furthermore, according to a study by Bryant and Cohen [22], while spent yeast from both multinational and craft breweries contain these acids, craft brewery spent yeast contains 6 times as much α - and β -acids: 2557 \pm 622 μ g/g in craft vs. 487 \pm 136 μ g/g in multinational. In addition to hops acids, spent brewers' yeast contains digestible proteins, several B-vitamins, and trace elements, which makes the yeast valuable as a nutritional supplement.

Raw spent brewers' yeast has been studied as a protein supplement for cattle [2], but the potential antimicrobial effects have not yet been compared with isolated hops acids. The first step toward determining the feasibility of using spent yeast from craft breweries as a source of antimicrobial compounds is to expose ruminal microorganisms to spent yeast *in vitro*. Two experiments were conducted to determine whether the residual hops secondary metabolites in spent craft brewers' yeast could inhibit methane production by rumen bacteria. The purpose of experiment 1 was to evaluate reproducibility using rumen digesta from a single steer and a simple substrate (fructose). The purpose of the second experiment was to replicate the results using rumen microorganisms from multiple animals (goats, $n = 4$) and a realistic substrate (grass hay).

2. Materials and Methods

2.1. Yeasts

Freeze dried craft brewers' yeast (CY) and bakers' yeast control (BY) were produced from spent byproduct from Gaelic Ale (Highland Brewing Company, Asheville, NC) and baking yeast (SAF Instant[®] Yeast-Red Label, Lasaffre Corporation, Milwaukee, WI), respectively. Spent CY was prepared as described elsewhere [22] with minor modifications. The chilled yeast slurry was taken from the fermentation tanks just prior to filtration and bottling. Slurries (approx. 10% solids) were stored at 4°C for less than 24 h before processing. The yeast were made non-viable by heating (62°C to 65°C, 3 min, open vessel). The slurry was chilled in an ice bath and centrifuged (2,850 × g) in an F9S-4 × 1000 y rotor for 10 min (Sorvall RC 6 Plus, Thermo Scientific) at 4°C to yield yeast pastes containing approximately 30% dry mater. In some cases, the pastes were re-suspended in 2 volumes of water and freeze-dried. The dried yeast was stored at -20°C. Non-viable dried baking yeast was prepared as described above starting with a 10% slurry in water of dry active yeast. Protein content was determined in triplicate by Bradford assay. The CY was approximately 43% protein, and the BY was not different. The amount of hops acids in the craft yeast preparation was determined via a previously described HPLC method [22] and was found to be 626 µg·g⁻¹ for iso-humulones, 4,129 µg·g⁻¹ for humulones and 76 µg·g⁻¹ in lupulone classes. No detectable hops acids were found in bakers' yeast.

2.2. Experiment 1

The basal suspension medium was prepared to contain (per L) 240 mg KH₂PO₄, 240 mg K₂HPO₄, 480 mg (NH₄)₂SO₄, 480 mg NaCl, 64 mg CaCl₂·2H₂O, 100 mg MgSO₄·7H₂O, and 600 mg cysteine·HCl; the pH was adjusted to 6.7 with NaOH. The suspension media was autoclaved (121°C, 20 min), then cooled under O₂-free argon.

Rumen fluid was collected from a slaughtered steer at Wells, Jenkins & Wells Abattoir (Forest City, NC). The rumen was opened with a razor blade and digesta removed by hand. Digesta (~2 L) was squeezed through 4 layers of cheesecloth into a 2 L Erlenmeyer flask, which was then capped with aluminum foil. The flask was kept warm, returned to the lab, and the flask was placed in a water bath at 39°C.

Eight flasks (100 mL) were prepared with freeze-dried yeast (four flasks CY 2% w/v, four flasks BY 2% w/v) and fructose at 1% w/v. Suspension media (5 mL) was added to each flask while gassing with O₂-free argon, except for the media-only control, which was filled with 10 mL media. The remaining nine flasks were then brought to 10 mL with rumen fluid and immediately capped with gas-tight rubber stoppers. The flasks were incubated in a water bath (39°C, 24 h). After the incubation was terminated, the flasks were stored unopened at ~4°C until methane analysis.

2.3. Experiment 2

Basal suspension medium was prepared as described above, except the broth was autoclaved (121°C, 20 min), cooled under O₂-free CO₂, and 4.0 g Na₂CO₃ was added (per liter) as a buffer. Instead of fructose, the fermentation substrate was timothy/orchardgrass, which was ground to pass through a 2 mm sieve. The nutritive content of the hay was (dry matter basis): 12.8% crude protein, 36.3% acid detergent fiber, and 56.3% neutral detergent fiber.

The University of Kentucky Animal Care and Use Committee approved animal husbandry and procedures involving the rumen fluid donor goats. The goats were maintained on same hay used in the media with free-choice access to water and a mineral mix. Rumen fluid was collected from 4 fistulated, 3-year-old Spanish goat wethers and filtered through cheesecloth into separate Erlenmeyer flasks, which were briefly held at 39°C.

Sidearm flasks (1 L, *n* = 8) were prepared with 60 g ground hay and 450 mL anaerobic suspension media in an anaerobic chamber (Coy, Grass Lake, MI; 95% CO₂, 5% H₂). Of these, 4 were prepared with 61 g pasteurized spent craft brewers' yeast (CY) paste and 4 with 61 g pasteurized bakers' yeast (BY) paste. The flasks were capped, labeled, and removed from the glove box. With the flasks under a CO₂ stream, rumen fluid from each goat (200 mL) was aliquoted into paired CY and BY flasks. The sidearm flasks were capped with ANKOM RF pressure monitoring units (ANKOM Technology, Macedonia, NY) and placed in a 39°C incubator.

Initial gas samples (6 mL) were taken from each flask using a 10 mL tuberculin syringe at approximately 1.5 hours into the fermentation and again at approximately 19.5 hours, at which point fermentation was terminated. Gas samples were injected into evacuated 9 mL crimp-top vials and stored at room temperature until analysis.

Fluid samples (2 mL) were collected, centrifuged at low speed to remove fiber, and supernatants were aliquoted and frozen until analysis.

2.4. Chemical Analyses

Methane was measured by GC-FID on an HP 6890 GC fitted with a Valco Instruments (Houston, TX) 30 m by 0.53 mm I.D. column packed with HayeSep-D porous polymer coated on 20 μm particles, a helium carrier flow rate of 4 ml/min, and oven maintained isothermally at 50°C. Injection volume was typically 1 ml, and methane eluted at 5 min and ethane at 11 min. A methane calibration standard, 850 ppm, was obtained from ShopCross, Greensboro, NC, however the amounts of methane produced by rumen fluid incubations were well above 1000 ppm so methane standard curves were prepared using house utility gas composed of methane (94.4%), ethane (3.6%), and higher hydrocarbons (0.2%) (PSNC Energy, Asheville, NC). Stoppered flasks (9 mL, 25 mL, 50 mL, 100 mL, 250 mL, 500 mL) were flushed with nitrogen to ambient pressure, capped, and 1 mL of the 94% methane standard was added to each with a gas-tight syringe, except for one other flask that was filled to ambient pressure with only the 94% methane. Headspace gas (1 mL) was removed from each incubation sample vial and standard curve vial using a 2 mL gas-tight syringe, and was injected without dilution into the instrument. The FID response was linear with $R^2 > 0.999$.

Short chain fatty acids (SCFA) were measured by GC-FID on an HP 6890 GC fitted with a 30 m by 0.32 mm Alltech Econocap Carbowax capillary column and an oven temperature gradient starting at 70°C ramping to 250°C over 23 min to capture well-separated peaks for all linear SCFAs from acetic to hexanoic acids, and also for isobutyric and isovaleric acids. Linear SCFAs were obtained from NuChek Prep (Elysian, MN) and isobutyric and isovaleric acids from Sigma-Aldrich. SCFAs were extracted with one volume of diethyl ether from acidified rumen fluid incubations [23] and 1 μl of the organic phase was injected. Hexanoic acid was used as an internal standard. Acetic acid, which had poor extraction properties, was also measured with an acetic acid UV test kit from Roche, #10148261035, obtained from R-Biopharm (Washington, MO).

A hand-held Vernier (Beaverton, OR) pH sensor using Vernier Logger Pro software was 2-point calibrated with pH 4.01 and 7.01 calibration solutions. Frozen Experiment 1 and Experiment 2 samples were thawed and agitated to mix contents. The samples were poured into narrow vials (7 mL) just large enough to fit the sensor. The sensor and vial were flushed with distilled water between each sample. Aliquots of bovine rumen fluid were taken to constant weight over a 90 min period in a 105°C oven and yielded a value of 1.6% dry matter.

2.5. Statistical Analyses

Gas Production curve analyses were performed using the MIXED procedure of SAS (v 9.3, SAS Institute, Inc. Cary, NY). The rate of gas production was analyzed as a continuous variable (1 - 20 h, 5 min increments; linear, quadratic and cubic regression coefficients), and treatment as a discrete variable using backward elimination stepwise regression analysis. Models containing interactions between treatments and regression coefficients were analyzed to determine significant ($P < 0.05$) linear, quadratic, and cubic regression coefficients for each treatment [24]. In the presence of a treatment \times time interaction, least square means for treatments were compared at each time point (1 - 20 h, 1 h increments) using the PDIFF option. Methane and SCFA data were analyzed in a one-way ANOVA with Tukey's test post hoc.

3. Results

3.1. Experiment 1

A broth medium containing fructose and either bakers' yeast (BY) or craft brewers' yeast (CY) was inoculated with bovine rumen fluid and incubated. Methane was produced in the sealed tubes with a high degree of reproducibility, and there was less CH₄ made in CY incubations than in BY ($P < 0.05$; **Table 1**). Less acetate and butyrate were produced in the CY incubations ($P < 0.05$; **Table 1**). However, propionate production was not affected ($P > 0.10$; **Table 1**).

3.2. Experiment 2

Gas was produced from ground hay by caprine rumen microorganisms during an overnight incubation (**Figure 1**).

Table 1. Methane and soluble products from fructose by bovine rumen microorganisms.

Product	Craft yeast treatment (CY)		Bakers' yeast treatment (BY)		Significance
	avg	sd	avg	sd	
Methane (ppm)	7670	274	10627	836	P < 0.05
Acetate (mM)	29.23	1.46	35.65	0.93	P < 0.05
Propionate (mM)	2.56	0.32	3.10	0.27	P > 0.10
Butyrate (mM)	2.56	0.29	3.23	0.27	P < 0.05
Ratio C3/C2	0.088		0.087		

avg, average; sd, standard deviation.

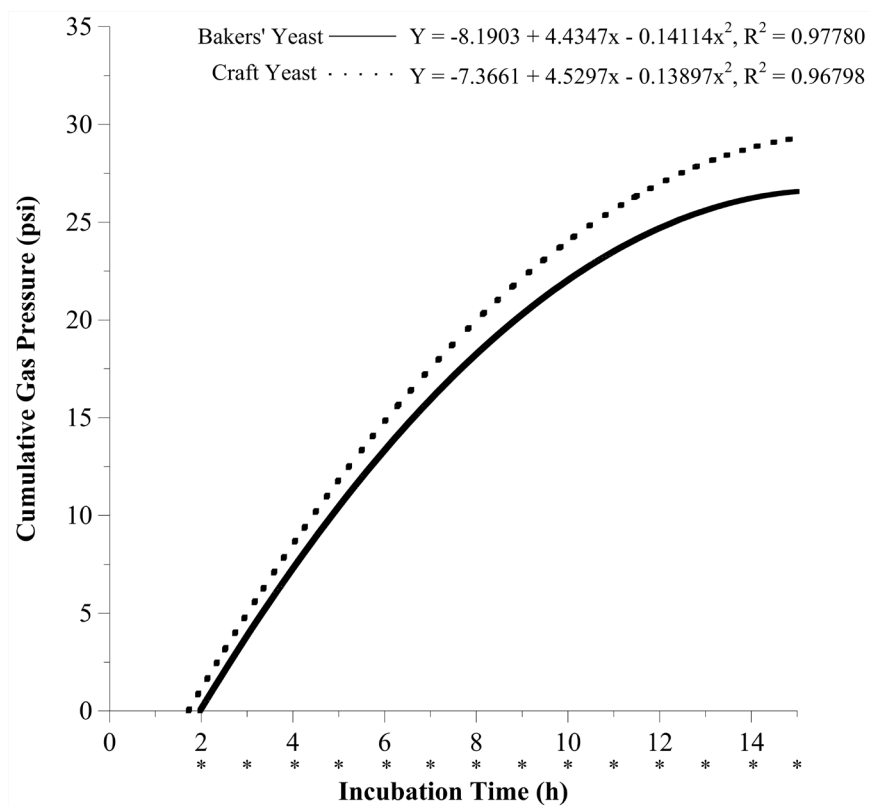


Figure 1. Cumulative gas production by caprine rumen microorganisms from ground hay with bakers' yeast control (solid line) or craft yeast treatment (hatched line). Asterisks indicate a significant difference between treatments within a time point ($P < 0.05$); Treatment: $P < 0.0001$, Pooled SEM: Treatment = 0.004152.

When means were separated out hourly from 1 - 20 h all except 1 h were significantly different between treatments (P values < 0.05) and gas production was greater in the CY treatments.

There was more variation in experiment 2 (4 animals, 1 incubation each), than in experiment 1 (1 animal, replicate incubations), which was expected. Once again, however, when methane was measured from samples taken at the end of the incubations, there was less CH_4 in the CY treatment than the BY treatment ($P = 0.079$; **Table 2**). Observationally, the rumen microorganisms from each goat made less CH_4 when fermenting with CY than with BY (**Figure 2**). Rumen microorganisms in both treatments produced acetate, propionate and butyrate. Less acetate was produced in CY than in BY ($P = 0.07$; **Table 2**). Propionate and butyrate were not affected by treatment ($P > 0.10$; **Table 2**).

Table 2. Methane and soluble products from ground hay by caprine rumen microorganisms.

Product	Craft yeast treatment (CY)		Bakers' yeast treatment (BY)		Significance
	avg	sd	avg	sd	
Methane (percent)	2.71%	1.69	6.94%	3.65	P = 0.079
Acetate (mM)	21.2	7.7	39.6	8.4	P = 0.07
Propionate (mM)	6.6	4.7	9.5	3.2	P > 0.10
Butyrate (mM)	1.5	0.6	3.4	3.8	P > 0.10
Ratio C3/C2	0.280		0.247		

avg, average; sd, standard deviation.

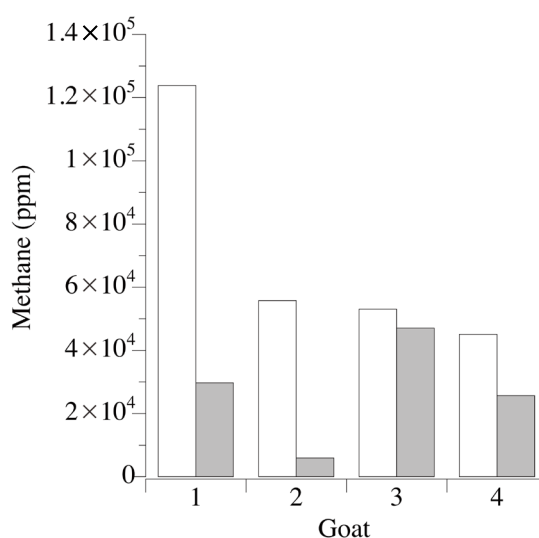
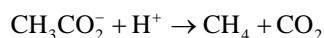


Figure 2. Final methane concentration in gas produced by caprine rumen microorganisms from ground hay with bakers' yeast control (open bars) or craft yeast treatment (filled bars) on a per animal basis. One-way ANOVA indicated that the treatments were different at the 10% level of confidence (P = 0.079).

4. Discussion

Experiment 1 was conducted in replicate with microorganisms from a single animal and showed product formation from a highly fermentable model substrate, fructose. Experiment 2 expanded by permitting animal variation and showing product formation from a natural substrate, the same hay in the donor animal diet. In both experiments, less acetic acid production and less methane production was observed in treatments that included spent craft brewers' yeast rather than the bakers' yeast control. Acetotrophic methanogens convert acetate to methane by cleaving the methyl group, following the general reaction [20]:



Another metabolic pathway for methanogenesis involves CO_2 reduction with H_2 , and it has long been recognized that membrane-active antimicrobials can impact these microorganisms [6] [7]. Experiments by Narvaez and colleagues [21] demonstrated that the addition of hops to rumen incubations decreased CH_4 production per unit of dry matter digested. Furthermore, hops extract and the ionophore monensin had an additive inhibitory effect on CH_4 production [25].

Rumen bacteria produced less acetate in the presence of hops beta-acids [18], which could be partially responsible for the decrease in methane observed. In the current study, caprine rumen microorganisms also made less acetate from ground hay in the presence of CY than in the presence of BY. However, it is important to note that Clostridia and many other Gram-positive Firmicutes produce H_2 . Thus, a decrease in methane is consistent

with the antimicrobial effects of hops acids on Gram-positive, H₂-producing bacteria in general, and many hops acid-sensitive bacteria with a classical Gram-positive cell envelope have been identified in animal gastrointestinal tracts [15] [18] [25]-[28]. It is likely that the hops acids in the CY inhibited the bacteria that produce H₂ and acetate. It is unclear if hops acids inhibited the methanogens directly.

American craft breweries often use more hops in recipes than large, multinational breweries, and recent results indicate that some spent craft brewers' yeast contain more than 3,000 ppm combined α - and β -acids [22], and most of this spent yeast is discarded, composted or digested. Thus, there are multiple potential benefits of spent craft yeast as a feed additive for ruminant livestock. First, *Saccharomyces cerevisiae* cells are a source of supplemental protein. Second, the hops acids delivered with spent craft brewers' yeast could help to increase feed efficiency by inhibiting the microbial pathways that lead to protein and energy losses in ruminants [15]. In addition, the reduction of enteric methane production could help to eliminate some of the greenhouse gas pollution created by ruminant fermentation [19], which accounts for approximately 3% of all greenhouse gas emissions originating in the U.S. [29], and 26% of all methane emissions [30].

5. Conclusion

Spent yeast is a rich source of protein that can be used as a feed supplement for livestock. We have shown that spent craft brewers' yeast, containing α - and β -acids from the hops plant, has additional potential benefit as a supplement. *In vitro*, supplemental craft yeast decreased the amount of methane produced by bovine and caprine rumen fermentation. Thus, there is now the potential for spent craft yeast, currently treated as waste, to become a value-added product that increases livestock efficiency.

Acknowledgements

We thank John W. Brock and Vivek Fellner for help with instrumentation and quantitative analysis of Methane. We thank Dana M. Emmert, Stephen F. Cartier, and Seth Cohen for helpful discussions. We thank Hollie Stephenson of Highland Brewing for spent craft yeast. B.E.H, G.E.A. and M.D.F. were supported by USDA-Agricultural Research Service. Proprietary or brand names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product, nor exclusion of others that may be suitable. USDA is an equal opportunity employer.

References

- [1] Barnett, J.A. (2000) A History of Research on Yeast 2: Louis Pasteur and His Contemporaries, 1850-1880. *Yeast*, **16**, 755-771. [http://dx.doi.org/10.1002/1097-0061\(20000615\)16:8<755::AID-YEA587>3.0.CO;2-4](http://dx.doi.org/10.1002/1097-0061(20000615)16:8<755::AID-YEA587>3.0.CO;2-4)
- [2] Grieve, D.G. (1979) Feed Intake and Growth of Cattle Fed Liquid Brewer's Yeast. *Canadian Journal of Animal Science*, **59**, 89-94. <http://dx.doi.org/10.4141/cjas79-011>
- [3] Newbold, C.J. (1996) Probiotics for Ruminants. *Annales de Zootechnie, INRA/EDP Sciences*, **45**, 329-335. <http://dx.doi.org/10.1051/animres:19960664>
- [4] Hobson, P.N. (1997) The Rumen Microbial Ecosystem. Springer Science & Business Media. <http://dx.doi.org/10.1007/978-94-009-1453-7>
- [5] Van Soest, P. (1994) Nutritional Ecology of the Ruminant. 2nd Edition, Comstock Publishing, Ithaca, 314.
- [6] Van Nevel, C.J. and Demeyer, D.I. (1996) Control of Rumen Methanogenesis. *Environmental Monitoring and Assessment*, **42**, 73-97. <http://dx.doi.org/10.1007/BF00394043>
- [7] Van Nevel, C.J. and Demeyer, D.I. (1977) Effect of Monensin on Rumen Metabolism *in Vitro*. *Applied and Environmental Microbiology*, **34**, 251-257.
- [8] Chen, M. and Wolin, M.J. (1979) Effect of Monensin and Lasalocid-Sodium on the Growth of Methanogenic and Rumen Saccharolytic Bacteria. *Applied and Environmental Microbiology*, **38**, 72-77.
- [9] Wallace, R. (2004) Antimicrobial Properties of Plant Secondary Metabolites. *Proceedings of the Nutrition Society*, **63**, 621-629. <http://dx.doi.org/10.1079/PNS2004393>
- [10] Gilchrist, M.J., Greko, C., Wallinga, D.B., Beran, G.W., Riley, D.G. and Thorne, P.S. (2007) The Potential Role of Concentrated Animal Feeding Operations in Infectious Disease Epidemics and Antibiotic Resistance. *Environmental Health Perspectives*, **115**, 313-316. <http://dx.doi.org/10.1289/ehp.8837>

- [11] Busquet, M., Calsamiglia, S., Ferret, A. and Kamel, C. (2006) Plant Extracts Affect *in Vitro* Rumen Microbial Fermentation. *Journal of Dairy Science*, **89**, 761-771. [http://dx.doi.org/10.3168/jds.S0022-0302\(06\)72137-3](http://dx.doi.org/10.3168/jds.S0022-0302(06)72137-3)
- [12] Patra, A.K. and Saxena, J. (2010) A New Perspective on the Use of Plant Secondary Metabolites to Inhibit Methanogenesis in the Rumen. *Phytochemistry*, **71**, 1198-1222. <http://dx.doi.org/10.1016/j.phytochem.2010.05.010>
- [13] Flythe, M. and Kagan, I. (2010) Antimicrobial Effect of Red Clover (*Trifolium pratense*) Phenolic Extract on the Ruminant Hyper Ammonia-Producing Bacterium, *Clostridium sticklandii*. *Current Microbiology*, **61**, 125-131. <http://dx.doi.org/10.1007/s00284-010-9586-5>
- [14] Teuber, M. and Schmalreck, A.F. (1973) Membrane Leakage in *Bacillus subtilis* 169 Induced by the Hop Constituents Lupulone, Humulone, Isohumulone and Humulinic Acid. *Archives of Microbiology*, **94**, 159-171.
- [15] Flythe, M.D. (2009) The Antimicrobial Effects of Hops (*Humulus lupulus* L.) on Ruminant Hyper Ammonia-Producing Bacteria. *Letters in Applied Microbiology*, **48**, 712-717.
- [16] De Keukeleire, D. (2000) Fundamentals of Beer and Hop Chemistry. *Química Nova*, **23**, 108-112. <http://dx.doi.org/10.1590/S0100-40422000000100019>
- [17] Schmalreck, A.F., Teuber, M., Reininger, W. and Hartl, A. (1975) Structural Features Determining the Antibiotic Potencies of Natural and Synthetic Hop Bitter Resins, Their Precursors and Derivatives. *Canadian Journal of Microbiology*, **21**, 205-212. <http://dx.doi.org/10.1139/m75-029>
- [18] Flythe, M. and Aiken, G. (2010) Effects of Hops (*Humulus lupulus* L.) Extract on Volatile Fatty Acid Production by Rumen Bacteria. *Journal of Applied Microbiology*, **109**, 1169-1176. <http://dx.doi.org/10.1111/j.1365-2672.2010.04739.x>
- [19] Moss, A.R., Jouany, J.P. and Newbold, J. (2000) Methane Production by Ruminants: Its Contribution to Global Warming. *Annales de Zootechnie*, **49**, 231-253. <http://dx.doi.org/10.1051/animres:2000119>
- [20] Ferry, J.G. (1992) Methane from Acetate. *Journal of Bacteriology*, **174**, 5489-5495.
- [21] Narvaez, N., Wang, Y., Xu, Z. and McAllister, T. (2011) Effects of Hops on *in Vitro* Ruminant Fermentation of Diets Varying in Forage Content. *Livestock Science*, **138**, 193-201. <http://dx.doi.org/10.1016/j.livsci.2010.12.028>
- [22] Bryant, R.W. and Cohen, S.D. (2015) Characterization of Hop Acids in Spent Brewer's Yeast from Craft and Multinational Sources. *Journal of the American Society of Brewing Chemists*, **73**, 159-164.
- [23] Noack, J., Timm, D., Ashok Hospattankar, A. and Slavin, J. (2013) Fermentation Profiles of Wheat Dextrin, Inulin and Partially Hydrolyzed Guar Gum Using an *in Vitro* Digestion Pretreatment and *in Vitro* Batch Fermentation System Model. *Nutrients*, **5**, 1500-1510. <http://dx.doi.org/10.3390/nu5051500>
- [24] Littell, R.C., Milliken, G.A., Stroup, W.W. and Wolfinger, R.D. (1996) SAS System for Mixed Models. SAS Institute Inc., Cary.
- [25] Narvaez, N., Wang, Y. and McAllister, T. (2013) Effects of Extracts of *Humulus lupulus* (Hops) and *Yucca schidigera* Applied Alone or in Combination with Monensin on Rumen Fermentation and Microbial Populations *in Vitro*. *Journal of the Science of Food and Agriculture*, **93**, 2517-2522. <http://dx.doi.org/10.1002/jsfa.6068>
- [26] Siragusa, G.R., Haas, G.J., Matthews, P.D., Smith, R.J., Buhr, R.J., Dale, N.M. and Wise, M.G. (2008) Antimicrobial Activity of Lupulone against *Clostridium perfringens* in the Chicken Intestinal Tract Jejunum and Caecum. *Journal of Antimicrobial Chemotherapy*, **61**, 853-858. <http://dx.doi.org/10.1093/jac/dkn024>
- [27] Harlow, B.E., Lawrence, L.M., Kagan, I.A. and Flythe, M.D. (2014) Inhibition of Fructan-Fermenting Equine Faecal Bacteria and *Streptococcus bovis* by Hops (*Humulus lupulus* L.) β -Acid. *Journal of Applied Microbiology*, **117**, 329-339. <http://dx.doi.org/10.1111/jam.12532>
- [28] Flythe, M.D., Aiken, G.E., Gellin, G.L., Klotz, J.L., Goff, B.M. and Andries, K.M. (2015) Hops (*Humulus lupulus*) β -Acid as an Inhibitor of Caprine Rumen Hyper-Ammonia-Producing Bacteria *in Vitro*. *Agriculture, Food and Analytical Microbiology*, **5**, 29-36.
- [29] US Environmental Protection Agency (2015) Sources of Greenhouse Gas Emissions: Agriculture. <http://www.epa.gov/climatechange/ghgemissions/sources/agriculture.html>
- [30] US Environmental Protection Agency (2015) Overview of Greenhouse Gases: Methane Emissions. <http://www3.epa.gov/climatechange/ghgemissions/gases/ch4.html>



Submit or recommend next manuscript to SCIRP and we will provide best service for you:

Accepting pre-submission inquiries through Email, Facebook, LinkedIn, Twitter, etc.

A wide selection of journals (inclusive of 9 subjects, more than 200 journals)

Providing 24-hour high-quality service

User-friendly online submission system

Fair and swift peer-review system

Efficient typesetting and proofreading procedure

Display of the result of downloads and visits, as well as the number of cited articles

Maximum dissemination of your research work

Submit your manuscript at: <http://papersubmission.scirp.org/>