IN VITRO RESPONSES OF YUCCA SCHIDIGERA EXTRACT ON RUMINAL METHANE PRODUCTION, FERMENTATION CHARACTERISTICS AND DEGRADABILITY

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SUMMARY

The present study was conducted in order to investigate the effects of Yucca Schidigera extract (YSE) at 25, 50 and 100 µl /75 ml culture ruminal fluid on in vitro ruminal fermentation characteristics, diet degradability and methane production using semi-automatic system for gas production (GP) assay. Three rumen-cannulated Barki sheep (58.0 ± 2.3 kg of BW) were used as inoculum donors. The culture fluid was anaerobically incubated with 300 mg of a mixture of concentrate and peanuts hay (50:50, w/w) at 39°C for 24 h. Three runs of GP were used for each assay. Results showed that YSE addition to the diets decreased GP (P<0.05) than those obtained by the control. Compared with the control, CH₄ production and NH₃-N concentration were decreased by 37 and 18.5% with 25 µl YSE. Truly degraded dry matter (TDDM) was similar among the different levels of YSE addition. While, truly degraded organic matter (TDOM) was decreased (P < 0.05) by 100 µl YSE addition but there were no differences among 25 and 50 µl levels of YSE addition. Ruminal acetate proportion was lower (P < 0.05) by 50 µl YSE while decreased by 25 and 100 µl YSE addition. The results indicated that YSE exhibited strong suppressing effect on ruminal methanogenesis, NH₃-N concentrations and protozoal populations which could modify the rumen fermentation patterns, may be a beneficial ruminal modifier for improving nutrient utilization and animal performance.

Keywords: Yucca Schidigera extract, in vitro ruminal fermentation and gas production.

INTRODUCTION

In the last decade there has been renewed and increasing interest in plant products and bioactive compounds as rumen modifiers, with most experiments primarily focused on changes in rumen fermentation, mainly in vitro rumen fermentation. Herbs and medicinal plants have been used for centuries for various purposes because of their antimicrobial properties (Davidson and Naidu, 2000). Plant secondary metabolites have been shown to modulate ruminal fermentation to improve nutrient utilization in ruminants Hristov et al. (1999). These compounds possess antimicrobial activity that is highly specific, which raises their possibility to target methanogens. Some of these products and compounds affect CH₄ production and stimulate microbial metabolism thereby increasing the extent of feed degradation in the rumen as well as the efficiency and yield of rumen microbial biomass. However, the effectiveness of these natural additives has varied based upon source, type and level of the active substance responsible for the effect (Sallam, 2012, and Patra, 2012).

One of these plants, Yucca Schidigera plant used as animal natural feed additives and source of the steroidal saponins (Cheeke, 1999). Moreover, Yucca Schidigera is a desert plant native to the arid deserts of the Mexican states of Baja California, Guerrero and Huajaca. It averages (4.5) m in height with (1) m leaves and is also known as “Spanish Dagger” or “Mohave Yucca” (Wang, 2000a). According to Wang et al. (2000b), steroidal saponins form complexes with cell walls of cellulolytic and amylolytic bacteria, which disrupt membrane function and cell growth of some bacterial genera. Saponins have an inhibitory action toward protozoa by affecting cell membrane integrity (Goel and Makkar, 2012) thereby reducing
their numbers in the rumen. Moreover, Wina et al. (2005) reported that raw yucca extract contained 44 g/kg of DM of steroidal saponins, which are the secondary plant glycosides with attached sugars (Wang et al., 2000b). The purpose of this study was to evaluate the effects of various levels of Yucca Schidigera extract (YSE) supplement on ruminal fermentation characteristics, diet degradability, and methane production using in vitro semi-automatic system.

MATERIALS AND METHODS

The chemical analysis and in vitro assays were carried out at Laboratory of Animal Nutrition, Department of Animal and fish Production, Faculty of Agriculture, Alexandria University (Egypt).

Experimental diets:

Yucca Schidigera extract (YSE) is a light brown fine natural powder manufacture entirely from the steam of the Yucca Schidigera plant by mechanical means (sun drying and grinding) and was cached from Free Trade Egypt Company, kafr el-dawar, Behira, Egypt. The basal diet used as a fermentation substrate was consisted of 50% peanuts hay (Arachis hypogaea) and 50% concentrate as 86.41, 12.78, 2.18, 45.82, 25.34 and 3.18% for OM, CP, EE, NDF, ADF and lignin. Different levels of YE was supplemented to the basal diet at 25, 50 and 100 µl /75 ml of culture fluid to 300 mg of incubated substrate.

Ruminal inocula donor and preparations:

Three adult rumen-cannulated Barki sheep with BW (58.0±2.3 kg) were used as inoculum donors kept at the Experimental farm of the Animal and Fish Production Department. Sheep were fed peanuts hay ad libitum plus 750 g/100 kg of BW of commercial concentrate mixture (145 g CP/kg DM). The chemical analysis of the concentrate mixture was 895, 145, 27, 382 and 226 g/kg for OM, CP, EE, NDF and ADF respectively. Ruminal liquid and solid contents (500:500 v/v) were collected separately before the morning feeding through the cannula using a stainless steel probe (2.5-mm screen) attached to a large capacity syringe and kept in water bath at 39°C saturated with CO₂ until inoculation took place.

In vitro gas production (GP) assay:

The readings of in vitro GP pressure in the bottles headspace were taken using semi-automatic system procedure equipped with a pressure transducer and a data logger (Pressure Press Data GN200, Sao Paulo, Brazil) according to Bueno et al. (2005). Ground samples (0.3 g) were put into numbered and pre-weighted dry Ankom filter bags (ANKOM, Technology Corporation, Fairport, USA) and were incubated in 120 ml serum bottles and head space 75 with 45 ml of diluted rumen fluid (15 ml inoculum + 30 ml of Menke’s buffered medium, MB9). The composition of MB9 was NaCl 2.8 g, CaCl₂ 0.1 g, MgSO₄.7H₂O 0.1 g, KH₂PO₄ 2.0 g and Na₂HPO₄ 6.0 g per 1 liter of distilled water. The pH was adjusted to 6.8 and CO₂ was flushed for 30 min (Onodera and Handerson, 1980). Once filled, bottles were sealed immediately with 20 mm butyl septum stoppers (Bellco Glass Inc, Vineland, NJ, USA), then, manually mixed and incubated in a forced air oven (FALC instruments S.R.L., Treviglio, Italy) at 39°C for 24 h. The bottles were shaken manually after the recording of the gas headspace (75 ml) pressure at 3, 6, 9, 12 and 24 h of incubation using a pressure transducer and data logger according to Bueno et al. (2005).

The gas volume (mL) = 4.974 × measured pressure (psi) + 0.171 (n = 500; r² = 0.99; unpublished data) where: V is gas volume (ml); p is measured pressure (psi). Three runs of GP were used for each assay. For each run, six bottles per treatment were prepared: three for truly degraded organic matter (TDOM) and truly degraded dry matter (TDDM) determination and the other three bottles for determining fermentation constituents. A corresponding six bottles consisted only of rumen fluid and buffer solution without substrate (blank) to correct the GP from the inoculum, and further, six bottles contained Berseem hay as an internal standard to correct the variation between inoculum.

Representative gas samples (2 ml) were collected from the bottles at distinct incubation times using 5 ml syringe and accumulated in 10 ml Vacutainer tubes (BD Vacutainer® Tubes, NJ, USA) to determine the CH₄ concentration. The methane was determined by gas chromatography (Model 7890, Agilent Technologies, Inc, Colorado 80537, USA) with three valve system using 1/8 inch packed columns having early back flush of the C6 components and equipped with a thermal conductivity detector. Separation was achieved using micro packed column with helium as carrier gas and a flow rate of 28.0 ml/min. The detector and column temperatures were 250°C and 60°C respectively. The test of linearity and calibration
were accomplished using a standard gas curve in the range of probable concentrations of the samples. The produced CH$_4$ was calculated according to the method of Longo et al. (2006) as follows: CH$_4$, ml = (Total GP, ml + Headspace, 75 ml) × CH$_4$ concentration (ml/ml). The net GP and CH$_4$ values were determined by correcting for the corresponding blank values.

**Ruminal degradability and fermentation constituents:**

After 24 h, all the bottles were placed in cold water (4°C) in order to stop the fermentation process. Three bottles for measuring the gas production (GP) and CH$_4$ and the other three bottles were for measuring pH, NH$_3$-N, protozoal counts and individual VFA.

For TDOM determination, half of those bottles were injected with 70 ml of neutral detergent solution and incubated at 105°C for 3 h as described by Blümmel and Becker (1997). The residue was filtered in pre-weighed crucibles, washed with hot water and acetone and oven dried at 105°C for 16 h. The flasks content was filtered in pre-weighed crucibles, washed with hot water then acetone and the residual DM and ash were determined. The TDOM was determined after ashing at 550°C for 4 h with correction for the corresponding blank. Three serum bottles containing only rumen fluid were incubated as blanks and used to compensate for GP in the absence of substrate. Three serum bottles with internal standard was used for adjustments the variation between the runs. Three runs were done for the same samples. The liquid phase of the other three bottles was used for the fermentation constituents and protozoal count assessments. Ruminal pH was measured directly within 2-3 min of sampling using a portable pH meter (GLP 21 model; CRISON, Barcelona, Spain). Ruminal NH$_3$-N concentration was measured calorimetrically by spectrophotometer (Alpha-1101 model; Labnics Equipment, California, USA) using commercial lab test described by Konitzer and Voigt (1963). Rumen protozoa number were microscopically counted in 2 ml of rumen fluid subsample was mixed with 4 mL of methyl green-formalin-saline (MFS) solution and stored in glass bottle at room temperature for later total protozoa counting. Total protozoa number was counting microscopically (Olympus biological microscope, model CX31RBSFA, Philippines) using Neubauer improved bright-line (Labor Optik, Lancung, UK) counting chamber according to the procedure described by Dehority et al. (1983).

Individual short chain fatty acids (SCFA) concentrations were determined as described by Abo-Zeid et al. (2017) using a gas chromatograph (GC) with some modifications. In brief, after thawing, an aliquot of 1.6 ml was prepared with 0.4 ml of 25% meta phosphoric acid (4:1 ratio) and centrifuged at 15,000 × g for 20 min and 4°C (K1015 Micro Prime; Centurion Scientific Ltd, Stoughton, Chichester, UK). The supernatant was used to determine SCFA concentrations with a GC (Thermo fisher scientific, Inc., TRACE1300, Rodano, Milan, Italy) fitted with an AS3800 auto sampler and equipped with a capillary column HP-FFAP (19091F-112; 0.320 mm o.d., 0.50 μm i.d., and 25 m length; J&W Agilent Technologies Inc., Palo Alto, CA). Hydrogen at 1.35 mL/min was used as carrier gas. Air, hydrogen and nitrogen fluxes (make up gas) were kept at 450, 40, and 35 mL/min, respectively. A 0.1μL aliquot was injected in split less mode for the entire run with 31.35 mL/min of H$_2$ flux (63.432 Pa). Injector and flame ionization detector (FID) temperatures were held isothermally at 250°C. Oven heating slope was 80°C (1 min), 120°C (20°C/min for 3 min), and 205°C (10°C/min for 2 min), with 9 min overall analytical time.

**Chemical analyses:**

Ground diets samples were chemically analyzed according to AOAC (2006) for DM (ID number 930.15), OM (ID number 942.05), CP (as 6.25 × N; ID number 954.01) and ether extract (EE). Sequentially in the same sample, neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined in filter bags and expressed exclusive of residual ash as described by Van Soest et al. (1991) by ANKOM 220 fiber analyzer unit (ANKOM Technology Corporation, Macedon, NY, USA).) was used without sodium sulfide or α amylase.

**Statistical analyses:**

Statistical analyses of the in vitro assay and the digestibility trial were analyzed to analysis of variance (ANOVA) by the generalized linear model procedure software package (SAS, 1996). The following model was assumed: $y_{ij} = \mu + F_i + e_{ij}$ Where: $\mu$ is the overall mean, $F_i$ is the treatment effect, $e_{ij}$ is the random error term. Differences among means were tested using Duncan multiple range test (Steel and Torrie., 1980). Differences were considered significant if $P< 0.05$. 

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RESULTS AND DISCUSSION

In vitro gas and CH₄ production:

Effects of different levels of YSE on GP and CH₄ production, TDDM, TDOM and PF after 24 h of in vitro incubation are shown in Table 1. The addition of YSE to the diets decreased (P<0.05) GP by 13.2% compared to the control. The addition of 25 µl of YSE exhibited the lowest CH₄ production than other levels and control diet (by 17 % ml/g DM and 26 % ml/g OM). Addition of YSE at levels of 25 and 50 had no effect (P>0.05) on TDOM after 24 h of incubation, while, reduced (P<0.05) with 100 µl of YSE addition to the diet. No significant differences for TDDM and PF were not for TDDM and PF were observed between the experimental diets. Our results are agreement with the previous reports that showed of responses of ruminal GP and CH₄ by supplementation of saponins or plants rich in saponins. It was revealed that in vitro supplementation with saponin-extracts from Yucca Schidigera (sarsaponins; steroidal saponins) and Quillaja saponaria (triterpenoid type saponins) decreased ruminal CH₄ production Mim et al. (2015) and Holtshausen et al. (2009).

Addition of yucca extract from 0 to 3.2 mg/ml reduced in vitro CH₄ and GP (Lila et al., 2003). In lactating cows, Ramirez-Restrepo et al. (2016) and Guyader et al. (2015), reported higher CH₄ emissions with tea saponins with linear reduction in ruminal protozoa number, which produce and supply large amounts of H₂ to methanogenic archaea (Morgavi et al., 2013, and Guyader et al. 2014). The current results are disagreement with McAllister and Newbold (2008) that the inhibition of methane production usually increases the concentrations of hydrogen, which is rechanneled to other hydrogen sinks such as propionate resulting in increased concentrations of propionate. The improvement of individual SCFAs proportion may be due to enhancing the microbial digestion by a certain essential oil, saponins and tannins compounds included in the medicinal plants. Our results were agreement with the results obtained by Lila et al. (2003) who reported that the increase in total SCFAs concentrations due to inclusion of sarsaparin in the fermentation media. Conversely, the production of acetate in the rumen results in large quantities of hydrogen and depends on the availability of reducing equivalents such as NAD⁺ (Busquet et al., 2005). The high partial pressure of hydrogen and high NADH/ NAD⁺ ratio in the rumen due to the inhibition of methanogenesis may result in a decrease in acetate production (Miller, 1995, and Busquet et al., 2005). Moreover, a number of studies have reported that saponins or plants rich in saponins decreased the methane production in the rumen. Saponin-extracts from Yucca Schidigera (sarsaponins; steroidal saponins) and Quillaja saponaria (triterpenoid type saponins) or these plants as such have been examined in different laboratories, which have been demonstrated to reduce methanogenesis both in vitro (Takahashi et al., 2000, and Holtshausen et al., 2009).

The toxic effect of saponins toward protozoa reported previously by 16% reduction (Hu et al., 2005), 51% of total bacteria (Guo et al., 2008) and some modification in ruminal SCFA profiles with a reduction of the acetate-to-propionate ratio (Reynolds et al., 2011). A greater H₂ production with tea saponins increased rumen acetate proportion, which is known to release H₂ in the rumen as the limiting substrate for methanogenic archaea (Janssen, 2010). Ramirez-Restrepo et al. (2016) reported that inclusion of tea saponins on the diet showed some modification of fermentation patterns toward nonglucogenic SCFA (acetate and butyrate) and might stimulate cellulytic bacteria proliferation at the expense of their potential inhibitory effect on protozoal multiplication.

Table 1: Effects of different levels of yucca extract supplement on gas (GP), methane (CH₄) production, truly degraded dry matter (TDDM), truly degraded organic matter (TDOM) and partition factor (PF) after 24 h of in vitro incubation (means ± SD).

| Item          | Control | 25     | 50     | 100            | YE ± µl | P-value |
|---------------|---------|--------|--------|----------------|---------|---------|
| GP (ml/g DM)  | 181.34±9.50ᵃ | 166.64±11.98ᵇ | 160.56±23.62ᵇ | 157.47±17.92ᵇ | 0.0003  |
| CH₄ (ml/g DM) | 11.85±0.93ᵃᵇ | 9.84±2.13ᵇ | 12.96±2.68ᵇ | 10.55±0.94ᵇ | 0.0373  |
| CH₃ (ml/g OM) | 13.24±1.64ᵃ | 9.83±0.14ᵇ | 12.50±1.75ᵃ | 12.41±2.72ᵃ | 0.0208  |
| TDDM (%)      | 76.49±3.04 | 77.47±4.06 | 75.52±2.84 | 75.28±2.52 | 0.4529  |
| TDOM (%)      | 70.53±1.52ᵃ | 69.78±0.97ᵃ | 70.49±1.40ᵃ | 66.26±3.40ᵇ | 0.0007  |
| PF (mg TDOM/ml GP) | 4.99±1.49 | 4.50±1.62 | 3.83±0.43 | 4.17±0.25 | 0.1837  |

Means with the same letter is not significant but a, b, c: Values in the same row with different superscripts differ significantly (P<0.05)
Ruminal pH, NH$_3$-N concentration, SCFA and protozoa count

The effects of YSE on ruminal pH, NH$_3$-N concentration, SCFA and protozoa count are presented in Table (2). No significant differences on ruminal pH among experimental diets were observed. Ruminal NH$_3$-N concentration was reduced by 18.5 and 9% with addition of 25 and 100 µl of YSE respectively compared with the control diet. Supplementation with YSE at levels of 25 and 50 had no effect (P>0.05) on acetate proportion and protozoal counts after 24 h of incubation, while reduced (P<0.05) with 100 µl of YSE addition to the diet. No significant differences were observed after 24 h of incubation in total SCFA, propionic, butyric, Iso-butyric, valric acids and acetate to propionate ratio. In the present study, acetate to propionate ratio tended to decrease (P = 0.091) with addition of YSE.

Table (2): Effects of different levels of yucca extract supplement on rumen pH, NH$_3$-N concentration, SCFA, protozoa count after 24 h of in vitro incubation (means ± SD).

| Item                        | Control      | 25            | 50            | 100           | P-Value |
|-----------------------------|--------------|---------------|---------------|---------------|---------|
|                             | YE, µl       |               |               |               |         |
| pH                          | 5.62±0.71    | 5.63±0.68     | 5.64±0.70     | 5.63±0.69     | 1.0000  |
| NH$_3$-N, mg/dl             | 25.09±2.88$^a$ | 20.46±2.73$^a$ | 25.64±1.68$^a$ | 22.92±1.94$^b$ | 0.0002  |
| Short fatty acids, mM (SCFA)|             |               |               |               |         |
| Total SCFA                  | 133.74±3.03  | 131.10±0.01   | 131.98±0.02   | 131.99±0.01   | 0.4396  |
| Acetic                      | 69.81±1.37$^a$ | 61.92±6.98$^{ab}$ | 61.76±2.16$^{ab}$ | 57.94±5.17$^{b}$ | 0.0641  |
| Propionic                   | 37.82±1.15   | 37.13±5.31    | 35.27±4.99    | 39.90±1.12    | 0.5334  |
| Butyric                     | 19.76±2.82   | 24.12±4.96    | 25.70±8.07    | 23.94±3.95    | 0.7501  |
| Valeric                     | 2.30±1.02    | 2.48±0.48     | 3.43±0.56     | 1.90±0.68     | 0.1410  |
| Iso-valeric                 | 2.14±1.09$^a$ | 3.52±1.24$^{ab}$ | 3.10±0.28$^{ab}$ | 4.79±0.84$^{a}$ | 0.0746  |
| Iso-butyric                 | 1.91±0.80    | 2.82±1.23     | 2.72±0.30     | 3.52±0.80     | 0.2150  |
| A: P ratio                  | 1.84±0.05    | 1.69±0.31     | 1.77±0.20     | 1.45±0.17     | 0.0910  |

Protozoal count, x10$^7$/ml

| Item                        | Control      | 25            | 50            | 100           | P-Value |
|-----------------------------|--------------|---------------|---------------|---------------|---------|
|                             | YE, µl       |               |               |               |         |
| Total                       | 5.00±0.41$^{ab}$ | 4.60±0.82$^{b}$ | 5.40±0.28$^a$ | 4.46±0.54$^b$ | 0.0676  |
| Diplodinium                 | 3.86±0.91$^a$ | 2.70±0.33$^{b}$ | 2.80±0.75$^{b}$ | 2.73±0.27$^{b}$ | 0.0121  |
| Entodinium                  | 1.14±0.76$^b$ | 1.90±0.70$^{ab}$ | 2.40±0.74$^a$  | 1.73±0.27$^{ab}$ | 0.0436  |
| Holotricha                  | 0.09±0.22    | 0.20±0.31     | 0.00±0.00     | 0.00±0.00     | 0.3760  |

Means with the same letter is not significant but a, b, c: Values in the same row with different superscripts differ significantly (P<0.05).

Changes in ruminal NH$_3$-N concentrations varied amongst studies that fed YSE. A decrease in ruminal NH$_3$-N concentration associated with addition of YSE in in vivo studies (Hristov et al., 1999, and Pen et al., 2006). Depressed ruminal NH$_3$-N concentration is associated with protozoal numbers inhibition, probably as a consequence of lowered bacterial lysis (Williams and Coleman, 1992). Ammonia-binding capability of the glycol fractions of YSE has been reported in in vitro assays (Wallace et al., 1994). According to Muhammad et al. (2002), YSE reduced NH$_3$-N production via inhibition of urease activity resulting in a decrease in ruminal NH$_3$-N concentration. Moreover, the addition of S. saponaria in Rusitec reduced ruminal protozoa counts by 54% and CH$_4$ production by 20% (Hess et al., 2003). As saponins are the natural detergents and possess membranolytic properties; they complex with sterols in protozoal cell membranes and cause cell lysis which modifies ruminal fermentation by suppressing ruminal protozoa and selectively inhibiting some bacteria Cheeke (2000). Therefore, the saponins-rich plant materials have potential to enhance the flow of microbial protein from rumen, increase the efficiency of feed utilization by decreasing methanogenesis. The addition of YSE increased the partitioning of the nutrients to microbial cells combined with reduction in CH$_4$ production per unit of feed degraded Goel et al. (2008). In the present study, the lower CH$_4$ production by YSE was attributed to their inhibitory effect on ruminal protozoa and cellulolytic bacteria growth. The antiprotozoal potential of YSE was confirmed in the current experiment.
CONCLUSION

Dietary supplementation with 25 µl of Yucca Schidigera extract (YSE) decreased in vitro CH₄ production, protozoal numbers and NH₃-N concentrations suggest that YSE may be possible to modify the rumen fermentation patterns. Further research studies are needed to evaluate their effects in in vivo nutrient utilization and animal performance and methane production.

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