



Effect of combining exogenous fibrolytics enzymes with *Saccharomyces cerevisiae* or *Eucalyptus* essential oil on the *in vitro* ruminal fermentation and digestibility of wheat straw

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ABSTRACT

This study aimed to determine the efficacy of exogenous fibrolytics enzymes (EFE) to create a synergy with *Saccharomyces cerevisiae* (SC) or essential oil (*Eucalyptus globulus*) (EO) to promote the *in vitro* ruminal fermentation and digestibility of wheat straw (WS). The EFE was a mixture (1:1, v/v) of β -1, 3-1, 4-glucanase and endo-1,4- β -D-xylanase produced by the *Trichoderma longibrachiatum*. The WS was supplemented with EFE (EFE1=1, EFE2=2 μ l/g DM), SC (SC1=0.5, SC2=1 mg/g DM), EO (EO1=30, EO2=60 μ l/g DM) and the combinations EFE1+EO1, EFE1+EO2, EFE2+EO1, EFE2+EO2 and EFE1+SC1, EFE1+SC2, EFE2+SC1, EFE2+SC2. Compared to the control, both EFE and SC alone or in combination, improved the rate and the extent of WS fermentation. The potential GP improved only with EFE2, SC1, and EFE2+SC2 by 31.7, 24.9, 37.4% and the rate Rmax by 36, 59.2 and 55.2%, respectively. The organic matter digestibility and the energetic use of WS improved with the highest doses EFE2 and EFE2+SC2. While the EO alone or combined with the EFE had a depressive effect on all fermentation and digestibility parameters. The EFE seems to be more beneficial for the fermentation and digestibility of WS than when it was combined with SC or EO. Therefore, there is no synergetic effect between EFE and SC or EO with the used doses on fibrous by-product like wheat straw.

Key words: Essential oil, Fibrolytics enzyme, *In vitro* fermentation, Live yeast, Wheat straw

Crop residues were traditionally used as a cheap source of feed for livestock especially in developing countries (Mugerwa *et al.* 2012). Cereal straws are considered as fibrous feed, with a complex fibrous structure which limits the cell wall digestibility and makes it a poor-quality feed in its natural state (Sun 2010). In this context, numerous number of researches have been done in order to upgrade their nutritional value through physical, chemical and biological treatments (Kumar *et al.* 2009). The use of live yeast as feed supplements for ruminants is not a new concept. It prevents rumen flora disorders, increases the dry matter consumption and stimulates the activity of ruminal microorganisms (Sontakke 2012) but the *in vivo* results have been unsatisfactory (Tadesse 2014). The use of several plant extracts also gained growing interest during the last decade. Indeed, the essential oils were used as a modifier of ruminal fermentation (Calsamiglia *et al.* 2007) because of their selective antimicrobial activities (Hristov *et al.* 2008). In recent years, there is a tendency to use the exogenous enzymes in ruminant nutrition to improve

ruminal fiber digestibility (Eun and Beauchemin 2007), to stimulate feed utilization and livestock productivity. There is little information about the synergetic effects between exogenous fibrolytics enzymes and another type of feed additive to enhance the ruminal use of fibrous diet. It was proved that each feed additive has a specific mode of action, therefore, a synergetic effect was expected (Calsamiglia *et al.* 2007). Therefore, the aim of this work was to study the effect of exogenous fibrolytics enzymes supplementation alone and in combination with live yeast (*Saccharomyces Cerevisiae*) or essential oil (*Eucalyptus Globulus*) on the *in vitro* fermentation, kinetics of gas production and digestibility of wheat straw.

MATERIALS AND METHODS

Substrate preparation and treatments: Samples from wheat straw (WS) bales were collected, chopped (5 cm) and dried overnight at 55°C. Then, it was ground in a mill to pass through a 1 mm sieve and stored for subsequent analysis.

In this experiment, three feed additives were assessed for their efficacy to improve the ruminal utilization of wheat straw. The exogenous fibrolytics enzymes (EFE) was a combination of cellulase and xylanase (1:1, v/v) (Dyadic® International, Inc. Jupiter, Florida) produced by

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Trichoderma longibrachiatum in liquid form. Our analysis of EFE activities showed that it contained 22760 ± 152 IU of xylanase, 1160 ± 107 IU of endoglucanase and 113 ± 6.4 IU of exoglucanase. The EFE was diluted by distilled water and then sprayed directly onto ground wheat straw samples which were, weighed in advance in glass serum bottles (200 mg) to serve as incubation substrate with two doses; 1 and 2 $\mu\text{l/g}$ DM (EFE1 and EFE2). The EFE supplementation was applied 20 h before the *in vitro* incubation to allow enzyme-substrate interaction (Beauchemin *et al.* 2003).

The live yeast (Society of animal nutrition, SNA[®]) contained approximately 10^{10} CFU of *Saccharomyces cerevisiae*/g DM live yeast (SC) as provided by the manufacturers. The SC was mixed with the ground wheat straw at two doses, viz. 0.5 and 1 mg/g DM (SC1 and SC2). Then, 200 mg from the mix (wheat straw + SC) was weighed into incubation bottles.

Eucalyptus globulus oil was purchased from Ecovillage, Tunisia and applied to the wheat straw directly in the incubation bottles at two different doses, viz. 30 and 60 $\mu\text{l/g}$ DM (EO1 and EO2) at the time of incubation. Dosages were selected based on previous *in vitro* experiment (Vilela *et al.* 2012).

A combination of exogenous fibrolytics enzymes complex and essential oil (EFE1+EO1, EFE1+EO2, EFE2+EO1, EFE2+EO2) or *Saccharomyces cerevisiae* (EFE1+SC1, EFE1+SC2, EFE2+SC1, EFE2+SC2) were also assessed in this study.

Chemical analysis: The wheat straw was analysed in triplicate to determine the chemical composition. The dry matter (DM) was determined by oven drying at 95–100°C, ash by incineration at 550°C and crude protein (CP) according to AOAC (1990). Neutral detergent fibre, acid detergent fibre, and lignin (ADL) were analysed as described by Van Soest *et al.* (1991) using an ANKOM²⁰⁰ Fibre analyser unit. The sodium sulphite and heat stable amylase were used to determine the NDF, and both NDF and ADF were expressed exclusively from residual ash.

The fibrolytic activity of exogenous enzymes were assayed in triplicate for endoglucanase, exoglucanase, and xylanase according to the procedure described by Wood and Bhat (1988) and Bailey *et al.* (1992) under pH 6.6 and 39°C to reflect rumen conditions.

In vitro ruminal fermentation: The *in vitro* incubations were carried out in 100 ml serum bottles as described by Colombatto *et al.* (2003) and Eun and Beauchemin (2007). The Official Animal Care Committee of the National School of Veterinary Medicine Sidi Thabet approved the experimental protocol. Ruminal fluid was collected from two Holstein cows fitted with permanent ruminal cannula before the morning feeding from different sites within the rumen. The obtained ruminal contents were filtered through three layers of cheese cloth and mixed into a pre-warmed insulated flask under continuous flushing with CO₂. The particle-free ruminal fluid was mixed with a buffer solution which was prepared in advance as described by Menke and Steingass (1988) (pH=6) with the ratio 1:2 (ruminal fluid:

buffer) to obtain the fermentation inoculum. Each incubation bottle was filled with 30 ml fermentation inoculum under a continuous stream of CO₂. Immediately after loading, bottles were closed hermetically with a rubber stopper and crimp seal caps and incubated at 39°C. Bottles containing rumen fluid and buffer medium without substrate were considered as a negative control (blanks) and used to correct the recorded gas production (GP). The fermentation essay was repeated three times to ensure the GP results and to minimise the experimental error. Each treatment was conducted in triplicate in each fermentation run.

The gas production was recorded after 2, 4, 6, 8, 12, 24, 48, 72, 96 h of incubation by using a pressure transducer connected to a visual displayer.

Statistical analysis: To describe the kinetics of gas production over time, the following Groot function was used (Groot *et al.* 1996).

$$GP[\text{ml}] = \frac{A}{1 + \left(\frac{B}{t}\right)^c}$$

Where A is the estimated potential GP (ml/g DM), B is the time of incubation at which the half of A has been produced (h), C is the sharpness of the curve. A, B and C were calculated using the residual least squares method using the reduced generalized gradient algorithm of the solver function in Microsoft Excel software. The maximum rate of GP (Rmax) and the time at which the maximum rate of GP is attained (Tmax) were calculated according to Yang *et al.* (2005) functions as;

$$R_{\text{max}}[\text{ml/h}] = AB^c C \left[\frac{T_{\text{max}}^{(c-1)}}{(1 + B^c \times T_{\text{max}}^{-c})^2} \right]$$

$$T_{\text{max}}[\text{h}] = B \left[\frac{C-1}{C+1} \right]^{\frac{1}{c}}$$

The metabolizable energy (ME) and the *in vitro* organic matter digestibility (DMO) were estimated according to the equations proposed by Menke and Steingass (1988) and volatile fatty acids (VFA) as proposed by Getachew *et al.* (1988) as:

$$ME [\text{MJ/kg DM}] = 2.2 + (0.136 \times GP_{24\text{h}}) + (0.057 \times \text{CP}) + (0.00286 \times \text{EE}^2)$$

$$\text{OMD} [\%] = 14.88 + (0.889 \times GP_{24\text{h}}) + (0.45 \times \text{CP}) + (0.0651 \times \text{Ash})$$

$$\text{VFA} [\text{mmol/200 mg DM}] = -0.00425 + (0.0222 \times GP_{24\text{h}})$$

where, GP_{24h} is the gas production (ml/200 mg DM) after 24 h of incubation, CP is the amount of crude protein (%DM), EE is the amount of ether extract (%DM) and ash is the amount of ash content (%DM).

The experimental was conducted in a completely randomized design, where the results were subject to least square analysis of variance by using the GLM procedure of SAS studio 3.6. The feed additive types, doses and the interaction between additive type $\times$ additive doses were

Table 1. Effect of different doses of exogenous fibrolytics enzymes, *Saccharomyces cerevisiae*, eucalyptus essential oil and their combinations on the *in vitro* gas production during 96 h of incubation, fermentation and digestibility parameters of wheat straw (n=3)

EFE (µl/g DM)	SC (mg/g DM)	EO (µl/g DM)	Gas production (ml/g DM)			Fermentation parameters					Digestibility parameters		
			24 h	48 h	96 h	A	B	C	Rmax	Tmax	ME	OMD	VFA
0	0	0	139.3 ^{cd}	147.6 ^{cde}	152.9 ^{cd}	153 ^{ced}	7.6 ^a	1.5 ^{cde}	12.5 ^d	2.8 ^{ab}	6.2 ^{cd}	41.4 ^{bc}	0.61 ^{bc}
0	0.5	0	177.4 ^{ab}	185.5 ^{ab}	191 ^{ab}	191 ^{ab}	6.9 ^{abc}	1.5 ^{efd}	17 ^{abc}	2.4 ^{bc}	7.2 ^{ab}	48.2 ^{ab}	0.78 ^{ab}
0	1	0	178.2 ^{ab}	184.5 ^{ab}	190.5 ^{ab}	191 ^{ab}	6.4 ^{bc}	1.4 ^{ef}	18.2 ^{abc}	1.9 ^{cd}	7.2 ^{ab}	48.4 ^{ab}	0.78 ^{ab}
0	0	30	116.4 ^e	123.9 ^f	131.4 ^e	131 ^f	4.5 ^d	1.1 ^h	12.3 ^d	0.3 ^f	5.6 ^{de}	37.4 ^{cd}	0.51 ^{cd}
0	0	60	117.9 ^e	124 ^f	130.2 ^e	130 ^f	5.1 ^d	1.2 ^{hg}	13.8 ^{abcd}	0.7 ^f	5.6 ^{de}	37.6 ^{cd}	0.52 ^{cd}
1	0	0	166.5 ^{abc}	174.5 ^{abcd}	179.1 ^{abc}	179 ^{abcd}	6.1 ^c	1.5 ^{cde}	17.8 ^{abcd}	2.2 ^{bc}	6.9 ^{abc}	46.3 ^{ab}	0.73 ^{ab}
2	0	0	187.8 ^{ab}	195.9 ^{ab}	201.4 ^{ab}	201 ^{ab}	6.2 ^c	1.6 ^{cd}	19.9 ^{abc}	2.4 ^{bc}	7.5 ^{ab}	50.1 ^a	0.82 ^a
1	0.5	0	167.4	175.8 ^{abcd}	181.2 ^{abc}	181 ^{abcd}	7.4 ^{ab}	1.7 ^{bc}	15.1 ^{bcd}	3.2 ^a	6.9 ^{bc}	46.5 ^{ab}	0.73 ^{ab}
2	0.5	0	174.3 ^{abc}	182.5 ^{abc}	188.7 ^{ab}	189 ^{abc}	6.4 ^{bc}	1.5 ^{efd}	18 ^{abcd}	2.2 ^{bc}	7.1 ^{ab}	47.7 ^{ab}	0.76 ^{ab}
1	1	0	159.5 ^{bc}	167.7 ^{bcd}	172.6 ^{bc}	173 ^{bcd}	7.4 ^{ab}	1.5 ^{cde}	14.4 ^{cd}	2.8 ^{ab}	6.7 ^{bc}	45 ^{ab}	0.7 ^{ab}
2	1	0	197.7 ^a	204.7 ^a	210.1 ^a	210 ^a	6.6 ^{bc}	1.5 ^{ed}	19.4 ^{abc}	2.4 ^{bc}	7.8 ^a	51.8 ^a	0.87 ^a
1	0	30	139 ^{cd}	145.4 ^{ed}	151.2 ^{cd}	151 ^{ed}	6.1 ^c	1.4 ^f	15.2 ^{bcd}	1.7 ^{cd}	6.2 ^{cd}	41.4 ^{bc}	0.61 ^{bc}
2	0	30	116.7 ^{ed}	121.8 ^{ef}	129 ^{ed}	129 ^{ef}	5.1 ^d	1.3 ^g	16.7 ^{abcd}	0.9 ^{ef}	5.6 ^{de}	37.4 ^{cd}	0.51 ^{cd}
1	0	60	92.3 ^e	97.8 ^f	101.3 ^{ef}	99 ^{fg}	3.1 ^e	1.7 ^b	19.5 ^{abc}	1.4 ^{ed}	4.9 ^e	32.9 ^d	0.4 ^d
2	0	60	86.2 ^e	88.9 ^f	92.4 ^f	89 ^g	2.8 ^e	2.1 ^a	21 ^{ab}	1.8 ^{cd}	4.9 ^e	32 ^d	0.39 ^d
P-value			***	***	***	*	*	NS	*	NS	***	*	*
SEM			20.77	21.4	21.5	NS	NS	NS	NS	NS	NS	NS	NS
Polynomial contrast													
Exogenous fibrolytic enzyme													
Linear			*	*	*	*	*	NS	*	NS	*	*	*
Quadratic			NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Live yeast													
Linear			*	*	*	*	NS	NS	*	NS	*	.	.
Quadratic			NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Essential oil													
Linear			*	*	*	*	*	**	NS	**	NS	NS	NS
Quadratic			NS	NS	NS	NS	*	*	NS	*	NS	NS	NS
Interactions													
Additive type			NS	.	NS	NS	***	***	***	***	**	***	***
Additive dose			NS	NS	.	NS	*	*	NS	NS	**	***	***
Additive type × dose			***	***	***	***	***	***	**	NS	*	**	**

EEF, Exogenous fibrolytics enzymes; SC, *Saccharomyces cerevisiae*; EO, Eucalyptus essential oil; A, Estimated potential gas production (ml/g DM); B, Time of incubation at which the half of A has been produced (h); T_{Max}, Time at which the maximum rate of gas production is attained (h); ME, Metabolizable energy (MJ/kg DM); OMD, Organic matter digestibility (g/kg); VFA, volatile fatty acids (mmol/200 mg ME, DM); SEM, Standard error of the mean. *P<0.05, **P<0.01 and ***P<0.001.

considered as fixed factors. Data from each of the three runs for the same sample were averaged. The mean values of each sample (i.e. three samples of each treatment (n=3)), were used as the experimental unit.

The model used was:

$$Y_{ij} = \mu + A_i + D_j + (A_i \times D_j) + e_{ij}$$

where Y_{ij} is each individual observation for a given variable, μ is the overall mean, A_i is the feed additive type effect (EFE, EO, SC, EFE+EO and EFE+SC), D_j additive dose effect and e_{ij} is the experimental error. ($A_i \times D_j$) is the interaction between additive types and doses. Duncan's test was used for means comparison (Duncan 1955). Treatments significance was declared at $P<0.05$. Tendencies were declared at $P<0.1$.

RESULTS AND DISCUSSION

The wheat straw used in this experiment contained 890

g DM/kg fresh weight, 55 g ash/kg DM, 32 g crude protein/kg DM, 739 g NDF/kg DM, 469 g ADF/kg DM and 51 g ADL/kg DM. It was considered relatively medium in quality with a chemical composition comparable to that published by Agabriel (2010). The gas production kinetics, the fermentation parameters and the energy utilization of wheat straw depended on the type of supplemented feed additive. Compared to the control, the recorded gas production during the 96 h of incubation ($P<0.05$), the rate (Rmax) and the extent (A) of ruminal fermentation improved linearly by the supplementation of EFE and SC alone regardless the dose level or combined especially with the highest doses (EFE2+SC2). The parameters like ME, OMD and VFA production were affected only with the EFE and EFE+SC supplementation. It increased linearly ($P<0.05$) with EFE2 by 21.4, 21 and 34.4% and EFE2+SC2 by 15.4, 25.1 and 42.6% respectively. The linear effect of EFE prove that an effective enzyme dose could improve the ruminal

fermentation efficiency by increasing the fermentable material available for the ruminal microbial population because of the hydrolytic activity of EFE (Nsereko *et al.* 2002). In addition, Mao *et al.* (2013) reported that the copy number of total ruminal bacteria increased with the cellulase supplementation of rice straw, which could explain the improvement of ruminal degradation of feed and the total VFA production (Giraldo *et al.* 2007). Despite the different mode of action of live yeast, it promotes the same fermentation parameters as the EFE. In fact, it's known that by oxygen scavenging, the live yeast creates a microenvironment that stimulates the growth of cellulolytic bacteria, the attachment to fibre particles, the rate of cellulose digestion (Sontakke 2012) and improves the ruminal cellulolytic activities (Denev *et al.* 2007).

However, compared to the use of EFE or SC alone, the effect of EFE+SC on the studied *in vitro* parameters was not significant and no synergy was detected between the EFE and the SC with all supplemented doses. Similarly, Kholif *et al.* (2017) did not observe any synergetic effect on production performances, nutrient utilization, ruminal and blood serum measurements in goats fed with a basal diet composed of Egyptian berseem clover and concentrate feed mixture 40:60. So, as the wheat straw was a lignified feed with limited nutrient composition, each feed additives maximized the straw utilization efficiency (Kholif *et al.* 2017) and no more extra benefits could be extracted by combining EFE and SC.

The EO supplementation decreased linearly the GP from 24 h of incubation, the potential GP ($P=0.03$), the required time to produce the half of potential GP (B, $P=0.01$) and the required time to reach the R_{max} (T_{max} , $P=0.007$) regardless of the dose level, but without affecting the fermentation rate. Despite the rapid use of fermentable carbohydrate by the microbial population at the beginning of the fermentation, the EO depress the growth and the activity of ruminal microbes because of the high antimicrobial activity of eucalyptus EO against pathogens. But it seems to be a potent inhibitor against ruminal bacteria especially the protozoa as reported by Nooriyan Soroor and Rouzbehan (2017). The digestibility of wheat straw was not affected by the EO addition, suggesting no disturbing effect on ruminal fermentation despite the decrease of total gas production. Therefore, we can hypothesize that the EO addition modifies the microbial population of ruminant without affecting the digestibility of the feed (Newbolda *et al.* 2007).

The combination between EFE and EO decreased the extent of wheat straw fermentation especially with the highest doses (EFE2+EO2) by 35.6% accompanied by a rapid increase of the fermentation rate by 68%, while, the T_{max} and B decreased by 35.7% and 63% respectively. Compared to the EFE, the EO depress the effect of EFE regardless of the used doses, which caused an antagonist effect on the *in vitro* fermentation and the digestibility of wheat straw.

To conclude, the EFE and SC supplementation promote

the *in vitro* ruminal digestibility of wheat straw. No synergism was observed between the exogenous fibrolytics enzymes and live yeast or *Eucalyptus* essential oil on *in vitro* measured parameters.

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REFERENCES

- AOAC. 1990. *Official Methods of Analysis*. 15th edn. Association of Official Analytical Chemists, Arlington, VA, USA.
- Agabriel J. 2010. Alimentation des bovins, ovins et caprins/ : les Tables Inra. Quae. p 312.
- Bailey M J, Biely P and Kaisa P. 1992. Interlaboratory testing of methods for assay of xylanase activity. *Journal of Biotechnology* **23**: 257–70.
- Beauchemin K A, Colombatto D, Morgavi D P and Yang W Z. 2003. Use of exogenous fibrolytic enzymes to improve feed utilization by ruminants. *Journal of Animal Sciences* **81**: 37–47.
- Beauchemin K A, Krehbiel C R and Newbold C J. 2006. Enzymes, bacterial direct-fed microbials and yeast: principles for use in ruminant nutrition. pp 251–284. Elsevier Ltd.
- Bowman G R, Beauchemin K A and Shelford J A. 2002. The proportion of the diet to which fibrolytic enzymes are added affects nutrient digestion by lactating dairy cows. *Journal of Dairy Science* **85**(12): 3420–29.
- Calsamiglia S, Busquet M, Cardozo P M, Castillejos L, Ferret A and Fandiño I. 2007. Essential oils as modifiers of rumen microbial fermentation. *Journal of Dairy Science* **90**(6): 2580–95.
- Denev S A, Peeva T, Radulova P, Stancheva N, Staykova G, Beev G, Todorova P and Tchobanova S. 2007. Yeast cultures in ruminant nutrition. *Bulgarian Journal of Agricultural Science* **13**: 357–74.
- Duncan D B. 1955. Multiple F and multiple “F” test. *Biometrics* **11**(4): 1955.
- Eun J S and Beauchemin K A. 2007. Enhancing *in vitro* degradation of alfalfa hay and corn silage using feed enzymes. *Journal of Dairy Science* **90**(6): 2839–51.
- Eun J S and Beauchemin K A. 2007. Assessment of the efficacy of varying experimental exogenous fibrolytic enzymes using *in vitro* fermentation characteristics. *Animal Feed Science and Technology* **132**(3–4): 298–315.
- Getachew G, Blümmel M, Makkar H P S and Becker K. 1998. *In vitro* gas measuring techniques for assessment of nutritional quality of feeds: a review. *Animal Feed Science and Technology* **72**: 261–81.
- Giraldo L A, Carro M D, Ranilla M J, Tejido M L and Mohamed A H. 2007. *In vitro* ruminal fermentation of low-quality forages as influenced by the treatment with exogenous fibrolytic enzymes. *Advanced Nutrition and Feeding Strategies to Improve Sheep and Goat* **267**(74): 263–67.
- Groot J C J, Cone J W, Williams B A, Debersaques F M A and Lantinga E A. 1996. Multiphasic analysis of gas production kinetics for *in vitro* fermentation of ruminant feeds. *Animal Feed Science and Technology* **64**(1): 77–89.
- Hristov A N, Ropp J K, Zaman S and Melgar A. 2008. Effects of essential oils on *in vitro* ruminal fermentation and ammonia release. *Animal Feed Science and Technology* **144**: 55–64.
- Jerbi A, Derbali A, Elfeki A and Kammoun M. 2017. Essential

- oil composition and biological activities of *Eucalyptus globulus* leaves extracts from Tunisia. *Journal of Essential Oil Bearing Plants* **20**(2): 438–48.
- Kholif A E, Abdo M M, Anele U Y, El-Sayed M M and Morsy T A. 2017. *Saccharomyces cerevisiae* does not work synergistically with exogenous enzymes to enhance feed utilization, ruminal fermentation and lactational performance of Nubian goats. *Livestock Science* **206**: 17–23.
- Kumar P, Barrett D M, Delwiche M J and Stroeve P. 2009. Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. *Industrial and Engineering Chemistry Research* **48**(8): 3713–29.
- Mao H L, Wu CH, Wang J K and Liu J X. 2013. Synergistic effect of cellulase and xylanase on *in vitro* rumen fermentation and microbial population with rice straw as substrate. *Animal Nutrition and Feed Technology* **13**(3): 477–87.
- Menke K H and Steingass H. 1988. Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. *Animal Research and Development* **28**: 7–55.
- Mugerwa S, Kabirizi J, Zziwa E and Lukwago G. 2012. Utilization of crop residues and agro-industrial by-products in livestock feeds and feeding systems of Uganda. *International Journal of Biosciences* **2**(4): 82–89.
- Newbolda C J, McIntosha F M, Williams P, Riccardo L and Wallace R J. 2007. Effects of dose and adaptation time of a specific blend of essential oil compounds on rumen fermentation. *Animal Feed Science and Technology* **132**(3–4): 186–201.
- Nooriyan Soroor M E and Rouzbehan Y. 2017. Effect of essential oils of eucalyptus (*Eucalyptus globulus labill*) and angelica (*Heracleum persicum* desf. ex fischer) on *in vitro* ruminal fermentation, protozoal population and methane emission using afshari sheep inoculum. *Journal of Agricultural Science and Technology* **19**(3): 553–67.
- Nsereko V L L, Beauchemin K A A, Morgavi D P P, Rode L M M, Furtado A F F, Mcallister T A A, Iwaasa A D D and Yang W Z Z. 2002. Effect of a fibrolytic enzyme preparation from *Trichoderma longibrachiatum* on the rumen microbial population of dairy cows. *Canadian Journal Of Microbiology* **20**: 14–20.
- Phakachoe N, Suksombat W, Colombatto D and Beauchemin K A Phakachoe. 2013. Use of fibrolytic enzymes additives to enhance *in vitro* ruminal fermentation of corn silage. *Livestock Science* **157**(1): 100–12.
- Van Soest P J, Robertson J B and Lewis B A. 1991. Polysaccharides in relation to animal nutrition. *Journal of Dairy Science* **74**: 3583–97.
- Vilela F G, Bueno I C, Netto A S, Canaes T, Freitas J E, Venturelli B C and Renno F. 2012. Effect of oregano, ginger and thyme oils on *in vitro* rumen fermentation and methane emission. *Journal of Dairy Science* **95**: 628.
- Sontakke U. 2012. Benefits of *Saccharomyces cerevisiae* as a feed additive in ruminants. *Engormix*. Available at <https://en.engormix.com/dairy-cattle/articles/saccharomyces-cerevisiae-in-ruminants-nutrition-t35453.htm>1–5.
- Sun R C. 2010. Cereal straw as a resource for sustainable biomaterials and biofuel: Chemistry, extractives, lignins, hemicelluloses and cellulose. Elsevier, 300p.
- Tadesse A, Fulpagare Y G and Gangwar S K. 2014. Effect of urea treatment on chemical composition and oxalate content of sugarcane tops. *International Journal of Science and Nature* **5**(1): 15–18.
- Tadesse G. 2014. Rumen manipulation for enhanced feed utilization and improved productivity performance of ruminants: a review. *Momona Ethiopian Journal of Science* **6**(2): 3–17.
- Wood T M and Bhat K M. 1988. Biomass Part A: Cellulose and Hemicellulose. *Methods in Enzymology* **160**: 87–112.
- Yang H J, Tamminga S, Williams B A, Dijkstra J, Boer H and Yang H J. 2005. *In vitro* gas and volatile fatty acids production profiles of barley and maize and their soluble and washout fractions after feed processing. *Animal Feed Science and Technology* **120**(1–2): 125–40.