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 39ºC 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, CH_4 production and NH_3-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 ($P < 0.05$) with 100 µl YSE compared with the control. Acetate to propionate ratio tended to decrease ($P = 0.091$) with addition of YSE. Total rumen protozoal number increased ($P< 0.05$) 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, NH_3-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 CH_4 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±2.3kg) 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/100kg 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 pre-warmed thermo insulated flasks and transported under anaerobic conditions to the laboratory (Bueno *et al.,* 2005). Pooled rumen contents were squeezed through four layers of cheese-cloth and kept in a water bath at 39°C saturated with CO_2 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, $CaCl_2$ 0.1g, $MgSO_4.7H_2O$ 0.1 g, KH_2PO_4 2.0 g and Na_2HPO_4 6.0 g per 1liter of distilled water). The pH was adjusted to 6.8 and CO_2 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 24h 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; r^2 = 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 CH_4 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 (4ºC) 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, protozoa 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, Lancing, 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 4ºC (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: $yij = \mu + Fi + eij$ Where: μ 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 $P< 0.05$.

RESULTS AND DISCUSSION

In vitro gas and CH_4 production:

Effects of different levels of YSE on GP and CH_4 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 CH_4 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 CH_4 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 CH_4 production Mim *et al.* (2015) and Holtshausen *et al.* (2009).

Addition of yucca extract from 0 to 3.2 mg/ml reduced *in vitro* CH_4 and GP (Lila *et al.,* 2003). In lactating cows, Ramirez-Restrepo *et al.* (2016) and Guyader *et al.* (2015), reported higher CH_4 emissions with tea saponins with linear reduction in ruminal protozoa number, which produce and supply large amounts of H_2 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 sarsaponin 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 H_2 production with tea saponins increased rumen acetate proportion, which is known to release H_2 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 cellulolytic 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	YE, µl			P-value
		25	50	100	
GP (ml/g DM)	181.34±9.50[a]	166.64±11.98[b]	160.56±23.62[b]	157.47±17.92[b]	0.0003
CH_4 (ml/g DM)	11.85±0.93[ab]	9.84±2.13[b]	12.96±2.68[a]	10.55±0.94[b]	0.0373
CH_4 (ml/g OM)	13.24±1.64[a]	9.83±0.14[b]	12.50±1.75[a]	12.41±2.72[a]	0.0208
TDDM (%)	76.49±3.04	77.47±4.06	75.52±2.84	75.28±2.52	0.4529
TDOM (%)	70.53±1.52[a]	69.78±0.97[a]	70.49±1.40[a]	66.26±3.40[b]	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 by18.5 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		YE, µl			
	Control	25	50	100	P-Value
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[ab]	20.46±2.73[c]	25.64±1.68[a]	22.92±1.94[b]	0.0002
Short chain 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±9.96	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±1.40[b]	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, $x10^5$/ml					
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* CH_4 production, protozoal numbers and NH_3-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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الإستجابات المعملية لمستخلص نبات اليوكا على إنتاج الميثان وخصائص تخمرات الكرش وهضم الغذاء

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الدراسة الحالية صممت لبحث تأثير إضافة مستخلص نبات اليوكا بمستويات (25 و 50و 100 ميكروليتر على سائل الكرش معمليا) وتأثيره على خصائص تخمرات الكرش وهضمية الغذاء وإنتاج الميثان بإستخدام تقنية السيمى أتوماتيك لتحليل إنتاج الغاز(GP). حيث تم استخدام 3 أغنام برقى مجهزة بفستيولا الكرش بوزن (58±2.2 كجم) كمصدر مانح لسائل الكرش المستخدم حيث تم تحضينه فى بيئة لاهوائية على 39 درجة مئوية لمدة 24 ساعة مع 300 مجم مادة علفية مكونة من (50% مخلوط مركز + 50% عرش فول سودانى). تم القيام بثلاث دورات لتحليل إنتاج الغاز معمليا وأوضحت النتائج أن إضافة مستخلص نبات اليوكا للمادة العلفية خفضت من إنتاج الغاز بشكل معنوى مقارنة بمعاملة المقارنة كذلك بالنسبة لإنتاج الميثان وتركيز الأمونيا تم خفضهما بشكل معنوى بنسبة 37% و 18.5% على الترتيب مع مستوى 25 ميكروليتر أما معامل هضم المادة الجافة الحقيقى (TDDM) لم يحقق أى فرق معنوى بين المعاملات مقارنة معاملة المقارنة بينما معامل هضم المادة العضوية الحقيقى (TDOM) إنخفض بشكل معنوى خاصة لمستوى 100 ميكروليتر من مستخلص اليوكا ولكن المستويين 25 و50 ميكروليتر لم تحقق فروق معنوية مقارنة بمعاملة المقارنة. أيضا نسبة حامض الأستيك الى حامض البروبيونيك مالت للإنخفاض (p=0.091) مع إضافة اليوكا مقارنة بمعاملة المقارنة وكذلك بروتوزوا الكرش الكلية إنخفضت بشكل معنوى مع مستوى 50 ميكروليتر بينما المستويين 25 و100 ميكروليترانخفضت ولكن بشكل غيرمعنوى.

أشارت النتائج الى أن إضافة مستخلص نبات اليوكا مؤثر قوى يحد من عملية انتاج الميثان بالكرش وتركيز امونيا الكرش وعشائر برتوزوا الكرش والذى من خلاله يمكن تعديل بيئة تخمرات الكرش بحيث يمكن إعتبارة معدل نافع لبيئة الكرش لتحسين الإستفادة من العناصر الغذائية وتحسن اداء الحيوان.