Egyptian J. Nutrition and Feeds (2010) 13 (3): 465-484

INFLUENCE OF INOCULANTS TREATMENT OF CITRUS PULP AND DRY YEAST SUPPLEMENTATION ON PHYTONUTRIENTS CONCENTRATIONS, RUMEN FERMENTATION AND COWS PERFORMANCE.

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(Received 24/6/2010, Accepted 10/11/2010)

SUMMARY

In order to study the effect of effect of inoculants (LAB) treatment and dried yeast (DY) supplementation on the concentration of phytonutrients (anti-nutritional factors) in citrus pulp (orange), two underground trenches (25 ton each) were filled with citrus pulp and chopped rice straw (4:1) on DM basis . No additives were introduced in the first trench while inoculants were added to the second trench. Four diets were studied: untreated silage plus concentrate feed mixture (CFM) (D1), D1 plus DY (D2), treated silage plus CFM (D3) and D3 plus DY (D4). Digestibility trials were carried out using three Barki rams for each diet, while three other females sheep fitted with permanent rumen fistula were used for rumen fermentation and in situ studies. Twenty lactating crossbreed Friesian cows in their third and fourth lactation season were used after the first week of lactation. Cows were divided into four similar groups according to live body weight (535 ± 11.46 kg) and previous milk records (10-12 kg/ day in average) (Five cows for each group) using (2 x 2) factorial designs. Sheep were offered silage ad libitum plus 600 g/head/d CFM for digestibility and fermentation trials, while cows were fed 8 kg/ head/ day of CFM. Dried yeast was added to CFM at the rate of 5 g/ head for sheep and 10 g/ head for cows. The results showed that: 1- phytonutrients had been decreased with inoculation treatment, 2- an accumulative effect of inoculants and DY on digestion coefficients was observed; less ruminal NH-N3 and more VFA concentrations was obtained; more effective degradability (ED) of DM and OM and more microbial protein (MP) synthesis was found and 3- Milk production expressed as 4% FCM was increased with inoculation and supplementation with DY and had about 187% more cash return. In conclusion adding inoculants during making citrus pulp silage with rice straw (4: 1 DM basis) decreased citrus pulp content of phytonutrients. Feeding treated silage with DY supplementation improved nutrients digestibility, animal performance and economic return.

Keywords: *citrus pulp, phytonutrients, silage, digestibility, degradability, lactating cows.*

INTRODUCTION

Good quality feeds are needed to sustain livestock growth, especially during the dry season. Crop residues, agro-industrial by-products feed resources which abound during the

dry season are being evaluated to assess their nutritive potential to support livestock productivity. The annual productions of vegetables and fruits by-products in Egypt were estimated to be about 3.5 million tons (Agriculture Economics, 2004). One of the industrial by-products is citrus pulp lifted from the orange juice industry. Citrus pulp composed of 60 – 65% peel, 30 – 35% pulp and 0 –10% seeds (DM basis). It contains good energy substrates for ruminal microbes, including both soluble carbohydrates and a readily digestible NDF fraction, but its high moisture content may limit its storage. Citrus pulp has been previously used as a high energy feed in rations of growing and lactating cattle (Solomon *et al.* 2000 and Miron *et al.* 2001). Several factors had been generally identified that may limits its utilization or incorporation as non-conventional feedstuffs in livestock rations. These include low protein content, high fiber, and imbalance of amino acids in addition to the presence of anti-nutritional factors or phytonutrients (McDonald *et al.* 1988 and Oluremi *et al.* 2007). Phytonutrients have significant negative effects on livestock production; included reduction in palatability, digestibility and utilization of nutrients, resulting in not only decreased production but also low quality of meat and milk products due to the presence of such hazardous residues (Amuchie, 2001). Ensiling process and biological additives may help in solving the anti-nutritional factors problems (Migwi *et al.* 2001). Ensiling had improved fermentation of several varieties of forage crops (Weinberg *et al.* 2003). However, inoculation with lactic acid bacteria to forage is needed to ensure consistent improvement in fermentation (Huisden *et al.* 2009). On the other hand, yeast is a rich source of vitamins, enzymes and has other co-factors that may improve appetite and rumen environment (Putnam *et al.* 1997 and Moallem *et al.* 2009). Supplementing animal diets with small amounts of yeast has been shown to improve rumen digestibility of nutrients especially crude fiber, microbial activities and animals performance and health (Dawson and Steen 1997 and Magalhães *et al.*, 2008).The objective of this study was to determine the effect of inoculants and dry yeast supplementation on phytonutrients concentrations, rumen fermentation and cows performance.

MATERIALS AND METHODS

The present study was conducted at Noubaria Experimental Station, Animal Production Research Institute, Agriculture Research Center, Egypt.

Silage preparation and its quality:

Citrus pulp of orange was collected from Edffina canning factory in Alexandria Governorate. Rice straw (RS) was chopped to about 1-2 cm in length. Chemical composition of citrus pulp and rice straw is presented in Table (1).

Table (1). Proximate chemical analyses of citrus pulp and rice straw (on DM %).

Item	Chemical composition,%					
	OM	CP	CF	EE	NFE	Ash
Citrus Pulp	94.83	11.03	16.65	10.95	56.20	5.17
Rice straw	86.64	3.41	38.74	1.64	42.85	13.36

Two underground trenches (25 ton each) were filled with the chopped materials (4:1, citrus pulp: RS, on DM basis). No inoculants were introduced in the first trench while an inoculants of Lactobacillus planetarium (4 X 10^6 CFU/g) and Enterococcus faecium (4 X 10^6 CFU/g) produced by Pioneer Hi-bred International, Inc (LAB) were added at the rate of 1g/ 2 L water / ton of materials in the second trench. The two trenches were covered tightly with plastic sheet after pressing the silage by a tractor. The silage was also covered with 25 cm of soil layer to guarantee anaerobic condition and left for 60 days. In order to determinate the silage quality, polyethylene bags (three were used for each kind of silage) were packed by 500 g of the chopped materials at the same mixed ratio pressed well and kept closed and left at room temperature for 60 days. When bags were opened, color and odor were directly examined .Values of pH, ammonia-N (NH_3-N), lactic, acetic, and butyric acids were determined in the extraction of silage. Values of pH were determined directly using Beckman pH meter. The concentration of ammonia nitrogen was determined using magnesium oxide (MgO) as described by Al-Rabbat *et al.* (1971). Determination of lactic and acetic acids was achieved using gas chromatography according to England and Gill (1983). Proximate analyses were performed according to A.O.A.C methods (1995).

Approximately 200 mg (DM) of ground samples of citrus pulp and its silage were extracted in 10 ml of aqueous acetone (7:3 v/v) in a water bath maintained at 39–40 °C for 90 min (Makkar, 2000). Total phenolic components (TPC) were assayed by Folin-Ciocalteu-reagent 2N (Sigma®–Aldrich, El-Safua Co., Alexandria, Egypt) based on known concentrations of tannic acid as the calibration curve (Sigma®–Aldrich) according to Makkar and Becker (1993). Condensed tannins (CT) were determined according to Makkar (2003). Saponins (SAP) were extracted and isolated according to Ahmad *et al.* (1990). Alkaloid (ALK) was determined according to (Arambewela and Ranatunge, 1991). Phytic acid (PA) concentration was measured according to Wheeler and Ferrel (1979). Total oxalate (TO) was analyzed using a titrimetric method (Moir, 1953). Flavonoids (FLA) were determination according to Boham and Kocipai (1994).

Live dried baker's yeast (DY) saccharomyces was collected from the Egyptian Company for starch, yeast and detergents. It contains yeast culture (live *Saccharomyces cerevisiae* (10^9 CFU/g) grown on a media of corn and supported by Vitamin B_1 (0.017 mg/ 100 g), Vitamin B_2 (0.004 mg/ 100 g) and Nichotenic acid (0.055 mg/ 100 g). Concentrate feed mixture (CFM) used consists of 33% yellow corn, 5% soybean meal, 20 % wheat bran, 17% rice bran,17% undecorticated cotton seed meal, 4.5% molasses, 2% limestone, 1%salt, 0.5% mineral mixtures. Table (2) illustrated the chemical composition of silages, CFM and DY.

Digestibility and nitrogen balance trials:

Digestibility and nitrogen balance trials were carried out using three Barki rams (41± 1.50 kg, a live body weight) for each diet. Each trial lasted for four weeks; the first three weeks as a preliminary period, followed by one week for feces and urine collection. Animals were offered silage *ad libitum* twice a day at 9.0 a.m and 4.0 p.m. plus 600 g/head/d CFM in order to meat their maintenance requirements according to NRC (1994).The four diets were: untreated silage plus concentrate feed mixture (CFM) (D_1), D_1 plus 5g/head/d DY added to CFM (D_2), treated silage plus CFM (D_3) and D_3 plus 5g/head/d DY added to CFM (D_4). Chemical composition of feeds, feces and urine was determined according to A.O.A.C methods (1995). Fiber fractions (NDF, ADF and ADL) were determined according to Van

Soest *et al.* (1991).

Table (2). Chemical composition of silage, CFM, and DY (% on DM basis).

Items	Untreated silage[A]	Treated silage[B]	(CFM)[C]	(DY)[D]
Chemical analysis:				
OM	84.12	83.95	90.06	94.58
CP	8.65	8.72	14.81	46.32
CF	30.22	28.56	12.65	3.12
EE	3.04	3.08	2.92	0.98
NFE	42.21	43.59	59.68	44.16
Ash	15.88	16.05	9.94	5.42
NDF	35.62	33.96	51.93	------
ADF	26.14	23.81	34.14	------
ADL	5.02	4.77	4.89	------
Hemicelluloses	9.48	10.15	17.79	------
Cellulose	21.12	19.04	29.25	------

A: (Citrus pulp+RS; 4:1) silage (untreated silage).
B: (Citrus pulp+RS; 4:1) silage treated by lactic acid bacteria inoculums.
C: concentrate feed mixture.
D: dried baker's yeast.

Rumen fermentation and In situ trials:

Three ruminally-canulated Barki ewes were used for testing the rumen fermentation and *in situ* trials for each diet. Rumen samples were withdrawn before feeding and 1, 3 and 6 hrs after feeding for *in vitro* incubation using the zero rate techniques as described by Carrol and Hungate (1954). Ruminal pH and NH_3-N values were determined as before. Total VFA's were determined by steam distillation as described by Warner (1964). Rumen volume was determined by colorimetric method of cr-EDTA before, 3 and 6 hrs after feeding (El-Shazly et al. 1976). The microbial protein synthesis (g MP/day) in the rumen of sheep fed the experimental diets was calculated using the model equation by Borhami et al. (1992) as follow: g MP/day = mole VFA produced / day X 2 X 13.48 X 10.5 X 6.25 / 100

where one mole VFA yield about 2 mole ATP (Walker, 1965), one mole ATP produce 13.48 Y_{ATP} (g DM microbial cell) Borhami *et al.* (1979), N % of dry microbial cell = 10.5 (Hungate, 1965).

Nylon bags technique (Mehrez and Ørskov, 1977) was used to determine DM, OM and CP degradability for silage. Two polyester bags (7X15 cm) with pore size of 45 μm were used for each incubation time. Approximately 5g of air-dried silage (ground to 2 mm) were placed in each bag. Bags were incubated in the rumen of each sheep and withdrawn after 3, 6, 12, 24, 48, 72 and 96 h. After the bags were withdrawn from the rumen, they were rinsed in tap water until the water became clear, then they were squeezed gently. Microorganisms attached to the residual sample were eliminated by freezing at - 20°C (Kamel et al. 1995). Zero-time washing losses (a) were determined by washing 2 bags in running water for 15 min. The degradation kinetics of DM, OM and CP were estimated (in each bag) by fitting the disappearance values to the equation P = a + b (1- e-Ct) as proposed by Ørskov and McDonald (1979), where P represents the disappearance after

time t. Least-squares estimated soluble fractions are defined as the rapidly degraded fraction (a), slowly degraded fraction (b) and the rate of degradation (c), respectively. The effective degradability (ED) for tested silages were estimated from the equation cited by McDonald (1981), ED = a + bc / (c + k), where k is the out flow rate.

Lactation trials:

Twenty lactating crossbreed Friesian cows in their third and fourth lactation season were used after the first week of lactation. Cows were divided into four similar groups according to live body weight (535 ± 11.46 kg) and previous milk records (10-12 kg/ day in average) (Five cows for each group) using (2 x 2) factorial designs. Each group was offered one of the experimental diets for (100 days). The cows were fed 8 kg/ head/ day of CFM according to NRC (2001). Silage was fed *ad libtum* and the actual amount of silage consumed was recorded daily. Diets were fed twice a day at 8.00 and 16.00. Cows were machine milked twice daily at 8.00 and 16.00. Milk yield were individually recorded weekly at morning and evening during all experimental period (100 days). Milk samples from consecutive evening and morning milking were taken and mixed in proportion to their yield; milk samples were collected biweekly during all experimental period. Milk samples (100ml) were kept at 4°C for latter analysis. Fat correct milk (4%) was calculated according to Gaines (1923) using the following equation:

$$FCM = 0.4\ M + 15.0\ F,$$ Where M = milk yield and F = fat yield

Milk fat percentage was determined according to Gerber's method as described by Ling (1963). Total solids percent (TS), total protein and ash were determined according to the standard methods of A.O.A.C. (1995). Lactose was determined according to a rapid method for the determination of lactose in milk and cheese described by John *et al.* (1957). Solid not fat (SNF) was calculated by differences. Energy of milk was calculated using the formula suggested by McDonald *et al.* (1978) as follow: Energy Kcal/ kg milk = (92.25 fat % + 49.15 SNF % - 56.4).

Blood samples were collected twice from all cows (one before the start of experiment and another at the end of the experimental period. Blood samples were obtained from the external jugular vein of each animal in the morning before access to feed and water. Serum was obtained by centrifugation of blood at 4000 rpm for 15-min and was stored at-20°C until the time of analysis. Various chemical parameters were calorimetrically determined using commercial kits; following the same steps as described by manufactures. Glucose concentration was determined according to Trinder (1969); total proteins (TP) was determined according to Armstrong and Carr (1964); albumin (A) was assayed according to Doumas *et al.* (1971); Globulin was calculated by subtracting the albumin value from total protein value; Cholesterol was detected according to Roeschlau *et al.* (1974); urea was detected according to Berthelot (1959) and the activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) according to Reitman and Frankel (1957) were performed.

Statistical analysis:

Data were statistically analyzed as (2 x 2) factorial designs using tow – way ANOVA design procedure of (SAS, 2000); the model describing each trait was assumed to be:

$$Y_i = \beta_0 + \beta_{1z1i} + \beta_{2z2i} + \beta_{3z1iz2i} + e_i$$

Where:

Y_i = outcome score for the i_{th} unit, β_0 = coefficient for the intercept,
β_1 = mean difference on inoculants, β_2 = mean difference on dry yeast,
β_2 = interaction of inoculants and dry yeast , z_{1i} = dummy variable for inoculants,
z_{2i} = dummy variable for dry yeast, e_i = residual for the i_{th} unit,

Separation among means was carried out by using Duncan's Multiple Range Test, (Duncan, 1955).

RESULTS AND DISCUSSION

Silage quality:

Fermentation characteristics of treated or untreated silages during the ensiling period indicated a successful processing (Table 3). Silages had good smell and were free from any signs of molds. Values of pH indicated good preserved silage as it decreased with advancing ensilaging period where it reached 4.02 and 3.96 at 8 weeks of treatment for untreated and treated silages, respectively. Nkosi *et al.* (2009) reported that pH value of inoculated silage with lactic acid bacteria (LAB) decreased compared with the untreated, where LAB produced organic acids through its growth which reduced pH value. Reduction of the DM, energy losses and solubilization protein (SP), increases silage bunker life was also observed. The changes in NH_3-N and VFA values indicated less rate of SP content, solubilization of true protein occurs in the silo due to the action of plant proteases enzymes (Beever *et al.* 1986). However, fermentation characteristics are in agreement with previous studies reported by Filya (2003); Okine *et al.* (2005) and Sun *et al.* (2009).

Table (3): Silage quality at the opening day.

Item	Untreated silage	Treated silage
DM,%	33.56	32.12
Acidity (meq/g)	7.22	8.95
pH	4.02	3.96
NH_3-N,% of DM	0.08	0.07
Lactic acid, % of DM	4.58	5.02
Acetic acid, % of DM	3.26	3.04
Butyric acid, % of DM	1.07	1.02

Concentrations of Phytonutrients:

The screening of phytochemical in the citrus pulp and its silage revealed the presence of total phenolic components, tannin, saponin, phytate, oxalate, alkaloids, and flavonoid (Table 4). It was clear that making silage could be a good process to reduce concentration of phytonutrients in citrus pulp. Inoculation with LAB had more influence in that respect, whereas, it resulted in less concentration of phytonutrients than the critical percentages. These could be explained by the role of LAB in solubilization of such chemicals in the

silage's bunker (Daliéa *et al.* 2010). Condensed tannin (CT) was dropped from 0.523to 0.282 with LAB treatment, while it only dropped to 0.326 after making silage without LAB treatment. This is lower than 1- 20% commonly found in cereals and legumes (Price and Butler 1980). In ruminants, dietary CT of 2-3% have been shown to impart beneficial effects because they reduce the wasteful protein degradation in the rumen by the formation of protein tannin complex (Al-Soqeer, 2008).The concentration of saponin in the pulp of the citrus was 0.043%, while it is not detected in its both silages. Saponins are bitter and reduce palatability of livestock feeds (Oluremi *et al.* 2007). However, saponin contents in the citrus pulp s was observed to be appreciably below 3% reported by Kumar (1991) which could be responsible for cattle weight losses when they grazed on alfonibrilla (Drymaria arenaroides). Phytate and oxalate levels in citrus pulp seems to be safe for livestock consumption, especially oxalate it was low comparing with 0.7% and 0.27% in cocoa and beet roots, respectively (Concon 1988). Its that oxalate can decrease the availability of dietary essential minerals such as Ca, where at high concentrations it can causes death in animals due to its corrosive effects, while in small amounts, it can causes a variety of pathological disorders including hyperoxaluria, pyridoxine deficiency, and calcium oxalate stones (Kumar 1991). The amount of flavonoids was ranged between 0.032and 0.057% LAB silage and citrus pulp, which is less than the critical levels. Flavonoids have been reported to inhibit enzymes in mammals (CSIRO 2004). The detected levels of alkaloids in citrus pulp were less than critical values as well.

Table (4). Concentrations of Phytonutrients in citrus pulp and its silage (%).

Compound	Citrus wastes	Untreated silage	Treated silage
TPC	0.637	0.413	0.376
CT	0.523	0.326	0.282
SAP	0.043	ND	ND
PA	0.087	ND	ND
TO	0.046	0.032	0.029
FLA	0.057	0.035	0.032
ALK	0.063	0.030	0.024

TPC, total phenolic components; CT, condensed tannins; SAP, saponins; PA, phytic acid; TO, total oxalate; FLA, Flavonoid; ALK, alkaloids,. ND: not detectable.

Digestibility and nitrogen balance trials:

Nutrients digestibility, nutritive value and nitrogen utilization of experimental diets are shown in Table (5). Inoculated silage and supplementation with DY resulted in higher ($P< 0.01$) nutrients digestibility compared to other silages. These could be related to the microbial activities which solubilizing of carbohydrate esters of phenolic monomers in the cell wall (Khampa *et al.* 2009).Yan *et al.* (1996) reported improvements in nutrients digestibility as a result of inoculants treatment or yeast supplementation. The improvement in digestion coefficients followed supplementation of DY could be related to its addition at

time of concentrate feeding. It could also due to higher feed intake as well as feeding values, being (1207g/d, 65.94 % TDN and 8.34 % DCP, respectively).

However, phytonutrients may alter the bacterial population in the rumen. Thus, they can affect the digestibility of dietary components and alter the end product of fermentation (Kumar 1991). Making silage resulted in hydrolysis of such phytonutrients which was reflected on its less effect on digestibility by animals. The increase of DMI by about 13.87 % could be due to the cumulative effect of DY supplementation and its combination with LAB, where it only 10.03 % for DY effect and 6.62 % for LAB effect. Results of nitrogen retained as a percentage of N-intake was obviously higher ($P< 0.01$) with inoculant's silage supplemented with DY (28.15%), than its individual effect (26.67 and 25.12%) for yeast and inoculants, respectively. The same trend was observed for N-utilization when it expressed as N-retained/ N-absorption (%). So, DY supplementation seems to be more effect on N utilization than LAB alone.

Table (5). Dry matter intake (g/h/d), digestibility coefficients, nutritive value and nitrogen utilization for sheep fed the experimental diets .

Item	Experimental diets							
	untreated Silage			Treated silage		P-valu		
	(D_1)	(D_2)	(D_3)	(D_4)	SEM	Inoc.[1]	DY [2]	Inoc.*DY[3]
DM intake (g/h/d):								
Silage intake, g	527^{c}	633^{a}	597^{b}	674^{a}	22.19	*	*	*
Concentrate intake, g	533	533	533	533	—	—	—	—
Total DMI,g	1060^{c}	1166^{a}	1130^{b}	1207^{a}	22.19	*	*	*
Roughage: concentrate ratio	50 : 50	54 : 46	53 : 47	56 : 44	—	—	—	—
Digestibility coefficients (%)								
DM	61.65^{d}	65.36^{b}	63.24^{c}	67.72^{a}	0.69	*	*	**
OM	63.54^{c}	66.58^{b}	66.01^{b}	70.44^{a}	0.78	*	*	*
CP	66.87^{d}	70.68^{b}	68.75^{c}	72.74^{a}	0.67	*	*	**
CF	59.69^{d}	63.43^{b}	61.33^{c}	66.74^{a}	0.80	*	*	**
EE	60.40^{b}	60.24^{b}	62.27^{a}	62.13^{a}	0.34	*	NS	*
NFE	70.89^{c}	71.52^{c}	75.75^{b}	76.96^{a}	0.79	*	NS	*
NDF	63.24^{b}	69.91^{a}	65.71^{b}	71.55^{a}	1.08	NS	*	*
ADF	60.18^{d}	66.89^{b}	64.02^{c}	69.78^{a}	1.09	*	*	**
Hemicellulose	74.03^{c}	76.17^{b}	73.68^{c}	78.12^{a}	0.62	NS	*	*
Cellulose	67.79^{d}	76.45^{b}	72.56^{c}	79.93^{a}	1.40	*	*	**
Nutritive values (%):								
TDN	60.82^{d}	62.16^{c}	63.87^{b}	65.94^{a}	0.58	**	**	**
DCP	7.86^{c}	8.21^{b}	7.97^{cb}	8.34^{a}	0.07	NS	NS	*
Nitrogen utilization (g/h/d):								
N-intake (g/d)	19.94^{b}	21.68^{a}	20.97^{ab}	22.32^{a}	0.34	NS	*	**
N-absorbed (g/d)	13.33^{c}	15.32^{ab}	14.42^{bc}	16.23^{a}	0.36	*	*	**
N-retained (g/d)	4.64^{d}	5.78^{b}	5.27^{c}	6.27^{a}	0.19	*	*	**
N-retained as % of N-intake	23.27^{d}	26.67^{b}	25.12^{c}	28.15^{a}	0.58	*	*	**
N-retained as % of N- absorbed	34.79^{d}	37.72^{b}	36.54^{c}	38.70^{a}	0.51	*	*	*

** P< 0.01, * P< 0.05 and N.S = Not significant., *^{abcd} means in the same row with different superscripts significantly differ (P< 0.05), D1: CFM + (CP+RS) silage, D2: CFM + (CP+RS) silage +DY, D3: CFM + (CP+RS) LAB silage, D4: CFM + (CP+RS) LAB silage +DY*

Ruminal fermentation:

Ruminal pH values were not significantly affected by the dietary silages and/or DY supplementation, while concentration of ruminal metabolites (NH_3-N, mg/100 mlR.L and VFA, meq/100 mlR.L) were significantly ($P< 0.05$ and $P< 0.01$, respectively) varied among the experimental diets (Table 6). D_1 had the higher NH_3-N concentrations (15.42) followed by D_3 (14.50) without significant differences between each other. On the other hand, supplementation with DY resulted in less NH_3-N concentration in the rumen of sheep fed the D_2 and D_4. Higher rate of ammonia-nitrogen production was observed with D_4 and D_2 (4.53 and 4.41, respectively). Both untreated and treated silage without yeast supplementation had quiet similar rate of production. Diets containing either untreated or treated silage supplemented with yeast had the higher rate of NH_3-N production. While those containing untreated or treated silage without yeast addition were showed the lower rate of NH_3-N production. Supplementing with yeast led to an increase in TVFA's concentrations. Lower ($P< 0.01$) concentrations obtained for D_1. No effect seem to be found for inoculants on the rate of VFA production , but DY supplementation and its effect of interaction with inoculants treatment had highly ($P< 0.05$) increased. The overall mean revealed that a high ($P < 0.05$) rate of out flow from the rumen was obtained with sheep fed $D_1$and D_3 compared to other two diets which showed almost similar rate of out flow. Average values of microbial protein synthesis (MP) ranged from 45.64 to 95.13 (g/d) for D_1 and D_4, respectively, it was lower ($P< 0.01$) for D_1 than other diets. The rate of out flow observed in this study with D_4 could be considered as suitable rate of out flow for efficient MP synthesis. Yeast supplementation increase numbers of ruminal cellulolytic bacteria and their activities, which could increase forages degradability and increase the flow rate of microbial protein as well and may alter the patterns of VFA's formations (Dawson and Tricarico, 2002). It also provides vitamins to support the growth of rumen fungi and stimulate utilization of hydrogen by ruminal acetogenic bacteria (Oeztuerk *et al.* 2005). Yeast is also observed to stimulate cellulolytic bacteria in the rumen, increase fiber digestion and flow of microbial protein from the rumen. The degradation of roughage components was improved due to the treatment effect of inoculation and / or the supplementation of the yeast as previously observed by Erasmus *et al.* (1992); El-Waziry *et al.* (2000) and Oeztuerk, (2009).

Table (6). Overall mean of rumen parameters of sheep fed the experimental diets.

Item	Experimental diets							
	untreated Silage		Treated silage		P-values			
	(D_1)	(D_2)	(D_3)	(D_4)	SEM	Inoc.[1]	DY[2]	Inoc.*DY[3]
PH	6.53	6.46	6.49	6.42	0.08	NS	NS	NS
NH_3-N concentration(mg/100mlR.L)	15.42[a]	13.11[b]	14.50[a]	12.85[b]	0.35	NS	*	*
Rate of NH_3-N production(mg/100 mlR.L)	3.04[b]	4.41[a]	3.09[b]	4.53[a]	0.22	NS	*	*
VFA concentration (meq/100 mlR.L)	8.83[c]	11.62[b]	11.16[b]	12.38[a]	0.41	*	*	**
Rate of VFA production (meq/100 mlR.L)	3.17[b]	4.43[a]	3.87[ab]	4.79[a]	0.22	NS	*	*
Rumen volume (L)	3.04[d]	3.56[b]	3.25[c]	4.04[a]	0.13	*	*	*
Out flow rate(%/hr)	6.44[a]	5.39[b]	6.24[a]	5.80[b]	0.13	NS	*	*
Microbial Protein Synthesis(g/h/day)	45.64[d]	75.72[b]	60.75[c]	95.13[a]	5.87	*	*	**

*** P< 0.01, * P< 0.05 and N.S = Not significant.*

[abc] means in the same row with different superscripts are significantly differ (P< 0.05).

Degradation kinetics:

In situ DM, OM and CP degradability are presented in Table (7). It illustrated that washing loss fraction "a" of DM, OM and CP for silages were not significantly different (P< 0.05).The degradable fraction "b" of DM and OM was not affected for inoculants treatment, while effective degradability was significantly affected. Meanwhile, DY and its interaction with inoculants treatment had positively affected (P< 0.05) for fraction "b" and effective degradability. However, DY supplementation had more individual effect than LAB; this could be due to the more nutrients digestibility. This finding agrees with those reported by Erasmus *et al.* (1992); Kamel *et al.* (2000) and El-Waziry and Ibrahim (2007), they reported an increase in protein flow from the rumen of sheep fed diet supplemented with yeast culture. When LAB and DY were combined together, the soluble and insoluble fractions increased and the effective degradability was also increased by about 17.42 and 15.48 for DM and OM of the treated materials against the untreated one, respectively. These could be due to the synchronization effect of DY and LAB together on the function of the cell wall of such materials and decreased concentrations of all phytonutrients. So, it means that it is easier to achieve a balance between rumen undegradable and degradable proteins when citrus pulp was conserved in silage with LAB and DY supplementation together. However, it seems that no effect of inoculants treatment, DY and their interaction on any of degradation kinetics and the effective degradability for crude protein.

Table (7): Degradation kinetics of DM, OM and CP for single roughage in sheep fed the experimental diets.

Item	Experimental diets							
	untreated Silage		Treated silage		P-values			
	(D_1)	(D_2)	(D_3)	(D_4)	SEM	Inoc.[1]	DY [2]	Inoc.*DY[3]
				DM				
a	31.42	34.16	31.70	35.96	2.05	NS	NS	NS
b	43.80^{b}	46.08^{ab}	42.93^{b}	48.97^{a}	0.79	NS	*	*
c	0.0537^{c}	0.0635^{ab}	0.0596^{bc}	0.0677^{a}	0.0016	NS	*	*
EDDM	59.52^{c}	65.45^{b}	60.25^{c}	69.89^{a}	2.43	*	*	*
				OM				
a	31.86	33.89	31.54	36.08	2.09	NS	NS	NS
b	43.13^{b}	45.96^{ab}	43.16^{b}	47.43^{a}	0.59	NS	*	*
c	0.0491^{c}	0.0553^{b}	0.0528^{b}	0.0601^{a}	0.0012	*	*	*
EDOM	58.63^{c}	63.68^{b}	59.06^{c}	67.71^{a}	1.05	*	*	*
				CP				
a	28.41	29.43	29.13	30.02	0.55	NS	NS	NS
b	36.14	37.75	35.90	37.87	0.46	NS	NS	NS
c	0.0512	0.0603	0.0535	0.0618	0.0013	NS	NS	NS
EDCP	51.19	54.63	52.13	55.51	2.06	NS	NS	NS

* P< 0.05 and N.S = Not significant.

^{abc} means in the same row with different superscripts are significantly differ (P< 0.05).

a: soluble fraction (%). b: potentially degradable fraction (%), c: rate of degradation (% h^{-1}).

ED: effective degradability= a + [bc/c + k], where k is the out flow rate assumed to be 0.03/ hr.

Milk yield and composition:

Feed intake of the experimental diets by dairy cows is presented in Table (8). Feed intake (kg/ head/ day) of diets containing treated silages were significantly higher ($P< 0.05$) than D_1. In addition, synergistic effect between microbiologically treatments increased roughages intake. The dry matter intake (DMI, kg/ head/ day) ranged between 13.18 and 14.92, whereas, higher ($P< 0.05$) DMI was obtained with cows supplemented with DY fed treated silage with LAB. Lower ($P< 0.05$) DMI was shown by cows fed untreated silage (13.18 kg/ head/ day). These results were in agreements with Wohlt *et al.* (1998); Dann *et al.* (2000); Boland (2002) and Marghany *et al.* (2005). They reported that yeast supplementation significantly increased DM intake by lactating cows. Also, Fraser *et al.* (2002); Owen (2002) and Okine *et al.* (2005) suggested that inoculants have positive effects on DMI compared with untreated silage. In general, cows fed on D_4 had highest ($P< 0.05$) daily milk yield and 4% FCM yield compared with other diets (Table 8). $D_1$was recorded the lowest milk yield and 4% FCM yield as well. D_2 and D_3 had intermediate values with significant differences. There were an improvement by about 10.43 % in milk yield and 11.74 % in 4% FCM yield as a result of inoculants treatment. Supplementation with DY resulted in an improvement in milk yield compared to the untreated silage. The corresponding improvement was 13.81% and 16.34 %, respectively. Schingoethe *et al.(* 2004); Beauchemin *et al.* (2003) and Jouany and Morgavi (2007) reported that yeast addition increased nutritional value of poor quality forages; improved feed intake and milk yield in dairy cows. This increase was much more compared to the finding of previous studies which showed an improvement between 1.4 to 3 kg/day for postpartum dairy cows fed direct-fed microbes (Erasmus *et al.* 1992; Sauvant 2005 and Nocek and Kautz 2006). In other studies, no significant effect was reported (Dann *et al.* 2000; Raeth-Knight *et al.* 2007). Improvement in milk yield was associated with an increase in fat and protein production, which agreed with that reported by Nocek and Kautz (2006). Cows fed D_4 had higher ($P< 0.05$) milk components (%) and yield (g). Lower milk composition (%) and yields (g/d) were found for cows fed D_1. Putman *et al.* (1997); Boland (2002) and Abdel-Khalek (2003) found that milk production and milk components of cows were improved significantly by adding yeast culture to the diets.

Effciency of milk production and economic evaluation:

When the milk efficiency was expressed as DMI or TDNI per kg 4% FCM produced, D_4 was more efficient, followed by D_2, then D_3 (Table 9), while the least efficiency one was found with D_1. Wholt *et al.* (1998) and Allam *et al.* (2001) reported that feed conversion was improved by yeast supplementation which confirms the obtained results. The cost of producing one kg milk ranged from PT. 105.94 to 123.64 for D_4 and D_1, respectively. So, D_4 was the cheapest diet in that concern, followed by D_2, then D_3. The economic return (L.E. / h/ d) or the profit above feeding cost was higher with yeast supplemented diets than other diets especially with silage treated with LAB.

Blood parameters:

Values of some blood constituents in the blood of cows consuming the different experimental diets are presented in Table (10). No significant differences were observed among groups concerning the entire blood constituent. Moreover, they were within the normal average as described by Stanek *et al.* (1992).

Table (8). Feed intake, milk yield and its constituents of lactating cross Friesian cows fed the experimental diets during lactating period.

Item	Experimental diets							
	Untreated Silage		Treated silage		P-values			
	(D_1)	(D_2)	(D_3)	(D_4)	SEM	Inoc.[1]	DY [2]	Inoc.*DY[3]
DM intake (kg/head/day):								
Silage intake, kg	6.07^c	7.34^b	7.21^b	7.80^a	0.21	*	*	*
Concentrate intake, kg	7.11	7.11	7.11	7.11	-	-	-	-
LDY intake, kg	---	0.01	---	0.01	-	-	-	-
DMI, kg	13.18^c	14.46^b	14.32^b	14.92^a	0.21	*	*	*
production (kg/ day) :								
Milk yield	11.25^c	12.72^b	12.35^b	14.13^a	0.36	*	*	*
4% FCM	10.05^c	11.58^b	11.13^b	13.05^a	0.37	*	*	*
Fat	0.37^d	0.43^b	0.41^c	0.49^a	0.02	*	*	*
Protein	0.33^d	0.40^b	0.36^c	0.46^a	0.02	*	*	*
Energy, Kcal	682.57^c	707.96^b	689.16^c	733.95^a	5.62	NS	*	*
Milk composition (%):								
Total solids	12.15^d	12.57^b	12.24^c	13.02^a	0.09	*	*	*
Solid not fat	8.86^c	9.17^b	8.90^b	9.53^a	0.08	*	*	*
Fat	3.29^c	3.40^b	3.34^b	3.49^a	0.03	*	*	*
Protein	2.93^b	3.15^a	2.95^b	3.23^a	0.05	NS	*	*
Lactose	5.20^c	5.31^b	5.22^c	5.58^a	0.04	*	*	*
Ash	0.73	0.71	0.73	0.72	0.02	NS	NS	NS

** P< 0.05 and N.S = Not significant.*

abcd means in the same row with different superscripts are differ significantly (P< 0.05).

Table (9). Nutrients intake, feed conversion and economic evaluation of daily milk production of cows fed the experimental diets during lactation period.

Item	Experimental diets							
	untreated Silage		Treated silage		P-values			
	(D_1)	(D_2)	(D_3)	(D_4)	SEM	Inoc.[1]	DY [2]	Inoc.*DY[3]
Nutrients intake(kg/h/d):								
DMI, kg	13.18^{b}	14.46^{ab}	14.32^{ab}	14.92^{a}	0.21	*	*	*
TDNI, kg	8.02^{c}	8.99^{b}	9.15^{b}	9.84^{a}	0.17	*	*	*
4% FCM, kg	10.05^{c}	11.58^{b}	11.13^{b}	13.05^{a}	0.37	*	*	*
Feed conversion (kg / kg):								
DMI / FCM	1.311^{a}	1.248^{b}	1.286^{ab}	1.143^{c}	0.03	*	*	*
TDNI/ FCM	0.798^{a}	0.776^{bc}	0.822^{ab}	0.754^{c}	0.01	NS	*	*
Economic evaluation:								
Daily feed cost, L.E	13.91	14.58	14.68	14.97	---	---	---	---
Price of daily milk yield, L.E	18	20.35	19.76	22.61	---	---	---	---
Economic return, L.E	4.09	5.77	5.08	7.64	---	---	---	---
Economic return,(h/d)%	100	141.07	124.20	186.79	---	---	---	---

* P< 0.05 and N.S = Not significant.

^{abc} means in the same row with different superscripts are differ significantly (P< 0.05).

** Calculation based on the following price in Egyptian pound (L.E.) per ton at 2009, concentrate feed mixture (CFM) =1400 L.E/ton, untreated silage =150 L.E/ton, treated silage =155 L.E/ton. One kg of live dried yeast10 L.E and One kg of raw milk1.60 L.E/kg.*

Table (10). Blood serum parameters of lactating cross Friesian cows fed the experimental diets during lactating period.

Item	Experimental diets							
	untreated Silage		Treated silage		P-values			
	(D_1)	(D_2)	(D_3)	(D_4)	SEM	Inoc.[1]	DY [2]	Inoc.*DY[3]
Glucose mg/dl	85.56	88.07	86.92	88.73	0.35	NS	NS	NS
Total protein (TP), g/dl	8.33	8.67	8.35	8.78	0.13	NS	NS	NS
Albumin(A), g/dl	4.61	4.85	4.63	4.94	0.11	NS	NS	NS
Globulin(G), g/dl	3.72	3.82	3.72	3.84	0.05	NS	NS	NS
A / G ratio	1.24	1.27	1.24	1.29	7.48	NS	NS	NS
Urea, mg/dl	43.24	42.88	43.02	41.16	0.41	NS	NS	NS
Cholesterol mg/dl	92.02	92.93	91.89	91.74	0.15	NS	NS	NS
GOT, u/l	32.45	32.77	32.50	32.83	0.16	NS	NS	NS
GPT, u/l	18.81	19.13	18.83	19.17	0.13	NS	NS	NS

N.S: Not significant.

CONCLUSION

Nutrition quality of feeds is critical in livestock development, the phytochemical examination of citrus pulp and its silage has shown that they contain Phytonutrients. It was observed that their concentrations in untreated or treated silage were lower than the levels at citrus pulp (below than critical values).Thus, no harmful effect to ruminants fed untreated or treated silage. Citrus pulp could be ensiled with rice straw (4:1, on DM basis) treated with LAB and supplemented with DY could be used as ruminant forage plus concentrate feed mixture during summer season. Inoculation of LAB seems to have more effectiveness in reducing these Phytonutrients to be below than the critical values. It is necessary to carry on more research for a long term feeding on such materials with analysis of metabolites; blood; milk and meat products of animals fed such materials. However, the supplementation of DY to cows fed silage treated with LAB could be beneficial in improving production of milk and milk fat.

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تأثير معاملة لب ثمار الموالح باللقاح البكتيرى وإضافة الخميره الجافه على تركيزات المثبطات الغذائيه وتخمرات الكرش وأداء الأبقار الحلابه.

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فى تجربه لمعرفة تأثير المعاملة بالملقح البكتيرى إضافة و الخميره الجافه على تركيز المركبات المضاده للتغذيه والتى تقلل من الاستفاده من مخلفات عصر البرتقال فى صورته بدون معامله ولدراسة تأثيرها على تخمرات الكرش وأداء الأبقار الحلابه و تم عمل كومتين سيلاج من مخلفات عصر البرتقال والمضاف إليها قش الارز بنسبة (4: 1) على أساس الماده الجافه حيث أضيف الملقح البكتيرى لإحداهما بمعدل 1جم /2لتر ماء /طن وتم تجربة أربع معاملات وهى الأولى سيلاج غير معامل بالملقح البكتيرى وبدون إضافة الخميره الجافه والثانيه سيلاج غير معامل بالملقح البكتيرى ومضاف إليها الخميره الجافه والثالثه سيلاج معامل بالملقح البكتيرى وبدون إضافة الخميره الجافه والرابعه سيلاج معامل بالملقح البكتيرى ومضاف إليها الخميره الجافه. وتم إجراء تجارب الهضم بإستخدام ثلاثة كباش برقى لكل عليقه بينما أستخدمت ثلاث نعاج مزوده بفستيولات لدراسة نشاط الكرش لكل عليقه بالإضافه إلى عشرون بقره فرزيان خليط حلابه فى موسم الحليب الثالث والرابع تم تقسيمها إلى أربعة مجاميع تجريبيه متماثله(خمسة أبقار فى كل مجموعه) بإستخدام التصميم المعاملى لإجراء تجارب اللبن والتى أستمرت 100 يوم .وقد تم تغذية السيلاج بصوره حره مع إضافة العلف المركز بمعدل 600جم/رأس يوميا فى تجارب الهضم وقياسات الكرش وبمعدل 8كجم/رأس يوميا فى حالة الأبقار الحلابه وإضافه الخميره الجافه إلى العلف المركز بمعدل 5جم/رأس أغنام و10جم/بقره .وقد أظهرت النتائج ما يلى:

1) أنخفاض محتوى السيلاج من جميع المركبات المضاده للتغذيه مع إضافة الملقح البكتيرى.
2) أدى التأثير التجميعى عندالمعامله بالملقح البكتيرى و أضافة الخميره معا على زيادة معنويه فى جميع معاملات الهضم مع أنخفاض معنوى فى تركيز الأمونيا فى الكرش مع زيادة تركيز الأحماض الدهنيه الطياره معنويا وزيادة معنويه لدرجة تحلل الماده الجافه و العضويه مع زيادة إنتاج البروتين الميكروبى معنويا .
3) هناك زياده معنويه ملحوظه فى كمية اللبن المعدل لنسبة دهن 4% فى حالة العليقة المحتوية على السيلاج المعامل باللقاح البكتيرى و المضاف إليها الخميرة الجافة مع زيادة العائد بحوالى 187%.

وبذلك يمكن النصح بإضافة الملقح البكتيرى عند عمل سيلاج مخلفات عصر البرتقال المخلوط مع قش الارز بنسبة (4: 1) على أساس الماده الجافه مع إضافة الخميرة الجافه للعليقه أثناء التغذيه قد يؤدى إلى أنخفاض تركيز المواد المضاده للتغذيه وتحسين الأداء الأنتاجى للأبقار مع أرتفاع المردود الاقتصادى لتلك العلائق .