

EFFECT OF PHYSICAL AND CHEMICAL TREATMENTS ON BERSEEM SILAGE QUALITY, NUTRITIVE VALUES, RUMEN FERMENTATION AND BLOOD METABOLITES OF RAMS

Abd El-Hady, M. A. A.¹ ; A. A. Gabr² ; S. A. El-Ayouty² and M. A. Z. Shahin²

¹Animal Production Res. Inst., Agric. Res. Center, Dokki, Giza, Egypt.

²Animal Production Dept., Fac. Agric., Mans. Univ., Egypt.

E-mail: magid_70@hotmail.com

ABSTRACT

The object of this study was to investigate the effect of wilting, chopping and formic acid treatment on berseem silage quality besides determination of digestibility, rumen fermentation and blood metabolites of rams fed tested rations. Berseem forage (at 3rd cut) was manually chopped at 2-3 cm of length and wilted for 0, 24 or 48 hr and thereafter ensiled with or without formic acid (0.5%) treatment in plastic barrels. At the same time, another quantity of un-chopped berseem forage was wilted for 24 hr and ensiled with or without formic acid treatment. Digestibility trials were conducted to evaluate the utilization of berseem silages using mature Ossimi rams. The results showed that DM of berseem silage increased by wilting to around 20% and 30% for 24 hr and 48 hr, respectively. The wilted silage for 24 hr and treated with formic acid (S4) had the lowest value of pH (being 3.87), the highest level of lactic acid (3.15%) the lowest level of acetic (1.57%) and butyric acids (0.0%) with Flieg's score 90. The lowest level of lactate and Flieg's score of quality, whilst the highest pH value was obtained in un-chopped berseem silage either treated or untreated with formic acid. Likewise, the highest count of *lactobacilli*, the lowest mold and *clostridia* were found in S4, which were positive correlated with fermentation parameters. Digestibility of DM, OM, CP, EE and CF was higher ($P<0.01$) in un-wilted than wilted silages, as well as, nutritive values had a similar trend. The intake of TDN, DCP and ME was higher ($P<0.05$) in wilted silage rations than un-wilted. Formic acid treatment improved ($P<0.05$) digestibility of most nutrients, whilst nutritive values did not differ significantly. The nutrients digestibility of un-chopped silage was lower than chopped silage except for CF and its fractions. The quality index (QI) value was ($P<0.05$) improved by wilting of silage. The lowest value of QI was found in un-chopped and un-wilted silages. Rumen liquor parameters were not significantly affected by wilting or formic acid treatment, except for NH₃-N and TVFA's which were higher in wilted than un-wilted silage. The highest ($P<0.01$) value of NH₃-N and TVFA's was showed in wilted for 24 hr and treated silage. The pH, eNDF and NH₃-N values were higher in un-chopped than chopped silage ration. Blood hemoglobin and hematocrite values, as well as plasma total protein and glucose ($P<0.01$) increased in wilted silage groups than un-wilted. Whereas, formic acid did not affect blood parameters. It could be concluded that, the lowest quality and digestibility was obtained with un-chopped silage either with or without formic acid treatment. Chopping and wilting berseem forage for 24 hr (about 20% DM) as pre-ensiled treatments with formic acid sprinkle produced the best quality silage and higher digestibility without any side effect on health of rams during the experimental period, which could be resulted in improvement growth performance of the animal.

Keywords: Berseem, silage, wilting, chopping, formic acid, digestibility, fermentation, rams.

INTRODUCTION

There is a great shortage in animal feedstuffs in Egypt especially throughout summer period which reflects on the productions of such animals (Shalaby *et al.*, 1989). Whereas, in winter season berseem clover (*Trifolium alexandrinum*) is the main forage as an annual multi-cut crop fed *ad libitum* as a common practice. This species has the advantage over other annual species of providing multiple harvests and high protein yield during the growing season. Some of this forage is dried in last cut to produce berseem hay for summer feeding, but the amount of hay is not enough and loss some of nutritive value and carotenoids. Therefore, silage making of berseem that is plentiful during the wet season is one of the solutions to feed shortages in the summer season (Khan *et al.*, 2006). Ensiling forages has several advantages relative to harvesting as hay. These include greater opportunity for mechanization, reduce labor cost and less chance of weather losses (Mustafa and Seguin, 2003). Unfortunately, ensiling berseem is not widespread in Egypt because of many problems affect silage quality. Where, leguminous fodders have high buffering capacity due to high moisture, minerals and protein contents, therefore pH drops slowly during ensiling and resulted in high nutrient losses (Bolsen *et al.*, 1996). Ensiling high moisture crops could result in *clostridia* fermentation that led to heavy loss of nutrients (Gary, 1992 and Matsuoka *et al.* 1993). Therefore, before ensiling lucerne or berseem fodder, moisture contents should be reduced either by field wilting or by the addition of some absorbent (Touqir *et al.*, 2007). Time of wilting has been investigated and produced extremely variable results due to weather conditions such as humidity, wind speed and ambient temperature prevailing at the time of the trial (McDonald *et al.*, 1991). Biochemical losses from respiration could be higher than losses from un-wilted silage and digestibility of the silage is reduced (Thomas and Thomas, 1985). Several workers reported that the optimum level of wilting was to arrive 20-30% DM of pre-ensiling forage (McDonald *et al.*, 1991; Suepea *et al.*, 2000; Touqir *et al.*, 2007).

The main goal of silage making is to preserve as much of the nutritional value of the original crop as possible. Preservation is achieved by acidity and by maintaining an oxygen-free (anaerobic) environment. Acids are produced by bacteria that convert fermentable carbohydrates into organic acids, predominantly lactic and acetic acids. Formic acid is widely used to accomplish this target and direct rapid pH drop of ensiled material and eventually the acidity level is adequate to inhibit or kill most bacteria and other microorganisms and consequently nutrient losses. At this pH if protected from exposure to air and water seepage, silage can be preserved for a long period (Touqir *et al.*, 2008). Addition of formic acid to silage material has been reported to have generally positive effects on fermentation (Haigh, 1988 and Snyman *et al.*, 1996). Fairbairn *et al.* (1992) found that the application of 4.5 L formic acid/ton fresh matter reduced proteolysis in alfalfa owing to rapid reducing pH. As well as, chopping of pre-ensiling forage is one of the main important factors affects silage fermentation and therefore its quality

(Woolford, 1984). It can lead to rapid fermentation, therefore fall in pH of forage. The chopping of ensiling forage increases silage intake in two ways: firstly, through improving the fermentation quality and, secondly, through increasing the rate of passage of food through the rumen, therefore the rumination is easier than in un-chopped silage (McDonald *et al.*, 1991).

In Egypt, the majority of silage is made from corn only, as a summer crop. Whereas, ensiling berseem forage as a winter crop is not wide due to lower quality and preservation, as well as the local previous studies on ensiling berseem was done as a whole plant without chopping. The question of this study was: how can be improved berseem silage quality and preservation? Therefore, the main object of this study was to investigate the effect of wilting and chopping as a pre-ensiling physical process with or without formic acid treatment on berseem silage quality, digestibility, rumen fermentation and blood constituents with sheep.

MATERIALS AND METHODS

This study was conducted at the Experimental Farm of the Animal Production Department, Faculty of Agriculture, Mansoura University. The analytical work was undertaken at the laboratories of department. The experimental groups were distributed as following:

- 1) **S1**: Berseem was ensiled without wilting or formic supplement.
- 2) **S2**: Berseem was ensiled without wilting but sprinkled with 0.5% formic acid.
- 3) **S3**: Berseem was ensiled after wilting for 24 hr without formic supplement.
- 4) **S4**: Berseem was ensiled after wilting for 24 hr and sprinkled with 0.5% formic acid.
- 5) **S5**: Berseem was ensiled after wilting for 48 hr without formic supplement.
- 6) **S6**: Berseem was ensiled after wilting for 48 hr and sprinkled with 0.5% formic acid.
- 7) **S7**: Berseem was ensiled after wilting for 24 hr as whole plants without chopping or formic supplement.
- 8) **S8**: Berseem was ensiled after wilting 24 hr as whole plants without chopping and sprinkled with 0.5% formic acid.

Ensiling process:

About 2 ton of 3rd cut fresh berseem (*Trifolium alexandrinum* L.) was collected from the field and divided into 8 equal portions for making silages with different treatments as above. The chopping process was carried out manually by knives (2-3 cm of length) and wilted for 0 (15% DM), 24 hr (18.7% DM) or 48 hr (28% DM) in sunny days during April, 2007. The un-chopped berseem was wilted for 24 hr and contained 21.8% DM. Berseem was well pressed manually by legs (to ensure air removal) in plastic barrels (200 liter) with or without sprayed formic acid (0.5% as fresh matter). Formic acid was diluted with little of water and sprayed every 20 cm layers of berseem. After full filling of each barrel, it was covered by plastic sheet and

packed by barrel covers and belt to maintain anaerobic condition of silo. All barrels were kept at room temperature for 2 months before feeding animals.

Evaluation of silage quality:

For judging the quality of berseem silage, physical characteristics like color, odor and smell of silages were examined. Samples of each silo were taken when opened for subsequent proximate chemical analysis, as well as fermentation characteristics of silage were examined. Silage extract was prepared by homogenizing 20 g wet material of each sample with 100 ml distilled water in warm blender for 10 min. (Waldo and Schultz, 1956). The homogenized samples were filtered through double layer of cheese cloth and filter paper. Then, the filtrate was used to determine silage pH directly by digital pH-meter with a combined electrode. Lactic acid was determined in silage juice spectrophotometry according to Barker and Summerson, (1941). Determination of acetic, propionic and butyric acid concentrations were measured chromatography according to Jayaprakasha *et al.* (2002).

The above chemical assessment were used to judge the quality of silages according to Flieg (1952) who developed a scheme upon which points are awarded according to the relative amounts of lactic, acetic and butyric acids in silage. So that, the higher proportions of lactic and acetic acids to butyric acid had the higher score and the better quality. This system has gained wide acceptance in Europe and Germany. Another fresh sample from every silo was taken and prepared for microorganisms differential counts in silages by the plate culture count method according to Gibson *et al.* (1958) and Cai *et al.* (1998) for development extensively *lactobacilli*, *clostridia*, *bacilli*, *entero-bacteria*, yeasts and molds.

Digestion trials:

Eight digestion trials were carried out at the experimental farm of faculty of agriculture, Mans. Univ., using three mature healthy Ossimi rams in each group with an average live body weight of 48.6 ± 0.87 kg and 2 years old to determine nutrients digestibility coefficients and nutritive values of the experimental silages. Each animal was confined in individual concrete pen for 15 days as an adaptation period followed by five days as a collection period. All rams were injected subcutaneously with anti-parasites at the beginning of the experiment. Animals were fed to cover 80% of their maintenance requirements (NRC, 1985).

The ingredients of concentrate feed mixture (CFM) which used were 60% of ground yellow corn, 20% soy bean meal (44% CP), 15% wheat bran, 1.5% premix, 2% calcium carbonate, 1% di-calcium phosphate and 0.5% sodium chloride, that were mixed manually. The chemical composition of CFM and berseem silages was illustrated at Table (1). The daily feed allowances were weighted and offered twice daily (at 8.0 AM and 4.0 PM) as 4.0 kg of every berseem silage plus 0.3 kg of CFM/head/day (as fresh matter). These amounts of berseem silage and CFM were constant in all groups. Drinking water was available to each animal all over the time. Samples of feeds were taken in the first and last of experiment and kept for later analysis. Feces samples were taken from the rectum during the collection period of each trial (5 days) and dried in forced air oven at 65°C for 48 hrs. Dried samples were composed for each animal and representative

samples were taken, ground and kept for chemical analysis. Acid insoluble ash was used as a neutral marker according to Van Keulen and Young (1977). Digestibility coefficients were calculated from the equations given by Schneider and Flatt (1975).

$$\text{DM digestibility \%} = 100 - [100 \times (\text{AIA\% in feed} / \text{AIA\% in feces})]$$

$$\text{Nutrient digestibility \%} = 100 - [100 \times (\text{AIA\% in feed} / \text{AIA\% in feces})] \times [\text{nutrient\% in feces} / \text{nutrient\% in feed}]$$

Proximate analysis of feedstuffs and feces was determined according to A.O.A.C. (1990). Samples of feeds and feces also were analyzed for fiber fractions according to the procedures of Van Soest, *et al.* (1991) to determine neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL). Cellulose was calculated as = ADF-ADL and Hemicellulose as = NDF-ADF. Non-fibrous carbohydrates (NFC) were calculated as: $\text{NFC\%} = \text{OM} - (\text{\%NDF} + \text{\%CP} + \text{\%EE})$ (Calsamiglia *et al.*, 1995). The quality index was calculated according to Moore (1994) as follow:

$$\text{RFQ (relative feeding quality)} = (\text{DMI\% of BW}) \times (\text{TDN\% of DM}) / 1.23$$

$$\text{QI (quality index)} = 0.0125 \times \text{RFQ} + 0.097$$

Rumen fermentation:

Rumen fluid samples were collected from all rams during the last day of collection period. The samples were taken by rubber stomach tube using gentle mouth suction. About 100 ml of rumen fluid was collected just before offering the morning feed (zero time) and consequently at 2, 4 and 8 hr post feeding (at timed interval). The collected samples were filtered through 4 layers of surgical gauze and were immediately used to determine rumen pH using digital pH-meter. The effective natural detergent fiber (eNDF) was calculated as = $(\text{pH} - 5.425) / 0.04229$ according to Fox *et al.* (2000). Then samples were stored in dried bottles at -20°C for measuring other parameters. Ammonia nitrogen (NH₃-N) concentration was measured according to Conway (1957) method. Total volatile fatty acids (TVFA's) in rumen liquor were measured according to stem distillation procedure as described by Warner (1964). Rumen buffering capacity was also determined according to (Jasaitis *et al.*, 1987) that total acidity was measured in the same sample by titration and expressed as 0.1N HCl ml equivalent required to reduce the original pH to 4.5.

Blood metabolites:

Blood samples were collected from each experimental animal after morning feeding in the 2nd day of collection period via jugular vein using heparinized tubes. Blood plasma was separated by centrifugation at 3000 r.p.m for 10 minutes and stored at -20°C until analyzed for the different blood parameters. Blood hemoglobin (Hb), hematocrite (HT), red blood cell counts (RBC's) and white blood cell counts (WBC's) were measured using auto blood counter (CD 1700 Speciment). Plasma glucose, total protein, creatinine and aspartate aminotransferase (AST) were determined colorimetric using commercial kits according to the procedures outlined by the manufacture.

Table (1): Chemical composition of CFM, silages and calculated experimental rations as affected by wilting and chopping with or without formic acid treatments of berseem silage.

Items	DM	Chemical Composition (% as DM)											
		OM	CP	EE	CF	NFE	Ash	NDF	ADF	ADL	Hemi.	Cellu.	NFC
Ingredients													
CFM	88.45	91.84	18.4	1.94	12.4	59.1	8.16	29.1	12.6	6.1	16.5	6.48	41.3
S1	15.66	83.15	17.1	2.32	34.7	29.0	16.85	43.1	27.1	7.4	16.0	19.7	18.2
S2	13.32	84.95	16.9	2.55	33.3	32.2	15.05	44.9	25.8	8.0	19.2	17.8	18.4
S3	19.88	84.46	16.4	2.69	35.7	29.7	15.54	45.5	28.4	7.3	17.1	21.1	17.3
S4	19.85	83.83	16.8	2.37	33.2	31.5	16.17	45.2	28.6	8.2	16.6	20.4	16.9
S5	31.95	84.23	16.1	2.65	33.6	31.9	15.77	46.0	28.9	8.4	17.0	20.6	17.5
S6	29.85	84.05	16.2	2.52	35.1	30.2	15.95	46.1	30.7	7.9	15.3	22.8	16.6
S7(un-Chopped s.)	20.32	83.76	16.0	2.65	34.6	30.5	16.24	49.7	35.0	7.9	14.6	27.1	12.7
S8(un-Chopped s.)	24.59	83.32	15.3	2.27	36.0	29.8	16.68	50.1	36.8	7.7	13.3	29.1	12.8
Experimental rations													
R1	20.74	87.00	17.5	2.21	28.0	36.0	13.00	38.9	22.8	7.01	16.2	15.8	25.1
R2	18.56	87.91	17.4	2.30	26.3	41.9	12.09	39.7	21.4	7.38	18.3	14.0	26.0
R3	24.66	87.81	16.9	2.50	29.9	38.5	12.19	41.4	24.4	6.98	17.0	17.4	23.3
R4	24.63	82.61	17.2	2.26	28.0	35.2	17.39	41.1	24.6	7.71	16.6	16.9	23.0
R5	35.89	81.38	17.0	2.53	29.9	31.9	18.62	43.1	26.1	7.99	16.9	18.1	21.6
R6	33.94	84.49	17.1	2.41	30.9	34.1	15.51	43.0	27.4	7.62	15.5	19.8	21.1
R 7	25.07	85.75	16.6	2.47	29.1	37.6	14.25	44.6	29.5	7.90	15.1	22.0	19.8
R 8	29.04	85.13	15.9	2.20	31.0	36.0	14.87	45.7	31.7	8.11	14.0	24.3	18.9

Statistical analysis:

Data of the study were analyzed using the General Linear Model (GLM) of SAS (2004). In chopped silage groups, data of nutrients digestibility, feeding values and blood parameters were analyzed using two ways classification model included wilting, formic and interaction between them. Whereas, rumen parameters was subjected using factorial design analysis of variance model included wilting, formic, time and their interactions. Data of un-chopped silages digestibility were analyzed using one way classification model included formic acid effect. Whereas, rumen parameters of un-chopped silages were analyzed two ways classification model included formic acid, time and their interactions. The differences among overall means were compared using Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Data of the chemical composition of CFM, berseem silages and experimental rations are presented in Table (1). The DM% of un-wilted silages (S1 and S2) with or without formic acid was the lowest values (15.66 and 13.32 %, respectively), whereas DM% was around 20% and 30% of berseem silages wilted for 24 and 48 hr, respectively. The CP of silage ranged from 14.8 to 17.1%. Wilted berseem silage (S3 and S4) for 48 hr had the highest value of NDF% and ADF% and the lowest value of NFC% as DM basis compared with the other treatments. The other chemical nutrients were

slightly varied among groups. Similar trend was found with their experimental rations. The composition of un-chopped silages (S7 and S8) was similar to those chopped silages except for fiber fractions. The un-chopped silage was higher in NDF% and ADL%, whilst the lowest percent of CP% was in un-chopped treated silage.

These data are agreed with those of Haigh and Mansbridge (1998) and Abou El-Enin, (2005) who ensiled perennial ryegrass and berseem clover (BC), respectively. Shrestha *et al.* (1998) and Mustafa and Seguin (2003) reported that NDF of BC silage ranged between 36.9 to 45.5% and ADF ranged 25.7 to 33%. In this respect, the results are in accordance to those findings by Touqir *et al.* (2007) who found similar most composition of berseem silage at different levels of DM (17.2, 20 and 30%).

Silage fermentation and quality:

Physical characteristics of wilted silage without formic acid treatment had a dark green color and a vinegar un-palatable smell. Whereas, wilted silage and treated with formic acid had a yellowish olive green color and palatable very good smell due to high lactic acid concentration. Berseem silage which neither un-wilted nor un-treated with formic acid had a dark green color, high moisture and low smell, while the treated silage had a better smell and a light green color. Opening the untreated silage with formic acid (S1, S3, S5 and S7, respectively) were covered by excessive layer of molds which excluded before feeding, whereas the treated silages were clarified from the molds.

Fermentation parameters of berseem silages and their evaluation are presented in Table (2). Silage wilted for 24 hr (S3) had the lowest value of pH (5.07) compared with untreated silages. The highest value of pH was recorded with wilted silage for 48 hr (without any additives) followed by un-chopped silages (S7 and S8). The decline of pH in chopped silages than un-chopped can be in direct response to release of organic acids contained in the cell sap (Woolford, 1984). Whereas, sprinkled formic acid was more effective than wilting in reducing pH value, therefore the lowest value of pH was 3.87 in wilted silage for 24 hr and treated with formic (S4) owing to the associated effect for wilting and formic acid treatments. However, increasing the wilting time for 48 hr with formic acid treatment (S6) had a worst effect on pH value of silage (being pH 4.49). Lactic acid concentration slightly increased by wilting only for 24 hr (S3) and quadratic by wilting and formic acid treatments (S4 and S6). The highest value of lactate was 3.15% followed by 3.06% for S4 and S6, respectively. The lowest value of lactate was recorded in un-chopped silage (0.92-0.99%). It is very difficult to compress the un-chopped forage materials especially berseem forage due to its tube stems. So that, it could not be remove the penetrated air completely resulted in bad fermentation, higher DM losses and higher pH value (Ruxton, 1972; Rees *et al.*, 1983).

Table (2): Effect of wilting and chopping with or without formic acid supplement on some fermentation characteristics and silage quality.

Treatment	pH	VFA's (% as DM)				TVFA's	Lactic/ TVFA's	Lactic/ Acetic	Fleig point	Classifica- tion
		Lactic acid	Acetic	Propi- onic	Butyric					
Chopped silage:										
S1	5.15	1.49	21.11	0.13	1.31	24.05	0.062	0.07	34	Poor
S2	4.05	2.57	3.01	0.15	0.06	5.79	0.444	0.85	65	good
S3	5.07	1.65	10.70	0.16	1.67	14.2	0.116	0.15	28	Poor
S4	3.87	3.15	1.57	0.14	ND	4.87	0.647	2.00	90	Very good
S5	5.80	1.04	2.94	0.13	0.04	4.15	0.250	0.35	48	Medium
S6	4.49	3.06	1.78	0.16	ND	4.99	0.613	1.72	86	Very good
Un-chopped silage:										
S7	5.67	0.92	22.11	0.37	1.94	8.55	0.11	0.17	15	Bad
S8	5.55	0.99	5.32	0.11	1.01	24.23	0.04	0.05	34	Poor

S1= un-wilted silage, no formic; S2= un-wilted, treated with formic; S3= wilted for 24 hr, no formic; S4= wilted for 24 hr, treated with formic; S5= wilted for 48 hr, no formic; S6= wilted for 48 hr, treated with formic; S7= un-chopped, no formic; S8= un-chopped, treated with formic acid; ND: not detected

The ratios of lactic: TVFA's and lactic: acetic had shown corresponding trend. So, the highest ratio was obtained with S4 silage (being 0.647 and 2.00, respectively) followed by S6 silage (0.613 and 1.72) and S2 silage (0.444 and 0.85). The result can be explained the greater values of *lactobacilli* count (Table 3) in wilted and formic acid treated silages (being 5.2 and 5.0 Log₁₀cfu/g for S4 and S6, respectively). The concentrations of acetic and butyric acids of silage were declined linearly with both wilting and formic acid treatment. The lowest value of acetic was in S4 followed by S6 silages and the highest value was showed with un-wilted and untreated silage with formic acid (S1). Likewise, butyric acid was not detected in both S4 and S6 but the highest value was in un-chopped and untreated with formic acid (S7) which indicate to the deterioration of protein and the lactate to butyrate by *clostridia* (McDonald, 1981). Whereas, propionic acid concentration was slightly differed among groups. The TVFA's was in the minimum levels in wilted and treated with formic silages, which indicate a good quality and more palatable silage, consequently improved feed intake (Jatkauskas and Vrotniakiene, 2006).

Table (3): Effect of wilting and chopping with or without formic acid treatment on microorganisms counts (log₁₀ CFU/g silage).

Items	<i>Lactobacilli</i>	Yeasts	Molds	<i>Entero-bacteria</i>	<i>Clostridia</i>	<i>Bacilli</i>
Chopped silage:						
S1	3.9	3.9	2.9	6.9	2.6	2.6
S2	4.1	4.1	1.8	4.6	2.1	2.7
S3	3.6	2.3	2.7	6.8	2.2	2.4
S4	5.2	2.5	1.4	4.5	2.0	2.2
S5	3.5	3.3	2.2	5	2.1	2.5
S6	5.0	3.9	1.7	4.7	1.9	2.2
Un-chopped silage:						
S7	3.0	2.2	2.4	6.0	2.5	2.4
S8	3.2	2.1	1.6	5.9	2.2	2.5

The Fleig point is a summative record, which used to evaluate the quality of silage. According to Fleig point (Table 2), the best quality of silage was S4 followed by S6 (being 90 and 86, respectively). While, the lowest value was recorded in un-chopped silage (S7; being 15), followed by S3. The formic acid treatment improved the quality score of un-chopped berseem but still poor quality. Corresponding results are in Table (3) which concluded that S4 and S6 had the lowest values of undesirable microorganisms as molds, *entero-bacteria*, *clostridia* and *bacilli spp.* so that, the *entero-bacteria* can be metabolite glucose to acetate and the *clostridia* can be metabolite lactate to butyrate leading to DM and energy losses by about 51 % and 18.4%, respectively (McDonald *et al.*, 1973). According to the results in Tables 2 and 3 it was found a positive correlation between acetic acid and *entero-bacteria* count as well as between butyric acid and *clostridia* count. These findings are in agreement with Suepea *et al.* (2000) who found that pH value of berseem silage with added corn meal was 4.21 and 7.54 when chopped and un-chopped, respectively. Moreover, Fleig point was 61 and 21, respectively. As well as, Abou El-Enin (2005) found that wilted berseem silage and treated with formic acid had a lowest value of pH and butyric acid concentration and the highest value of lactic acid concentration and consequently the highest value of Fleig-zimmer point as very good silage. Zhang (2011) found that chopping of guinea grass at 3 cm was beneficial to lactic acid fermentation and all fermentation parameters except NH₃-N content compared with 1 and 6 cm of length. In accordance findings, Rowghani and Zamiri (2009) found that corn silage treated with formic acid was significantly ($P<0.05$) higher in lactic acid, total acids concentration and higher ($P>0.05$) in lactic: acetic ratio compared with inoculants treated or untreated silage. Likewise, the earlier investigation showed lucerne silage treated with 1.5 - 6.0 l/t had greater levels of lactic acid and lower values of pH, acetic, propionic and butyric acid concentration (Barry *et al.*, 1978) which are in accordance with the present results. In this field, Polan *et al.* (1998) reported that direct-cut alfalfa plus formic acid had more favorable fermentation as well as a lower pH, greater lactic acid and greater water soluble carbohydrate compared with wilted alfalfa plus anhydrous NH₃. Similar results were found by Etman *et al.* (1995) who found the pH value of berseem silage treated with formic acid was 3.9 compared with molasses (pH 4.62). Jatkauskas and Vrotniakiene (2006) illustrated that formic acid treatment to ensiled red clover-grass mixture (3:1) reduced ($P<0.01$) pH values (4.30 vs. 4.51) and elevated ($P<0.01$) lactic acid concentration (4.32 vs. 3.15%), as well as reduced acetic (1.3 vs. 2.5%) and butyric acids (0.014 vs. 0.3%). Touqir *et al.* (2007) showed a similar values of pH and lactic acid values for berseem silages treated with different levels of DM and molasses. They proposed that the best values of pH and lactic acid were in 30% DM, whilst in the present study the best values were at 20% DM of silage treated with Formic acid. Fransen and Strubi (1998) reported that wilting was an effective treatment in young napier grass to lower pH value compared with fresh young napier grass silage. They also found that the pH value of direct cut silage of this grass was 5.09 while wilted silage had a pH 4.72. Gordon *et al.* (1999) found that un-wilted ryegrass and treated with

formic (19.4% DM) had better values of pH and lactic acid than wilted and treated with formic ryegrass (46% DM).

The results are in accordance with Suepea *et al.* (2000) who reported that wilted berseem clover silage (25% DM) had the lowest value of butyric (being 0.005%) and pH 4.21 with Flieg score 75 compared with score 70 in wilted berseem silage (36% DM). Carpintero *et al.* (1969) concluded that formic acid silage results in highly negative correlation between level of addition and proteolysis together with deamination. However, wilting is important factor for ensiling the high moisture forages legumes as berseem to increase DM, Henderson *et al.* (1972) found that during a 31 hr with DM content of 32%, 20% of initial protein was hydrolyses. There is general agreement that the extent of proteolysis is increased by extending the wilting period and, more important, by wilting under humid conditions (McDonald *et al.*, 1991). So that, the best period for wilting chopped berseem was 24 hr. during spring season under humid local conditions, together with 0.5% formic acid treatment at ensiling process.

Nutrients digestibility:

Data of nutrients digestibility are shown in Tables (4 and 5). The un-wilted berseem silage ration with or without formic acid treatment was the highest ($P<0.05$) significantly of DM, OM, CP, EE and CF% digestibility. The differences between 0 hr and 24 hr were not significant. This may be related to total dry matter intake (TDMI) which was the lowest value in un-wilted silage and resulted in increase the rate of passage of the feed (Shaver *et al.*, 1986). As well as, the biochemical losses from respiration and deamination of wilted silage could be higher than losses from un-wilted silage and digestibility of the silage is reduced (Thomas and Thomas, 1985). Whereas, NFE, NFC and fiber fraction did not differ significantly among wilting groups.

Otherwise, formic acid treatment of silage ration increased ($P<0.05$) the digestibility of most nutrients compared with untreated silage except for CP% that reduced ($P<0.05$) by treatment, which are in agreement with Handerson *et al.* (1982). Digestibility of ADF, ADL and hemicellulose tend to be higher in treated silage with formic acid than untreated but without significant differences. The nutrients digestibility was not affected significantly by interaction between wilting and formic acid treatment. Generally, the digestibility of nutrients in un-chopped silage ration was lower (not statistically) than chopped silage except for CF and its fractions. Treated un-chopped silage with formic acid was slightly higher in most nutrients digestibility than untreated un-chopped silage ration.

Table (4): Nutrients digestibility and feeding values of experimental rations as affected by wilting and chopping with or without formic acid treatment of berseem silage with sheep.

Items	Chopped silage									Un-chopped silage			
	Wilting (hr)				Formic			Significance			-F	+F	SE
	0	24	48	SE	-F	+F	SE	W	F	WxF			
TDMI, kg	0.845	1.06	1.50	-	1.16	1.11	-	-	-	-	1.08	1.25	-
TDMI/BW%	1.73 ^c	2.19 ^b	3.11 ^a	0.03	2.40	2.28	0.03	**	**	*	1.96	2.47	0.06**
Digestibility%													
DM	66.8 ^a	65.3 ^{ab}	63.5 ^b	0.64	64.4	66.0	0.52	*	*	NS	63.3	62.3	0.77
OM	72.0 ^a	71.7 ^a	69.4 ^b	0.48	70.3	71.7	0.39	**	*	NS	67.3	66.9	0.87
CP	69.8 ^a	70.1 ^a	67.2 ^b	0.51	70.4	67.7	0.42	**	**	NS	65.5	62.5	1.26
EE	70.1 ^a	71.1 ^a	67.3 ^b	0.74	68.4	70.7	0.60	**	*	NS	68.2	69.2	0.95
CF	59.3 ^a	59.9 ^a	56.9 ^b	0.53	57.9	59.5	0.43	**	*	NS	58.8	61.6	1.42
NFE	82.4	81.7	82.2	1.19	80.9	83.3	0.97	NS	NS	NS	74.2	74.9	1.37
NDF	59.8	59.1	57.2	0.96	57.5	59.8	0.78	NS	*	NS	59.5	60.3	1.39
ADF	39.8	40.5	40.9	0.97	39.3	41.5	0.80	NS	NS	NS	42.8	46.4	1.46
ADL	17.1	16.5	17.0	1.68	16.9	16.8	1.37	NS	NS	NS	18.3	21.3	0.88
Hemice.	85.4	86.3	84.0	1.22	84.1	86.4	0.99	NS	NS	NS	92.1	91.6	1.46
Cellulose	51.0	50.8	50.7	1.34	48.9	52.6	1.10	NS	*	NS	51.2	54.0	1.87
NFC	92.5	95.3	96.6	1.36	93.5	96.2	1.11	NS	NS	NS	84.7	88.4	1.29
Nutritive value %													
TDN%	62.9 ^a	61.4 ^b	60.4 ^b	0.39	61.2	61.9	0.32	**	NS	*	58.0	57.5	0.74
TDNI, kg/d	0.531 ^c	0.651 ^b	0.907 ^a	0.004	0.71	0.68	0.003	**	**	*	0.625	0.719	0.01**
DCP%	12.2 ^a	11.9 ^a	11.4 ^b	0.09	12.1	11.6	0.07	**	**	NS	10.9	9.9	0.20*
TDN/CP	3.61	3.60	3.54	0.02	3.57	3.60	0.02	NS	NS	**	3.49	3.62	0.046
ME, Mcal/kg	2.24 ^a	2.18 ^b	2.15 ^b	0.014	2.18	2.20	0.01	**	NS	**	2.06	2.05	0.026
MEI, Mcal/d	1.89 ^c	2.32 ^b	3.23 ^a	0.01	2.53	2.42	0.01	**	**	*	2.22	2.56	0.03**
NE ¹ , Mcal/kg	1.42 ^a	1.38 ^b	1.36 ^b	0.01	1.38	1.40	0.01	**	NS	*	1.30	1.29	0.018
RFQ	88.4 ^c	109.1 ^b	152.7 ^a	1.31	119.3	114.2	1.07	**	*	*	92.5	115.6	3.75*
QI	1.20 ^c	1.46 ^b	2.00 ^a	0.016	1.58	1.52	0.01	**	*	*	1.25	1.54	0.047*

¹NE (Mcal/kg) = (TDN% x 0.0245) – 0.12 (NRC, 2001); TDMI= total dry matter intake ; -F= without formic ; +F= with formic

^{a, b and c} least square means in the same row for chopped silage with different superscript differ significantly (P<0.05)

*P<0.05 ; **P<0.01 ; NS= non-significant

The nutritive value (TND, DCP, ME and NE) of un-wilted berseem silage ration was significantly greater (P<0.01), whilst it was the lowest (P<0.01) TDNI, MEI, relative feeding quality (RFQ) and quality index (QI) values compared with wilted silage rations. The lowest value of TDNI and MEI in un-wilted silage could be due to lower DMI. Nutritive value was affected (P<0.01) by interaction between wilting and formic acid treatment, which was greater in un-wilted silage treated with formic acid (Table 5). The un-chopped silage ration had lower (not statistically) nutritive value and QI compared with chopped silages. The index of quality measures the voluntary intake of TDN above maintenance. When forage is fed without supplemental energy or protein, QI is related to the available TDN for the gain (Moore and Kunkle, 1995). Therefore, the treatment with formic plus pre-ensiled wilting improved the efficiency of ensiled berseem.

Table (5): Nutrients digestibility and feeding values of experimental rations as affected by the interaction between wilting and formic acid treatment of berseem silage.

Items	Chopped silage						SE	
	Wilting (hr)	0		24		48		
	Formic acid	-F (R1)	+F (R2)	-F (R3)	+F (R4)	-F (R5)		+F (R6)
BW		49.0	48.7	49.0	48.0	48.0	48.7	-
TDMI, kg/d		0.89	0.80	1.06	1.06	1.54	1.46	-
TDMI/BW%		1.82	1.64	2.16	2.21	3.22	3.00	0.046
Nutrient: digestibility, %:								
DM		65.9	67.6	64.6	65.9	62.6	64.4	0.91
OM		71.1	72.9	70.9	72.4	68.9	69.8	0.68
CP		71.1	68.5	71.4	68.7	68.6	65.9	0.72
EE		68.5	71.7	69.6	72.7	67.0	67.7	1.04
CF		58.3	60.3	58.6	61.1	56.9	57.0	0.74
NFE		81.3	83.4	81.4	82.1	80.1	84.4	1.68
NDF		58.4	61.2	58.1	60.1	56.1	58.3	1.36
ADF		39.4	40.2	39.6	41.4	39.1	42.9	1.38
ADL		16.8	17.4	15.8	17.1	18.1	16.1	2.37
Hemicellulose		85.2	85.7	84.9	87.8	82.3	85.7	1.73
Cellulose		49.4	52.2	49.1	52.5	48.3	53.2	1.90
NFC		91.2	93.9	93.4	97.2	95.8	97.4	1.92
Feeding value:								
TDN%		61.4	64.4	61.9	60.8	60.3	60.5	0.56
TDNI, kg/d		0.55	0.51	0.66	0.64	0.93	0.88	0.01
DCP%		12.4	11.9	12.1	11.8	11.7	11.2	0.122
TDN/CP		3.52	3.71	3.66	3.54	3.54	3.55	0.032
MEI, Mcal/d		1.95	1.83	2.34	2.29	3.31	3.14	0.02
ME, Mcal/kg		2.19	2.29	2.21	2.17	2.14	2.15	0.02
NE, Mcal/kg		1.38	1.46	1.40	1.37	1.36	1.36	0.014
RFQ		90.9	85.8	109.0	109.2	157.8	147.5	1.852
QI		1.23	1.17	1.46	1.47	2.07	1.94	0.023

The obtained results are in agreement with those reported by Etman *et al.* (1995) who found that digestion coefficients of all nutrients of berseem silage treated with 0.4% formic acid were elevated compared with that containing 3% molasses. Similar results have been reported by Mayne and Steen (1990); Kokkonen *et al.* (2000); Jatkauskas and Vrotniakiene (2006) and Sarwar *et al.* (2005). Whereas, Aksu *et al.* (2005) illustrated that there were no significant effect of formic acid treatment for DM, OM, NDF or ADF digestibility in corn silage. The obtained results are consistent with Gordon *et al.* (1999) who found that the un-wilted ray grass silage had higher ($P < 0.05$) digestibility of DM, OM and GE. Similar results were found by Wilkins (1984) and Rohr and Thomas, (1984). However, other workers have not generally recorded such major negative effects of wilting on digestibility where rapid wilting techniques have been adopted (Yan *et al.*, 1996 and 1998; Dawson *et al.*, 1998). Donaldson and Edwards (1976) showed that DM, OM, CP digestibility and ME were higher in un-wilted grass silage than wilted, as well as formic acid treatment improved digestion coefficient of wilted silage.

Rumen Fermentation:

The data of rumen liquor parameters are presented in Tables (6 and 7). There was no significant interaction between sampling time and either wilting or formic for any characteristics measured except for pH and eNDF, therefore it was not showed in tables. The pH values of rumen liquor and calculated eNDF were not affected significantly by wilting or formic acid

treatment. Whereas, the interaction between wilting and formic was significant ($P < 0.05$), since the highest value of pH and eNDF was in R6 and the lowest value was in R4 than other groups (Table 7). The lowest ($P < 0.01$) values of pH and eNDF was in wilted group for 24 hr at 0, 2 and 4 hr (Fig 1) and it was elevated in the last time of sampling. The pH values ranged from 7.4 to 6.6 with significant differences ($P < 0.01$) among sampling times. Ammonia-N concentration was significantly ($P < 0.01$) higher in group fed silage ration wilted for 24 hr than others, also it was elevated ($P < 0.01$) by formic acid treatment (16.4 vs. 14.7 mg/dl). The interaction between the main factors was not significant. Similar trend was observed with TVFA's, which was increased by wilting time ($P < 0.01$) and formic acid treatment ($P > 0.05$). The highest ($P < 0.01$) value of TVFA's was in R4 group and the lowest value was in R1 (being 7.28 vs. 4.20 mEq/dl; Table 7). Buffering capacity of rumen was not affected by wilting, formic or their interactions, which related with rumen pH values. The results showed that the decreasing pH values after feeding at 2 hr owing to increase $\text{NH}_3\text{-N}$ and TVFA's and thereafter pH was returned to elevate with declining $\text{NH}_3\text{-N}$ and TVFA's (Fig. 1 and 2). The pH value and calculated eNDF% were higher in un-chopped silage than chopped. Whereas, rumen parameters except for buffering capacity were tend to be higher in un-chopped silage and treated with formic acid compared with that untreated.

Table (6): Rumen liquor parameters affected by wilting, chopping with or without formic acid treatment of berseem silage with sheep.

Items	Chopped Silage											Un-chopped Silage				
	Wilting (hr)				Formic			Significance				-F	+F	SE		
	0	24	48	SE	-F	+F	SE	W	F	T	WxF				WxT	FxT
pH	6.98	6.90	6.99	0.026	6.94	6.97	0.02	NS	NS	**	*	**	NS	7.06	7.13	0.03
eNDF	36.7	35.0	36.9	0.61	35.9	36.4	0.49	NS	NS	**	*	**	NS	38.8	40.4	0.65
$\text{NH}_3\text{-N}$ (mg/dl)	14.4 ^c	16.7 ^b	15.6 ^a	0.35	14.7	16.4	0.28	**	**	**	NS	NS	NS	16.4	17.1	1.47
TVFA's, mEq/dl	4.33 ^b	6.14 ^a	6.22 ^a	0.25	5.40	5.76	0.21	**	NS	**	**	NS	NS	5.22	5.83	0.56
Buff. Capac. (mg/dl)	7.60	7.16	7.67	0.21	7.30	7.65	0.17	NS	NS	**	NS	NS	NS	7.95	7.54	0.34

^{a, b and c} least square means in the same row within wilting levels with different superscript differ significantly ($P < 0.01$)

* $P < 0.05$; ** $P < 0.01$; NS= non-significant ; W = wilting ; F = formic ; T = sampling time

Table (7): Rumen liquor parameters affected by interaction between wilting and formic acid treatment of berseem silage with sheep.

Items	Chopped Silage							±SE
	Wilting (hr)	0		24		48		
		Formic	-F (R1)	+F (R2)	-F (R3)	+F (R4)	-F (R5)	
pH		7.01	6.94	6.93	6.88	6.89	7.07	0.036
eNDF		37.4	35.9	35.6	34.3	34.8	39.0	0.86
$\text{NH}_3\text{-N}$ (mg/dl)		13.0	15.7	16.4	17.1	14.9	16.4	0.49
TVFA's (mEq/dl)		4.20	4.33	5.00	7.28	6.78	5.67	0.36
Buff. Capac.(mg/dl)		7.49	7.72	6.91	7.41	7.51	7.84	0.29

Fig. (1): Showed rumen pH value and eNDF% as affected by interaction between wilting and sampling time (1=0 hr; 2= 2 hr; 3= 4 hr and 4= 8 hr after feeding).

Fig. (2): Showed rumen NH₃-N and TVFA's concentrations as affected by interaction between wilting and sampling time (1=0 hr; 2= 2 hr and 3= 4 hr after feeding).

The obtained results are in accordance with those of Abou El-Enin (2005) who used different additives during ensiling berseem included formic acid. Similar values of rumen pH, eNDF, NH₃-N and TVFA's were found by Sharaf (2008), when he ensiled corn stover as a whole plant or without ears with different additives. In accordance with the present study, Jatkauskas and Vrotniakiene (2006) proposed that red clover silage treated with formic acid did not affect significantly rumen pH, TVFA's and NH₃-N values. Despite a substantial difference in silage pH, the rumen pH (on average 6.94) was not affected by the diet, probably due to the buffering capacity of saliva. In general, the levels of rumen pH recommendation ranges for high proteolytic (pH 6-7) and cellulolytic (pH 6.2- 6.8) activities (Hassen *et al.*, 2009). Contrary findings were reported by Baytok *et al.* (2005) who found that rumen NH₃- N decreased ($P < 0.05$) significantly by formic acid treatment of corn silage. The rumen NH₃ values recorded for rams fed berseem silages at all sampling times in excess of 5 mg/dl, which has been reported to maximize microbial protein synthesis (Woolford, 1984). According to Satter and Roffler (1975) values of NH₃-N lower than 2.5 – 5 mg/dl rumen fluid may inhibit

rumen activity, while Ørskov (1982) suggested a rumen NH₃-N concentration of 20-24 mg/dl for maximum fermentation rate. Deswaysen (1980) reported that in un-chopped silage the fermentation of a mat of interwoven particle in the rumen delays the separation and backflow of small particles into the reticulum and, as well as, ruminations delayed. Since the rate of particle reduction of un-chopped silage is slower than that of chopped material, the reticulo-rumen retention time of digesta is longer, that explain the lower pH, greater TVFA's and consequently higher digestibility of chopped and treated silage.

Blood metabolites:

Data in Table (8) presents blood hematological and metabolites of experimental rams as affected by wilted, formic, fasting time and interaction among them. The hemoglobin (Hb) concentration and hematocrit% (HT) were higher (P<0.01) significantly in wilting groups than un-wilting. Whereas, red blood cell counts (RBC's) was greater (P<0.01) in un-wilting group than others, as well as white blood cell counts (WBC's) was tend to be higher in unwilting group than others but without significant differences among groups. On the other side, formic acid treatment did not affect significantly on any hematological criteria. Plasma total protein and glucose concentrations were higher (P<0.01) significantly in wilting groups than un-wilting. The highest value of T. protein and glucose was in second group which wilted for 24 hr (being 7.76 and 46.8 mg/dl, respectively). Whereas, T. protein and glucose did not differ significantly by formic acid treatment. The interaction between wilting and formic did not affect plasma glucose levels, but tend to be effective (P=0.05) with T. protein (Fig 3).

Table (8): Blood metabolites of experimental rams as affected by wilting with or without formic acid treatments of berseem silage rations before and after feeding.

Items	Chopped Silage							Significance		
	Wilting (hr)				Formic			Wilt.	Form	WxF
	0	24	48	SE	-F	+F	SE			
Hb	10.5 ^b	11.3 ^a	11.3 ^a	0.13	11.2	10.9	0.10	**	NS	NS
HT	31.6 ^b	34.4 ^a	34.7 ^a	0.49	33.8	33.4	0.39	**	NS	NS
RBC's	4.51 ^a	3.90 ^b	3.86 ^b	0.09	4.05	4.13	0.074	**	NS	NS
WBC's	5.28	4.92	4.95	0.39	5.21	4.88	0.32	NS	NS	NS
T. protein	6.56 ^b	7.76 ^a	7.39 ^a	0.17	7.14	7.32	0.14	**	NS	NS
Glucose	39.2 ^b	46.8 ^a	44.6 ^a	1.65	42.1	45.0	1.35	**	NS	NS
AST	78.2	79.4	84.8	2.95	81.9	79.7	2.41	NS	NS	NS
Creatinine	1.10 ^a	0.82 ^b	1.04 ^a	0.04	1.03	0.94	0.03	**	NS	*

^a and ^b test square means in the same row for chopped silage with different superscript differ significantly (P<0.01)

*P<0.05 ; **P<0.01 ; NS= non-significant

Plasma aspartate aminotransferase (AST) was not affected significantly by main factors. Whereas, plasma creatinine was affected (P<0.01) significantly by wilting for 24 hr and the interaction (P<0.05) between wilting and formic (Fig 3). Creatinine levels ranged from 0.82 to

1.10 mg/dl and were within the normal level of sheep. The results of blood metabolites are in the normal range according to Mohamed and Selim (1999) and indicated that all animals were in a good nutritional status. Data are in coincidence with the findings of Eid (1998); Allam *et al.* (1997) and El Shaer *et al.* (2001). There were no adverse effect on kidney and liver function as well as blood hematology (Hb, HT and WBC's), this indicated that the animals were in a good health as a result of inclusion of a good fermented berseem silage.

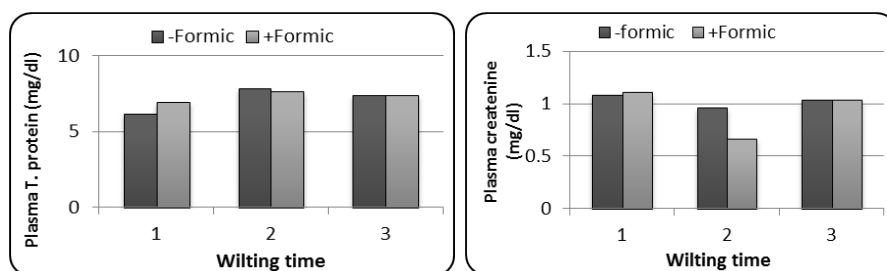


Fig. (3): Plasma T. protein and creatinine levels of rams as affected by wilting x Formic interaction (1= 0 hr; 2= 24 hr and 3= 48 hr wilting time).

Conclusion:

It could be concluded that, the lowest quality and digestibility was in un-chopped silage with or without formic acid treatment. Chopping and wilting berseem forage for 24 hr (about 20% DM) as pre-ensiled treatments with 0.5% formic acid sprinkle produced the best quality silage and higher digestibility without any side effect on health of rams, which could be resulted in improvement growth performance of the animal.

REFERENCES

A.O.A.C. (1990). Association of Official Analytical Chemists Methods of Analysis. 16th ed. Washington, D.C., USA.

Abou EL-Enin, Ebtehaq I.M. (2005). Nutritional evaluation of different treatments in silage production for ruminants feeding. PhD Thesis Ain Shams University.

Allam, S.M.; Hanfy, M.A.; Goma, I.A. and Abdou, A.M. (1997). Effect of ammoniation on date seeds utilisation in dairy rations. In: Proceeding of the 6th Conference on Animal Nutrition, El Minia (Egypt), 17-19 November 1997.

Aksu, E.B.T.; Karsli, M.A. and Muruz, H. (2005). The effects of formic acid, molasses and inoculant as silage additives on corn silage composition and ruminal fermentation characteristics in sheep. Turk J Vet Anim Sci. 29: 469-474.

- Barker, S. B. and Summerson, W. H. (1941). The colorimetric determination of lactic acid in biological material. *J. Biol. Chem.* 137: 537–554.
- Barry, T.N.; Cook, J.E. and Wilkins, R.J. (1978). *Journal of Agricultural Science, Cambridge*, 91:701-715. C.F. McDonald *et al.* (1991).
- Baytok, E.; Aksu, T.; Karsli, M.A. and Muruz, H. (2005). The Effects of Formic Acid, Molasses and Inoculant as Silage Additives on Corn Silage Composition and Ruminal Fermentation Characteristics in Sheep. *Turk J Vet Anim. Sci.*, 29: 469-474.
- Bolsen, K.K.; Ashbell, G. and Weinberg, Z.G. (1996). Silage fermentation and silage additives: Review. *Asian-Aust. J. Anim. Sci.* 9:483-489.
- Cai, Y.; Benno, Y.; Ogawa, M.; Ohmomo, S.; Kumal, S. and Nakase, T. (1998). Influence of *Lactobacillus* spp. From an inoculant and of *Weissella* and *Leuconostoc* spp. From forage crops on silage fermentation. *Appl. Environ. Microbiol.*, 64 (8) 2982–2987.
- Calsamiglia, S.; Stern, M.D. and Firkins, J.L. (1995). Effects of protein source on nitrogen metabolism in continuous culture and intestinal digestion *In Vitro*. *J. Anim. Sci.*, 73: 1819.
- Carpintero, M. C.; Holding, A. J. and McDonald, P. (1969). Fermentation studies on lucerne. *J. Sci. of food and Agriculture*, 20:677-681.
- Conway, E.F. (1957). *Micro-diffusion analysis and Volumetric Error*. Rev. Ed. Lock wood, London.
- Dawson, L.E.R.; Ferris, C.P.; Steen, R.W.J.; Gordon, F.J. and Kilpatrick, D.J. (1998). The effects of wilting grass prior to ensiling on silage intake. *Grass and Forage Sci.*, submitted.
- Deswaysen, A.G. (1980). Proceeding of the British Grassland Society Occasional Symposium No. 11, Brighton 345-349.
- Donaldson, E. and Edwards, R.A. (1976). Feeding value of silage: Silages made from freshly cut grass, wilted grass and formic acid treated wilted grass. *J. The Science of food and Agriculture*, 27:536-544.
- Duncan, D.B. (1955). Multiple range and multiple F tests. *Biometrics*, 11: 1.
- Eid, E.Y. (1998). Effects of organic wastes utilization in ruminant feeding on animal performance under desert conditions. MSc Thesis, Faculty of Agriculture, Zagazig University, Egypt.
- El-Shaer H.M., Fahmy, A.A.; Abdul Aziz, G.M Shalaby, A.S. and Abd El-Gawad, A.M. (2001). Nutritional evaluation of non- conventional fattening diets fed to sheep under arid conditions of Egypt. Proceeding of International Conference, Tunisia, 8- 10 Nov.
- Etman, K.E.I.; Mostafa, M.R.M; El-Sayes, M.F. and Gaafar, E.M. (1995). Nutritional studies on silage made from Egyptian clover with or without additives. *Egypt J. Appl. Sci.*, 10 (8):264-273.
- Fairbairn, R.L.; Alli, I. and Phillip, L.E. (1992). Proteolysis and amino acid degradation during ensiling of untreated or formic acid-treated Lucerne and maize. *Grass Forage Sci.*, 47:382-390.
- Flieg, O. (1952). The question of the evaluation of silage. *Landwirtschaftlicheforschung*, 3: 169-176.

- Fox, D.G.; Tylutki, T.P.; Van Amburgh, M.E.; Chase, L.E.; Pell, A.N.; Overton, T.R.; Tedeschi, L.O.; Rasmussen, C.N. and Durbal, V.M. (2000). The net carbohydrate and protein system for evaluating herd nutrition and nutrient excretion. Anim. Sci. Mimeo 213, Dpt. Anim. Sci., Cornell Univ., 130 Morrison Hall, Ithaca, New York 14853.
- Fransen, S.C. and Strubi, F.J. (1998). Relationships among absorbents on the reduction of grass silage effluent and silage quality. J. Dairy Sci. 81: 2623.
- Gary, M. (1992). Ensiling Process. In: Silage Manual (Ed. M. Bjorge and H. Bjorge). Edmonton, Alberta. pp. 14-17.
- Gibson, T.; Stirling, A.C.; Keddie, R.M. and Rosenberger, R. F. (1958). Bacteriological changes in silage made at controlled temperatures. J. gen. Microbiol. 19, 112-129.
- Gordon, F.J.; Dawson, L.E.R.; Ferris, C.P.; Steen, R.W.J. and Kilpatrick, D.J.(1999).The influence of wilting and forage additive type on the energy utilization of grass silage by growing cattle. Anim. Feed Sci. and Tech., 79:15-27.
- Haigh, P.M.(1988). The effect of wilting and silage additives on the fermentation of autumn made grass silage ensiled in bunkers on commercial farms in south wales. Grass Forage Sci., 43:337-345.
- Haigh, P.M. and Mansbridge, R.J. (1998). The Effect of Formic Acid with Formalin on Grass Silage Fermentation and the Performance of Dairy Cows. 69:255-259.
- Hassen, A.; Van Niekerk, W.A. and Bechaz, F.M. (2009).Silage fermentation attributes and certain rumen parameters in sheep fed two grass silages harvested at different stages of maturity. South African Journal of Animal Science. 39 (1):229-233.
- Handerson, A.R.; McDonald, P. and Anderson, D.H. (1982).The effect of silage additives containing formaldehyde on the fermentation of ryegrass ensiled at different dry matter levels and on nutritive value of direct cut silage. Animal Feed Science and Technology. 7:303-314.
- Henderson, A.R, McDonald, P. and Woolford, M.K.(1972).Chemical changes and losses during the ensilage of wilted grass treated with formic acid. J. Sci. Food. Agr., 23: 1079-1087.
- Jasaitis, D.K.; Wohlt, J.E. and Evans, J.I. (1987). Influence of fed ion content on buffering capacity of ruminant feedstuffs in vitro. J. Dairy Sci., 70:1391.
- Jatkauskas, J. and Vrotniakiene, V. (2006). Effects of silage fermentation quality on ruminal fluid parameters. BIOLOGIJA. 2006. Nr. 4. P. 65–71.
- Jayaprakasha, G.K. and Sakariah, K.K. (2002). Determination of organic acids in leaves and rinds of *Garcinia indica* (Desr.) by L.C. Journal of Pharmaceutical and Biomedical analysis. 28:379-384.
- Khan, M. A.; Sarwar, M.; Nisa, M. ; Khan, M. S.; Iqbal, Z.; Lee, W. S.; Lee, H. J. and Kim, H.S. (2006). Chemical composition, *in situ* digestion kinetics and feeding value of Oat grass (*Avena sativa*) ensiled with molasses for *Nili-Ravi* Buffaloes. Asian-Aust. J. Anim. Sci. 19:1127-1133.

- Kokkonen, T.; Tuori, M; Leivonen, V. and Syrjala-Qvist, L. (2000). Effect of silage dry matter content and rapeseed meal supplementation on dairy cows. *Animal Feed Sci. and Tech.*, 84:213-228.
- Matsuoka, S., Yonezawa, S.; Ishitabi, H.; Osanai, K. and Fujita, H. (1993). The effect of moisture content on aerobic deterioration of grass silage. *Proc. World Conf. Anim. Prod. Edmonton, Canada.* pp. 100-101.
- Mayne, C.S. and Steen, R.W.J. (1990). Recent research in silage additives for milk and beef production. *Sixty-third Annual Report of the Agric. Res. Inst. Of Northern Ireland*, pp. 31-42.
- McDonald, P. (1981). *The biochemistry of silage.* John Wiley and Sons, New York, NY.
- McDonald, P.; Henderson, A.R. and Ralton, I. (1973). Energy changes during ensilage *Journal of the Science of feed and Agriculture.* 24:827-834.
- McDonald, P; Henderson, N. and Heron, S. (1991). *The biochemistry of silage.* (2nd Ed.), Aberystwyth, UK, Chalcombe Publications.
- Mohamed , H.A. and Selim, H.M. (1999). *Hand book of Veterinary internal Medicine.* Dpt. Anim. Medicine, Faculty of Vet. Medicine, Zagazig Univ.
- Moore, J.E. (1994). Forage quality indices: development and application. P. 977-998.
- Moore, J.E. and Kunkle, W.E. (1995). Improving forage supplementation programs for beef cattle. Pp. 65-74. In 6th Annual Florida Ruminant Symposium.
- Mustafa, A.F. and Seguin, P. (2003). Ensiling characteristics, ruminal nutrient degradabilities and whole tract nutrient utilization of berseem clover (*Trifolium alexandrinum* L.) silage. *Canadian J. Anim. Sci.*, 147-152.
- National Research Council (2001). *Nutrient requirements of dairy cattle, seventh Revised Edition.* National Academy Press, Washington, D.C.
- National Research Council (NRC) (1985). *Nutrition requirements of sheep.* 6th revised Ed. National Academy Press. Washington. D.C.
- Ørskov, E.R., (1982). *Protein Nutrition in Ruminants.* Academic Press, New York.
- Polan, C.E., Stieve, D.E. and Garrett, J.L. (1998). Protein preservation and ruminal degradation of ensiled forage treated with heat, formic acid, ammonia, or microbial inoculant. *J. Dairy Sci.*, 81: 765-776.
- Rees, D.V.H.; Audsley, E. and Neale, M.A. (1983). *J. Agric. Sci. Cambridge.* 100:601-605. C.F. McDonald *et al.* (1991).
- Rohr, K. and Thomas, C. (1984). Intake, digestibility and animal performance. In: 'Efficiency of Silage Systems: A Comparison Between Unwilted and Wilted Silages' (ed. E. Zimmer and R.J. Wilkins), *Landbauforschung Volkenrode, Sonderheft* 69:64-70.
- Rowghani, E. and Zamiri, M. J. (2009). The effects of a microbial inoculant and formic acid as silage additives on chemical composition, ruminal degradability and nutrient digestibility of corn silage in sheep. *Iranian J. Vet. Res., Shiraz Univ.*, 10(2):110-118.
- Ruxton, I.B. (1972). M.Sc. Thesis, University of Edinburgh. C.F. Woolford, M.K. (1983).

- Sarwar, M.; Khan, M.A.; Nisa, M. and Touqir, N.A. (2005). Influence of berseem and lucerne silages on feed intake, nutrient digestibility and milk yield in lactating *Nili* buffaloes. *Asian-Aust. J. Anim. Sci.* 18:475-478.
- SAS (2004). SAS User's Guide, SAS (Statistical Analysis System) Institute, Cary, NC.
- Satter, L. D., and Roffler, R. E. (1975). Nitrogen requirement and utilization in dairy cattle. *J. Dairy Sci.* 58:1219.
- Shalaby, A. S.; Abdel-Aziz, G.M.; Rammah, A.M. and Beshay, M.G. (1989). Fodder beet, a new forage in Egypt. 2- Nutritional evaluation of fresh fodder beet (*beta Vulgaris L.*) and its silage with or without roughages. The 3rd Egyptian-British Conf. On Animal, Fish and Poultry Production, 7-10 Oct., Alex. Univ., Egypt.
- Sharaf, K.M.I. (2008). Studies on the use of silage in animal feeding. M.Sc. Thesis, Mans. Univ.
- Shaver, R.D.; Bytes, A.J.; Satter, L.D. and Jorgensen, N.A. (1986). Influence of amount of feed intake and forage physical form on digestion and passage of pre-bloom alfalfa hay in dairy cows. *J. Dairy Sci.* 69: 1545.
- Schneider, B. H. and Flatt, W. P. (1975). The evaluation of feeds through digestibility experiments. The University of Georgia press Athens, 3: 602.
- Shrestha, S. A., Hesterman, O. B., Squire, J. M., Fisk, J. W. and Sheaffer, C. C. (1998). Annual medics and berseem clover as emergency forages. *Agron. J.* 90: 197–201.
- Snyman, L.D., Joubert, H.W. (1996). Effect of maturity stage and method of preservation on the yield and quality of forage sorghum. *Anim. Feed Sci. Techn.*, 57: 63-73.
- Suepea, S.; ChiHsin, L.; WenWei, K.; RueyHshung, B. and JengBin, L. (2000). Forage production and silage making for berseem clover. *J. Taiwan Livestock Research*, 33:105-110.
- Tauqir, N.A.; Nisa, M.; Sarwar, M. and Bhatti, S.A. (2008). Impact of varying moisture levels, different additives and fermentation periods on nutritive value of leguminous and non-leguminous fodder silages in lactating *nili-ravi* buffaloes. *Pak. J. Agric.*, 45:386-402.
- Thomas, C. and Thomas, P.C. (1985). Factors affecting the nutritive value of grass silages. p. 223-256, in: D.J. Cole & W. Haresign (eds) *Recent Advances in Animal Nutrition*. London: Butterworths.
- Touqir N. A.; Khan, M.A.; Sarwar, M.; Nisa, M.; Lee, W. S.; Lee, H. J. and Kim, H. S. (2007). Influence of Varying Dry Matter and Molasses Levels on Berseem and Lucerne Silage Characteristics and Their In situ Digestion Kinetics in *Nili Buffalo Bulls*. *Asian-Aust. J. Anim. Sci.* Vol. 20, No. 6 : 887 – 893.
- Van Keulen, J. and Young, P.A. (1977). Evaluation of acid insoluble ash as a natural marker in ruminant digestibility studies. *Journal of Animal Science*, 44: 282-287.
- Van Soest, P.J.; Robertson, H.B. and Lewwis, B.A. (1991). Method of dietary fiber and non-starch polysaccharides in relation to animal material. *J. Dairy Sci.* 74: 3583.

- Waldo, D.R. and Schultz, L.H. (1956). Lactic acid production in the rumen. *J. Dairy Sci.*, 39:1455.
- Warner, A.C.I. (1964). Production of volatile fatty acids in the rumen, methods of measurements. *Nutr. Abst. & Rev.*, 34:339.
- Weinberg, Z.G. and Ashbell, G. (2003). Engineering aspects of ensiling. *Biochemical Engineering Journal* 13: 181-188.
- Wilkins, R. J. (1984). A review of the effects of wilting on the composition and feeding values of silage. In: E. Zimmer and R. J. Wilkins (Ed.). *Efficiency of silage systems: a Comparison Between Wilted and Unwilted Silages*. Landbauforschung Volkenrode, Sonderheft 69:71-72.
- Woolford, M.K. (1984). *The Silage Fermentation*. Marcel Dekker, New York, USA.
- Yan, T.; Patterson, D.C.; Gordon, F.J. and Kilpatrick, D.J. (1998). Effects of bacterial inoculation of unwilted and wilted grass silages. 1. Rumen microbial activity, silage nutrient degradability and digestibility. *J. Agric. Sci., Cambridge*, 131:103-112.
- Yan, T.; Patterson, D.C.; Gordon, F.J. and Porter, M.G. (1996). The effects of wilting of grass prior to ensiling on the response to bacterial inoculation 1. Silage fermentation and nutrient utilization over three harvests. *Anim. Sci.*, 62:405-417.
- Zhang, J. (2011). Effects of chopping length and cellulase addition on ensiling of guinea grass (*Panicum maximum* Jacq.). *Grassland Sci.*, 57: 113-115.

تأثير المعاملات الفيزيائية والكيميائية على جودة سيلاج البرسيم ومعاملات الهضم وتخمرات الكرش ومكونات الدم في الكباش
ماجد عبدالهادى عبدالعزيز عبدالهادى¹، أحمد عبدالرازق جبر²، السيد أحمد العبوطى² و محمد على زين العابدين شاهين²
¹معهد بحوث الانتاج الحيوانى - مركز البحوث الزراعية - مصر
²كلية الزراعة - جامعة المنصورة - مصر

أجرى هذا البحث بهدف دراسة تأثير فترات التجفيف الشمسى (الذبول) و التقطيع و المعاملة بحامض الفورميك على جودة سيلاج البرسيم ومعاملات الهضم وتخمرات الكرش ومكونات الدم في الكباش. تم جمع كميات من البرسيم (حشة ثالثة) وقطع يدويا بطول 2-3 سم وجفف لمدة صفر (طازج) ، 24 ، 48 ساعة ثم تم الكبس مع المعاملة بحامض الفورميك (0.5%) او بدونه في براميل بلاستيك سعة 200 لتر. في نفس الوقت تم جمع كميات أخرى من البرسيم وجففت لمدة 24 ساعة ولكن بدون تقطيع وتم الكبس مع أو بدون المعاملة بحامض الفورميك (0.5%). وقد أجريت تجارب هضم على سيلاج البرسيم بمعاملاته المختلفة لتقييم معاملات الهضم والقيمه الغذائية على كباش أوسيمى تامة النمو. وقد أشارت النتائج الى أن نسبة المادة الجافة في سيلاج البرسيم ازدادت الى 20% ، 30% في المعاملات 24 ، 48 ساعة تجفيف على التوالي. السيلاج المجفف 24 ساعة ومعامل بالفورميك كان أقل في قيمة رقم الحموضة (3.87) ، وكان أعلى تركيز في حامض اللاكتيك (3.15%) وأقل تركيز في حامض الخليك (1.57%) وحامض البيوتيريك (صفر%) مع درجة 90 مع مؤشر Flieg score . وقد كان أقل تركيز لحامض اللاكتيك وأعلى معامل حموضة مع أقل درجة من مؤشر Flieg score للجودة كان مع سيلاج البرسيم الغير مقطع سواء المعامل بالفورميك

أو بدون معاملة. كذلك كان أعلى عدد من بكتيريا حامض اللاكتيك وأقل عدد من بكتيريا الكلوستريديا والفطر في سيلاج البرسيم المقطع المجفف 24 ساعة ومعامل بالفورميك مما يوضح الارتباط مع نتائج تخمرات السيلاج الكيماوية. وكانت معاملات هضم المادة الجافة، المادة العضوية، البروتين الخام، مستخلص خالي الأزوت، والألياف الخام أعلى معنويا ($P < 0.01$) في علائق السيلاج الغير مجفف عن المجفف، وكذلك القيمة الغذائية أخذت نفس الاتجاه. في حين ان المأكول من مجموع المركبات المهضومة والبروتين المهضوم والطاقة الممتلئة كان أعلى معنويا ($P < 0.05$) في علائق السيلاج المجفف عنه في الغير مجفف. كذلك المعاملة بحامض الفورميك حسنت ($P < 0.05$) من معاملات هضم أغلب العناصر الغذائية في حين أن القيمة الغذائية لم تتأثر بالمعاملة. وكانت معاملات الهضم للعناصر الغذائية أقل في علائق السيلاج الغير مقطع عنه في السيلاج المقطع فيما عدا الألياف الخام ومكوناتها. وقد تحسن معامل الجودة (Quality Index) ($P < 0.05$) بمعاملات التجفيف لسيلاج البرسيم. وكان أقل معامل للجودة في السيلاج الغير مقطع والغير مجفف. ولم تتأثر قياسات سائل الكرش بالتجفيف أو المعاملة بالفورميك ماعدا الأحماض الدهنية الطيارة والأمونيا نيتروجين التي كانت أعلى معنويا ($P < 0.01$) في علائق السيلاج المجفف عن الغير مجفف. وقد كان أعلى تركيز للأحماض الدهنية الطيارة والأمونيا نيتروجين في السيلاج المجفف 24 ساعة ومعامل بالفورميك. وكان معامل الحموضة والألياف المتعادلة الأكثر استفادة وتركيز الأمونيا أعلى في السيلاج الغير مقطع عن المقطع. وقد كان تركيز هيموجلوبين الدم، والهيماتوكريت و البروتين الكلى والجلوكوز ازداد معنويا ($P < 0.01$) في مجموعة السيلاج المجفف عن الغير مجفف. في حين أن حامض الفورميك لم يؤثر على قياسات الدم. يمكن استخلاص من هذه الدراسة أن السيلاج الأقل جودة وأقل معامل هضم كان في الغير مقطع مع أو بدون معاملة بحامض الفورميك. عمليات التقطيع والتجفيف للبرسيم لمدة 24 ساعة (20% مادة جافة) قبل عملية السيلجة مع المعاملة بحامض الفورميك أدى الى انتاج سيلاج عالي الجودة وذو معامل هضم جيد بدون تأثيرات سلبية على صحة الكباش أثناء فترة التجربة مما قد ينتج عنه تحسن في معدلات نمو وأداء الحيوان.

قام بتحكيم البحث

كلية الزراعة – جامعة المنصورة
مركز البحوث الزراعية

أ.د / محمود يوسف العايق
أ.د / هدى محمد الحسينى

