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Abstract

As Ensiling practice requires an anaerobic environment, it is important to ensure an anaerobic environment for lab-scale silo as well as allow the release of fermentation gases from silo. The effect of using water lock on laboratory silos was studied. Three sealing methods (metal lid with water lock, metal lid without water lock and metal lid with water lock later replaced by metal lid without water lock) were studied. Silos were filled with lucerne to examine the extent of oxygen passing through the water lock. Two removable rubber stoppers were added with two replicates to observe the effect of air stress in laboratory silos. In addition, empty silo jars were made to explore how much oxygen could pass through the water lock.

As expected the silage was badly preserved because of low DM, low WSC with high buffering capacity and oxygen inlet into silo. Data from experiment proved that some oxygen passed through the water lock but it did not have a significant effect on fermentation quality. Fitting a water lock on the top of water lock was proved to be effective to seal the laboratory silo.

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Dedication

To my teacher, Thomas Pauly, for his enormous labor and cordial help throughout this research work that will be haunting in my heart. Author is grateful to his parent, brothers, sister and sister in law for their inspiration. Etmee, Nafis and Naima, these three are my all-time motivation.

Contents

1 Introduction

The Silage is a process of preserving forage for ruminants and horses particularly for winter season (‘t Mannetje, 2000). The basic aim of silage making is to provide forage (or roughage) to animals when fresh forage is not available. In order to do that, it is indispensable to make sure that anaerobic conditions prevail during the fermentation process and inhibit undesirable microorganisms from dominating the microbiological process (McDonald et al., 1966, Driehuis & Elferink, 2000). Most of the research on the fermentation process is made with laboratory scale silos for the benefit of silage industry (Henderson & McDonald, 1984).

Laboratory silos are used around the world to examine the effect of different treatments on silage fermentation, aerobic stability or weight losses. Many different types of laboratory silos were used for this purpose. For example, glass cylinders were used by Archibald (1946), sealed glass jars by Autrey et al. (1947), metal cans by Nevens (1933), vacuum-packed polythene bags by Johnson et al. (2005) and more recently polypropylene (PP) bags were used by Čabarkapa et al. (2010). With the advantages of cost effectiveness and rapid response to get the result, a glass preserving jar is frequently used in laboratory research around the world recommended by the German Agricultural Society (DLG) (Wieringa, 1960, Weinberg et al., 1993, Cussen et al., 1995). Like other types of silos, the glass jar silo with water lid and its sealing method was served as two purposes; release of gaseous product from silage material and exclude silage materials from oxygen (Cullison, 1960). To avoid the ingress of air in a silo, a special U-shaped valve filled with oil or mercury was fitted on the lid of glass jar silos described by Babcock and Russell (1901) and Peterson et al. (1925). Nowadays, the glass jar laboratory silo used by many scientists is made of an ordinary glass jar and a water lock (syn.: water lock, water lock or air lock or water vulve) which is attached to the top of silo lid. According to Čolović et al. (2010), the water valve is very comprehensible and constructive to prevent any air inlet as well as releasing fermentation gases.

The objective of this study was to estimate the quantity of oxygen that passed through the water lock of our laboratory silos and how this valve might affect

weight losses in silos during silage fermentation and the following storage period.

2 Background

2.1 Effect of oxygen during ensilage

Oxygen is pondered as one of the most detrimental components because it deteriorates silage quality. The effect of oxygen on silage is well documented in several publications. When air penetrates into a silo, the fermentation quality of silage is deteriorated and results usually in badly preserved silage (Woolford, 1990b). Air-infiltrated silage is often associated with undesirable microorganism (listeria, enterobacteria, clostridia, yeast and moulds), undesirable chemicals e.g. toxins, and lower acidity attributed to have a low level of lactic acid and a comparatively high level of butyric acid and ammonia-N. When the animal is fed silage with unknown levels of some of these fermentation products, it may lead to health hazard (Kitamoto et al., 1999, Wilkinson, 1999). The role of oxygen in four different phases of ensiling was described below (Pahlow et al., 2003, Merry et al., 1997).

Initial aerobic phase
Respiratory and enzymatic processes of the plant material characterize this first stage of the ensiling process. Some oxygen usually is lockped in the herbage thereby the respiration continues until the oxygen is depleted. Sprague (1974) showed that only 10 % oxygen was remained after 15 minutes and less than 0.5% remained after half an hour post sealing in wet silage. Air filtration can delay plasmolysis (a process that describes the collapse of plant cells and the release of plant juice) and the onset of pH reduction in silage. Greenhill (1964) showed that oxygen can delay the development of lactic acid bacteria. With immediately sealed lucerne forage, it took 5.5 hours at 25°C to start fermentation whereas it took 72 hours if oxygen leaked into it. Additionally, the pH of the immediately sealed silage dropped to pH 5.05 after 72 hours at 25°C while the air-infiltrated silage reached pH 8.5 (Greenhill, 1964).

Therefore, any delay in sealing is detrimental to silage quality; and the situation is even worse for forage legumes such as lucerne. Lucerne combines a low level of soluble carbohydrates with a high buffering capacity, which increases the risk of insufficient lactic acid production and a pH too high to inhibit clostridia and the production of butyric acid. This is one of the common reasons to get high butyric acid in air infiltrated silage (Takahashi, 1968, McDonald & Henderson, 1991).

Air infiltration after ensiling

The final product of silage is significantly influenced by air infiltration during ensiling (Rees et al., 1983). Badly consolidated silage is expected to get a higher butyric acid and ammonia-N contents, higher DM loss and a lower content of lactic acid than that of well-consolidated silage (McDonald, 1960). Moreover, penetrating air into silage can enhance the temperature of silage leading to changes in the chemical structure of proteins such as ester links, thioester links and amide links (McDonald, 1982; Ford, 1975).

Stable silage phase

This is the longest period of the process. If sufficient amount of lactic acid is produced to lower the pH below the critical pH, the fermentation will stop and the crop can be stored until the next season. If the critical pH level is not reached, a so called 'secondary fermentation' may occur, which is characterized by an increasing activity of clostridia, which transform lactic acid to butyric acid and results high ammonia levels and high DM losses (Jonsson, 1991; Knicky, 2005). If oxygen penetrates into silos, the activity of lactic acid bacteria is restricted thus leading to an insufficient acidification. Therefore the ammonia level increases and high DM losses occur (Woolford & Cook, 1978).

Feed out phase

After opening the silo, the silage environment changes suddenly from anaerobic to aerobic. Usually undesirable microorganisms such as yeast and occasionally acetic acid bacteria, which remain inactive in the absence of oxygen, dominate at this stage (Knicky, 2005; Moon & Ely, 1979). If this process proceeds, nutrient losses will be high because many aerobic microorganisms proliferate within a short period. Apart from the nutrient losses, sometimes pathogenic microorganism might grow, which might pose a health risk for the animals (Lindgren et al., 1985; Woolford, 1990a; Wilkinson, 1999). In this phase, it is not desirable to exposure on air for a longer period (Zimmer, 1980).

2.2 Fermentation characteristics (oxygen vs. other factors)

It is important to know about the silage fermentation product to assess silage quality. Good silage quality is characterized by a high amount of lactic acid, a low amount of ammonia and butyric acid. The level of pH depends on the DM content of the silage (dry or wet). To gets well fermented silage, we must ensure an anaerobic environment in the silo. Other than oxygen, silage quality can be degraded by unsuitable ensiling practices, composition of the fresh crop, and many more (McDonald et al., 1966).

Usually lactic acid is considered to be the most important fermentation product of silage and makes up approx. 60-70% of all acids in good quality silage. Some of the common reasons to get very low lactic acid in silage are discussed below. First of all, fermentation is severely restricted by high DM contents (>50% DM) (Kung & Shaver, 2001). Secondly, we cannot ignore the fact that, the low temperatures in Sweden could be a vital factor to have low lactate level particularly when silage is harvested in late season. A minimum of 6°C is needed for the successful fermentation for whole crop corn (Pauly, 2010). Moreover, air-infiltration usually leads to an oxidation of lactic acid where aerobic organism can grow and produce CO_2 (Cai et al., 1999). There is always a chance to get a low lactic acid level with high butyric acid in silage as clostridia can convert lactate into butyrate at a high pH (Kung & Shaver, 2001). Although clostridia, mostly responsible for producing butyric acid, are highly anaerobic, some research recorded clostridia fermentation occurred with low DM level when silage was exposed to air (Lingvall & Lättemäe, 1999). Jonsson (1991) stated that clostridia grew in anaerobic niches in air-infiltrated silage and that this was the probable reason for getting high butyric acid levels in silage.

Many researchers noted that the minimal level of water soluble carbohydrate (WSC) concentration for a successful fermentation varies from 25 to 35 g/kg fresh herbage, not to mention that it is also influenced by temperature, moisture content and buffering capacity (Dijkstra, 1957, Haigh & Parker, 1985, Zhang, 2002, Liu et al., 2011). If the buffering capacity is high in the forage and the amount of WSC low, there is not sufficient substrate left to produce enough lactic acid. Usually, there is a possibility of clostridia fermentation when a crop with low DM and low WSC content is ensiled (Wilkins et al., 1971, Jones, 1991). On the other hand, the amount of WSC remained in silage does not have a direct role to restrict fermentation during air exposure (Pahlow, 2005; Wrobel, 2008). Glucose can reduce the production of solute that might restrict fungal fermentation even though glucose could provide more subtract for aerobic deterioration (Pahlow, 2005; Wrobel, 2008).

The development of yeasts is very common when silage is exposed to air (Woolford & Wilkie, 1984, Moon et al., 1980, O'Kiely & Muck, 1992). However, some researchers found that there was no relation between air filtration in silage and yeast production (Ohyama & McDonald, 1975, Woolford & Cook, 1978). Most research has suggested that aerobically stable silage contained some compounds that inhibit yeast growth (O'Kiely & Muck, 1992). Other factors that could inhibit yeast growth are the levels of lactic acid and acetic acid (Moon, 1983). From the model of aerobic stability described by Courtin and Spoelstra, (1990b), it is known that aerobic stability depends on the population of microorganism in the forage as well as the chemical composition of the fresh crop (Courtin & Spoelstra, 1990b).

Jones (1991) stated that ammonia-N content should be less than 0.10 of total N content of crop. According to Luchini et al. (1997), the DM content is inversely correlated to ammonia-N content of silage. Other factors like high temperatures could play a role to exacerbate the fermentation quality. For example, when the temperature is between 20-40°C, high concentrations of ammonia-N and low concentration of lactic acid are expected (Garcia et al., 1989, Kim & Adesogan, 2006).

The aim of silage making is to ensure the production of a highly nutritious feed with high fermentation quality and long aerobic stability for our farm animals. To achieve this goal it is important to ensile the fresh forage as quickly as possible. However, leguminous crop with low DM content may delay wilting making crop difficult to ensilage (Haigh & Parker, 1985). Wilting can improve the fermentation quality of silage; and is a common practice around the world (Liu 2011).

2.3 Importance of water lock

Picture 1. A water lock filled with water (blue) fitted on a metal lid of a glass jar.

A water filled gas release valve or water lock can be fitted on the lid of a laboratory silo to observe the fermentation activity in the silo (Picture 1). The water column in the water lock is 55 mm in height and 12 mm in width. The total surface area in contact with air is 2.70 cm^2. The water lock is filled with demineralized water to minimize growth of algae and other organisms during storage and the water content is approx. 12 mL. Using a cap (red color in pic 1) on a water lock can prevent evaporation of water in water lock. The advantage of the water lock is to facilitate the release of the fermentation gases (mainly CO_2) and to exclude air or oxygen from entering into the silo (O'Kiely & Wilson, 1991). In addition, it is easy to observe the change of the water level in the water lock and draw conclusions on the microbial activity in the silo. Initially the microbial activity is very high in the silo producing a large amount of fermentation gases which bubble through the water lock. Because of pressure created by the fermentation gases, the water column in water lock is pressed down at one side and lifted up on the opposing site of the water lock (see Picture 1). When the silage fermentation ceases after a few weeks, the water column comes nearly in level as gas production ceases too. Indeed, the water level in the water lock can show whether the silo is tight or not. If the silo is not sealed properly, the two water columns in the water lock are in level as gases leave the silo by other ways rather than the water lock. Fitting a water lock on the silo lid makes it possible to distinguish between an air tight and an untight silo. That will reduce the variation among replicate silos and decrease the random error of the experiment.

2.4 Oxygen solubility in water

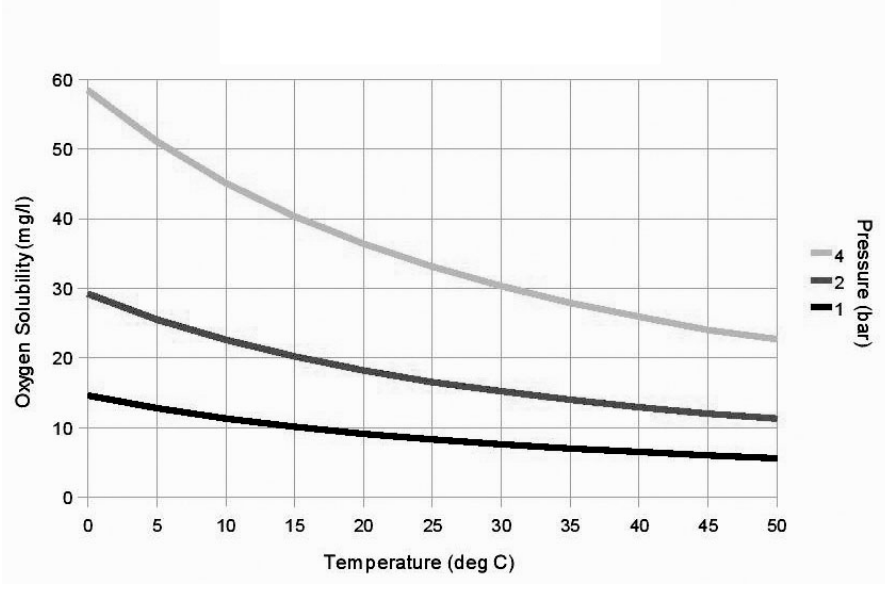
According to Kramer (1987), the saturation point or maximum amount of dissolved oxygen in water is 10 mg O_2/L whereas the saturation point for dissolved oxygen in air is 299 mg O_2/L at 15°C. Therefore, oxygen is constantly dissolved in water to maintain the balance of saturation point in water (Downing & Truesdale, 1955). Oxygen in water follows Fick's first law meaning that the movement of oxygen in water relies on the diversity of diffusion coefficient (Weast, 1988). The diffusion coefficient in water (2.1×10^{-5} cm^2/second at 25°C) is much lower than in air (about 0.2 cm^2/second at 25°C) as the viscosity of water is 1.002 centipoise at 20°C whereas the viscosity of air is approximately 0.18 centipoise (Winslow et al., 1932). The flux of oxygen into water is increases when oxygen concentration in air or air pressure is increased (Carpenter, 1966). Because of the low diffusion coefficient for oxygen in water, the oxygen penetration depth is very low. According to Finn (1954), the depth oxygen can penetrate into an agar-based culture media used for microbiological analyses is only approx. 1 mm. Below 1 mm depth, the oxygen level gets too low to support life of any aerobic microorganism.

2.5 Factors affecting oxygen solubility

Solubility of oxygen in water is greatly influenced by some environmental and biological factors. The most important environmental factors include temperature, humidity and air pressure whereas biological factors include concentration of gases as well as solutes present in water. Among all factors, we find only temperature which could make an effect on our experiment is discussed below.

Temperature is the most important factor for the solubility of oxygen in water. Benson & Krause Jr (1984) showed that if the temperature rises from 15°C to 25°C, oxygen solubility decrease by 18%. The solubility of oxygen in water is proportional to increasing of temperature ranging from 0-35°C (Downing & Truesdale, 1955). The effect of temperature on oxygen solubility is shown in Graph 1.

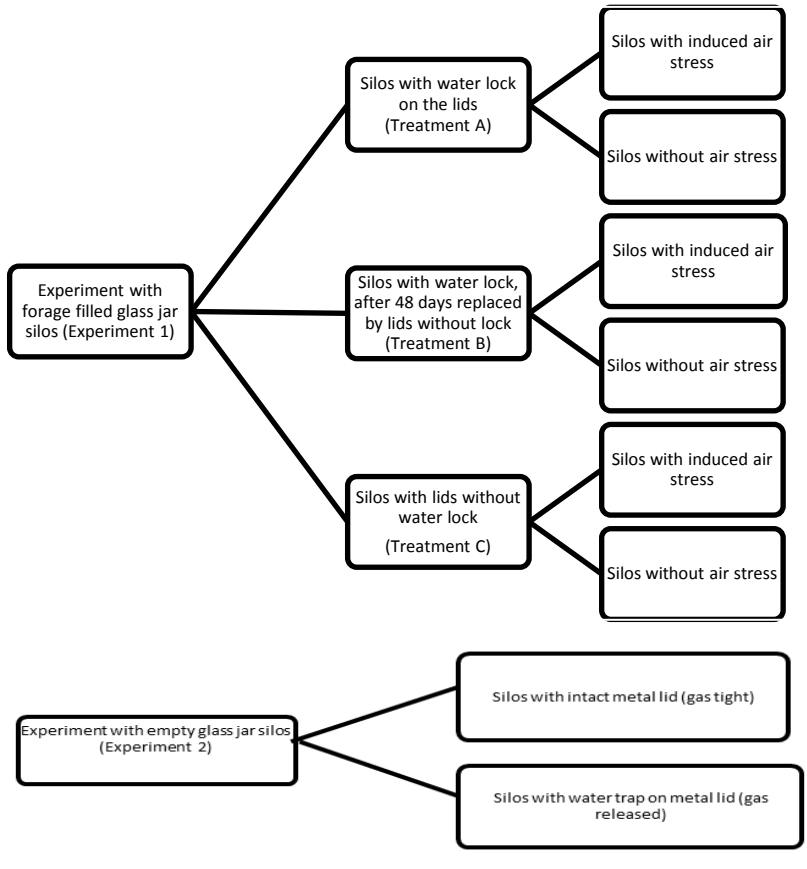
Graph 1. Oxygen solubility in water as affected by temperature and air pressure (1 bar = 1000 mbar = 1000 hPa) (www.engineeringtoolbox.com)



3 Materials and methods

According to our objectives, two experiments were carried out: One with laboratory silos filled with wilted lucerne to determine losses during ensilage period. The other experiment was done with CO_2 filled silos without silage to measure the oxygen ingress in our glass jar silos. The whole experimental plan is shown below in Figure 2.

Figure 2. The experimental design of the project



3.1 Experiment 1

This ensilage experiment was conducted at SLU, Kungsängen Research Centre in Uppsala, Sweden. In the first week of June, lucerne (*Medicago sativa*) was harvested with a scythe on a sunny day (temperature around 15° C) near Uppsala (59°57' N. 17°33' E). After wilting for approx. 2-3 hours, the crop was chopped with a stationary chopper. The average chop length was about 5 cm suitable for ensilage in laboratory silos (1800 ml glass jars). The plant material was then spread out on a clean plastic sheet (3 m * 2 m) layering approx. 15 cm thick and mixed thoroughly 8-10 times so that the forage was homogenized before sampling and ensiling. Three 40 g samples were taken from the plant material and merged into one composite sample for analysis of the fresh plant material.

Glass silos and lids were sprayed with ethanol (75%) and then left to dry. Silos were filled with 820 g FM forage and sealed with either a lid with a water-filled water lock or a gas-tight metal lid without any water lock (see Picture 2). In order to assess how much weight loss occurred during ensilage the 3 following sealing treatments were made (3 silos per treatment):

A) Silos sealed with a water lock lid for the whole storage period (0-180 days);
B) Silos sealed with a water lock lid for the first 48 days then lids were replaced by lids without water lock until the end of experiment (48-180 days);
C) Silos sealed with lids without water lock throughout the entire experiment (0-180 days).

Picture 2. Silo lids with and without water lock (left); Perkin Elmer gas chromatograph (middle); CO_2 filled silos for experiment 2 (right).

Another 2 silos per treatment were made with silos that had one hole just above the bottom and one in the lid. Holes (6 mm) were sealed with rubber stoppers (see picture 3). This type of silos is usually used in ensiling experiments in which a controlled air stress is applied. The idea with including these silos in the experiment was to examine the possible effect of ingress of oxygen on weight losses among silos with and without holes.
All silos were stored in-doors at room temperature (18-22°C) for 180 days before they were opened and sampled. During storage silos were weighed at 14 occasions to determine weight losses. The weight losses were calculated by using following formula:

Picture 3 Silo lid sealed with two rubber stopper (pink marking)

$$Weight\ loss = \frac{Initial\ silo\ weight - Silo\ weight\ after\ days}{Initial\ DM\ percentage \times Silo\ in\ weight} \times 100$$

After 48 days when we changed the water lid to metal lid in treatment B, the following formula was used (valid only for treatment B):

$$Weight\ loss = A + \frac{Silo\ weight\ after\ changing\ lid - Silo\ weight\ after\ days}{Initial\ DM\ percentage \times Silo\ in\ weight} \times 100$$

Where, A=weight loss in lid changing day.

3.2 Experiment 2

The purpose of this experiment was to measure how much oxygen passed through the water-filled air lock of our laboratory silos. Two types of silos with 3 replicates each were used as in Experiment 1 (i.e. lids with and without water tap). However, silos were not filled with forage but evacuated with CO_2. Both types of lids had two butyl rubber stoppers (Ø 12 mm) inserted on each lid to facilitate the collection of gas samples with a syringe and a hypodermic needle. Gas samples were analyzed for oxygen concentration at 3 occasions within a 24 day period. To collect samples for oxygen measurement in a gas chromatograph, special glass vials (76*28 mm and 21.1 mL) were used. For sample preparation, vials were recapitulated (vials were evacuated with N_2 gas and sealed with the help of recapitulated machine). After evacuation, these vials were taken to the silos to collect gas samples. Before taking gas samples,

some of CO_2 was flushed into the silos for about 2-3 minutes to avoid that any air entered the silos when the gas samples (5.0 ml) were taken (under pressure). The reason to do that when 5 ml gas sample was taken water level went down and created bubble permitting air inlet due to under pressure. Mistakenly, the injection of CO_2 was not performed at the first sampling occasion. A medical plastic syringe (10 mL) with a thin hypodermic needle was used to collect gas samples from the laboratory silos. The gas samples were injected directly (within approx. 5 seconds) into the vials. These samples were brought to the Department of Microbiology at the Swedish University of Agricultural Sciences (SLU) where they were analyzed within 6 hours after collection. Two samples from each treatment (in total 4) replicates were thrown away because of faulty handling.
A gas chromatograph from Perkin Elmer, Clarus 500, with an ECD-detector for the determination of the oxygen concentration was used (see Picture 1). A headspace auto sampler, Turbomatrix 110, was also connected to inject samples into the gas chromatograph. The initial column oven temperature was 80°C; then it was reached to 110°C within 10 min and finally attained to 320°C. The flame-ionization detector temperature was 320°C; the carrier gas was H_2 at a pressure of 550 kPa and a flow rate of 1 mL/min. A known standard (21% O_2) was used to check the values that we got out from this analyzer. After about 2 hours, the result was automatically printed out. The amount of oxygen in silo jar was calculated by using a dilution factor. The dilution factor is (22.1+5)/5=5.42 where the glass vial volume=22.1ml, sample volume=5 ml. Then the value picking out from gas chromatograph was multiplied with the dilution factor to get the oxygen concentration found in glass jar silo.

3.3 Statistical analysis

Experiment 1:
A randomized two-factorial ANOVA was conducted to compare the difference among the three treatment means. The models used for statistical analyses are given below.

$$Y = \text{Sealing} + \text{Air infusion} + \text{Error}$$
$$\text{d.f.}: 17 = 2 + 1 + 2 + 12$$

If the interaction (Sealing x Air) is not significant, it can be removed from the model; then

$$\text{d.f.}_{error} = 12 + 2 = 14$$

Experiment 2:
A randomized single factor ANOVA was conducted to compare the difference between the two treatment means. The models used for statistical analyses are given below.

$$Y = \text{Sealing} + \text{Error}$$
$$\text{d.f.}: 3 = 1 + 2$$

If the interaction (Sealing x Air) is not significant, it can be removed from the model; then

$$d.f._{error} = 2 + 1 = 3$$

Significance levels:
Significant difference was measured at the level of 0.05 level (p-values=0.05). If the P value is higher than 0.05, superscript letters are used. If a parameter (e.g. DM) is not significant, no superscript letters are used.

4 Result

4.1 Chemical and fermentation quality of silage

The chemical composition and the fermentation quality of ensiled lucerne are presented in Table 1. Lucerne was late cut therefore permitting low fiber and CP content. Moreover, it contained very low amount of WSC and very high buffering capacity. As it was not wilted, the DM content of crop was quite low that made it difficult to ensile. The value of ADF and NDF was average of this crop.

The fermentation products in Table 1 indicated that the silage was badly preserved and no significant difference was found between the treatments ($p>0.05$). The lactic acid concentration was very low in our silage ranging from 0.16 to 0.26% of FM (from 2 to 3% of FM considered as acceptable level). Ammonia-N content was significantly higher in value compared with well-preserved silage (<8 of total N). Interestingly, treatment C experienced comparatively higher lactic acid and lower ammonia-N concentration than other type of treatments indicated the treatment C was comparatively better fermented than others. Because of low DM content, it is anticipated to have high amount of acetic and butyric acid in silage. Butyric acid in silage is often produced by clostridia fermentation. The content of acetic acid and butyric acid was ranging from 0.4 to 0.6% of FM and from 0.8 to 2% of FM, respectively, which is not the acceptable value with low DM content (22%). The acceptable value for butyric acid is up to 0.3% of FM. However, the butyric acid content of treatment B (water lock replaced by metal lid) was within the accepted value. Production of 2.3-butanediol and ethanol was comparatively stable, but the ethanol content of silo with water lock was about 50 % higher (but still not significantly diferent) compared to of other types of silos indicated there may be some more oxygen inlet into it.

Table 1. Experiment 1: Chemical composition of the fresh crop and fermentation quality of silage.

	Fresh lucerne	Lucerne silage		
		Treatment A	Treatment B	Treatment C
Dry matter (% FM)	24.7	23.0[a]	22.8[a]	22.1[a]
Chemical composition (%-DM)				
Ash	9.0	ND	ND	ND
Crude protein	14.1	ND	ND	ND
NDF[b]	40.7	ND	ND	ND
ADF[c]	33.6	ND	ND	ND
WSC[d]	4.9	<0.1	<0.1	<0.1
Buffering capacity(g lactic acid/kg DM)	81.8	ND	ND	ND
Fermentation products (% FM)				
Lactic acid	ND	0.16[a]	0.19[a]	0.26[a]
Acetic acid	ND	0.60[a]	0.34[a]	0.44[a]
Propionic acid	ND	0.14[a]	0.09[a]	0.17[a]
Butyric acid	ND	1.21[a]	0.79[a]	1.83[a]
Ethanol	ND	0.34[a]	0.23[a]	0.22[a]
2.3-butanediol	ND	0.34[a]	0.19[a]	0.23[a]
Ammonia-N (% N)	ND	25.7[a]	22.2[a]	23.5[a]

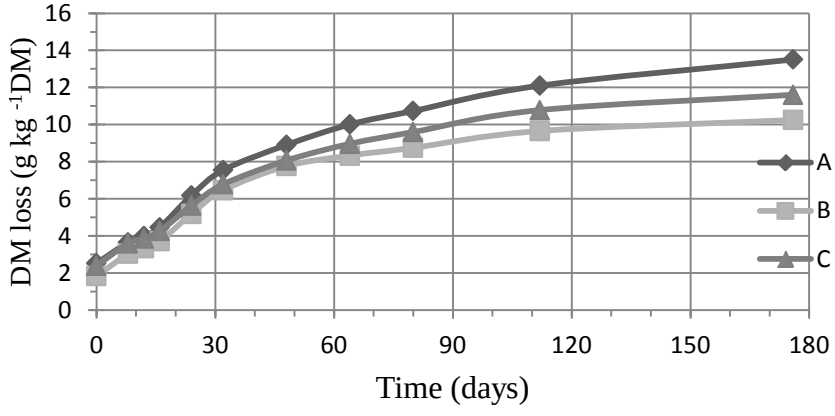
[a] not significant [b]Neutral detergent fiber [c]Acid detergent fiber [d]Water soluble carbohydrates [ND] not determined

4.2 Experiment 1: DM loss during ensilage

Determining of DM loss is an important criterion to evaluate silage during ensilage period. Graph 2 shows how the DM loss changed over ensiling period. There were no significant differences in final DM loss after 176 days storage

between treatments although the silos with water lock had numerically higher DM loss($p=0.99$). After The slight change in DM loss in the treatment where the lid was changed after 48 days (B) indicates that this new lid was tighter than the ones that was adapted from the beginning of the experiment (C).

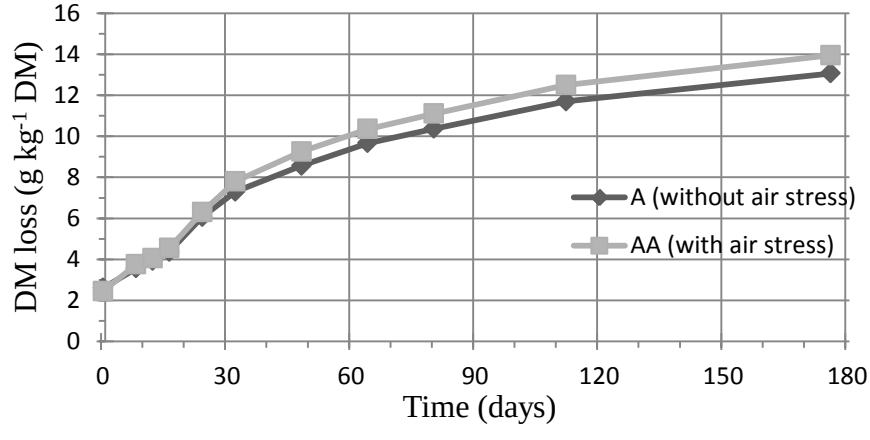
Graph 2. Experiment 1: Difference of DM loss between treatments during ensiling period (silo with water lock=A. Silo with water lock later changed to metal lid=B and silo with metal lid=C)



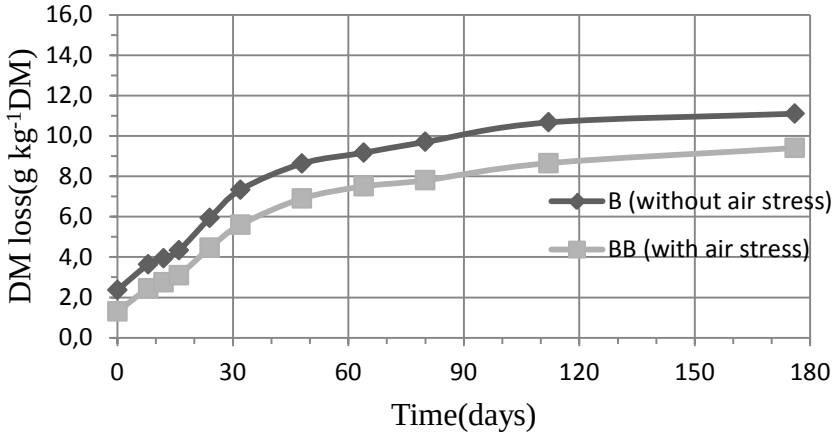
4.3 Experiment 1: The effect of air stress in laboratory silo

Difference in DM losses within treatments with or without air stress is depicted in Graph 2, 3 and 4. In all treatments, there was no significant differences in DM loss between silos with and without rubber stopper ($p>0.05$).

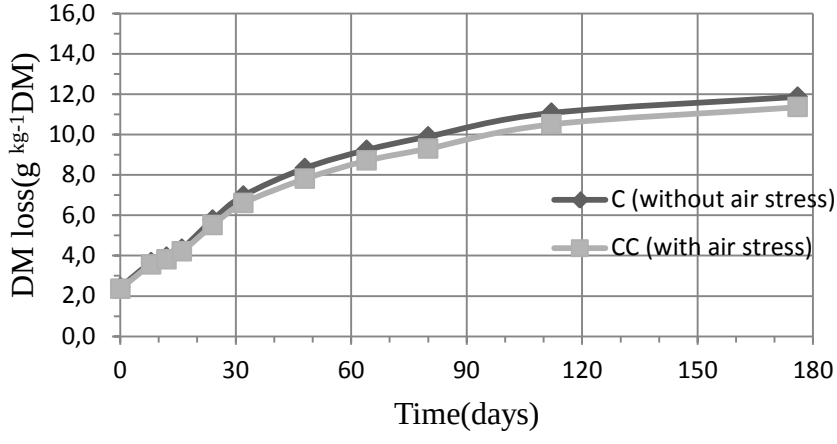
Graph 3. DM loss during ensilage between silos with (AA) and without air stress (A) within treatment A (water lock silo)



Graph 4 DM loss during ensilage between rubber stopper silo and without rubber stopper silo with treatment B (lid with water lock later replaced by lid without lock)



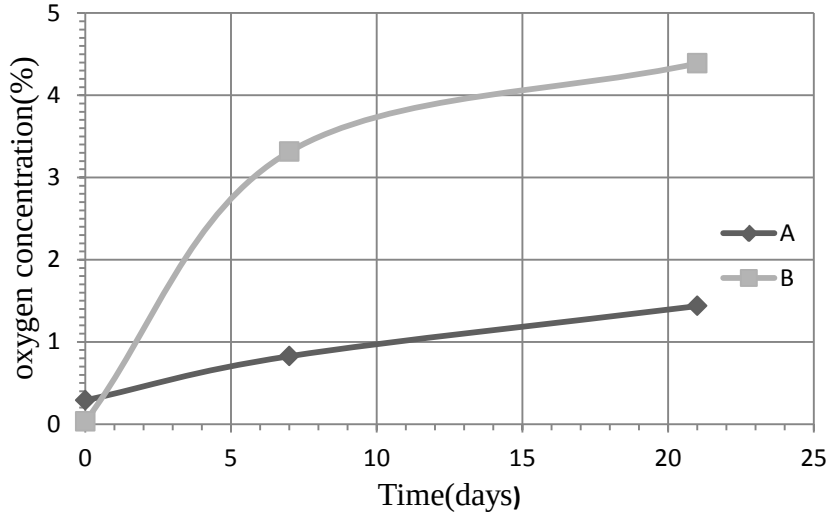
Graph 5 DM loss during ensilage between rubber stopper silo and without rubber stopper silo with treatment C (lids without water lock).



4.4 Experiment 2: Estimation of the amount of oxygen entering the silo

The only oxygen leakage that could occur during ensilage was through the water lock. Graph 6 presents the oxygen concentration in evacuated silos without water locks, i.e. with silos with water lock (B) and unaltered (intact) metal lids (A). In this experiment, the amount of oxygen entering silos with water lock (B) was not significantly higher (*p=0.13*) than silos without water lock (A). After 21 days oxygen levels in silos with water locks were approx. 3 times higher than in silos without water locks. We assume that about 8 ml O2/day or 40 ml air/day entered in the first 7 days. However, the rate of oxygen entered only about 2 mlO2/day or 10 ml air/ day from 7 to 21 days.

Graph 6. The concentration of oxygen (% of silo gas) in silos without (A) and with water locks (B). Each point represents the mean of 2 replicate silos.



5 Discussion

The aim of this experiment was to identify weather oxygen is leaking out through the water lock or not and to determine the effect of air ingress into silage. The aim was also to identify the amount of oxygen passing the water lock. No significant differences were detected, but the result of the chemical composition as well as the result of the DM losses, indicate that some more oxygen may have leaked into silos with water lock (treatment A).
The fermentation product of our silages indicated that the silages were generally badly preserved. Our silage experienced considerably lower amount of lactic acid and a higher amount of butyric acid and ammonia-N level than is considered to be good quality silage (Kung & Shaver, 2001). A striking feature in this experiment was that the fermentation losses were about the same also in silos completely sealed (treatment C). In a completely closed silo the only weight losses possible are in terms of heat loss. This could be the case since the temperature was not measured. The other possible explanation is that also the lids without water locks were not tight but let out the overpressure occurring when gas was produced in the fermentation process.

Other external factors which could play an important role to exacerbate the silage quality like the ambient temperature were monitored during the ensiling and storing period. However, the average temperature during our experiment was not extreme enough to play a role for fermentation. In addition, the research work was done in laboratory-controlled room so that temperature could not play a big role to change fermentation process. Another factor like rubber stoppers, we fixed two of these on the top of laboratory silo to examine if any oxygen could inlet into it. However, data from this experiment showed that the rubber stopper did not have a significant effect on fermentation loss or DM loss. Indeed we can say that oxygen did not pass through the rubber stopper or if passed through, it was minimal in amount. The crop that ensiled was not wilted and late cut. As a result, the chemical composition and the fermentation quality were not satisfactory. Nevertheless, at the end the experiment could be questioned why we did not ensile the best crop to get a satisfactory fermentation. The most satisfactory answer would be that our aim to measure the oxygen inlet into the silo, not to get the best possible fermentation quality of silage. Thereby, we did not take care to have a good quality crop ensiled in our experiment.

The point to fit a water lock on the top of lid is proved to beneficial in laboratory silage research. First of all, a water lock can not only protect air inlet into silo but facilitate to remove the gaseous product from silo. Then, it is difficult to prove during laboratory research that silo is completely sealed; and many of research works were questioned or rejected in past because of getting

wrong data attributed to oxygen inlet into it. However, placing a water lock on the top of metal lid can easily solve this difficulties as water level in water lock indicates weather the silo is properly sealed or not. On the other hand, the disadvantages to use a water lock is to contribute weight loss occurred by evaporating water from water lock. But if the water lock weight is deducted from the silo weight before starting experiment, the methodological error will be corrected. And fitting simple cap on the top of water lock might help to prevent water to evaporate from water lock. Secondly, data from our experiment showed that some oxygen passed through the water lock and inlet into it even though the impact of ingress in air in silo was not significant. A limited O_2 inlet into silo might be considerable because large, farm scale silos are not gas-tight. As the impact of O_2 passed through the water lock is negligible, it might have less variation among replicates to reduce error in laboratory research confirmed by our experiment in which the difference among replicates were not significant.

As far as I know, this is the first time someone worked with water lock of laboratory silo. Subsequently, there is no paper published about the oxygen passed through the water lock. But oxygen continuously dissolves in water due to pressure difference between air and water. Our experimental result from experiment 2 showed some oxygen dissolved into water of the water lock during ensilage and the difference in the amount of oxygen between silos with water lock and without water lock was not significant($p=0.13$). And the difference was wider as days progressed proves that there is oxygen leaking into the silo through the water lock. When we flushed some CO_2 into empty silo to minimize under pressure, there was no O_2 left in silo, just CO_2. But when all CO_2 goes through the water lock by diffusion or under pressure created by gas, some oxygen might inlet into it because of over pressure as there is nothing left in empty silo. As time progress, more and more oxygen come into it and filled the place of CO2, probable reason to get more oxygen as time goes on in empty silo. The O_2 transport is driven by the difference in concentration. This difference disappear gradually in the empty silos we used in Experiment 2, but when the silos are filled with silage the present bacteria continuously will consume the entering O_2 and maintain the difference in O_2 concentration between inside and outside the silo.

As we know from culture media that could be compared with silo which is also anaerobic, oxygen can pass through only 1mm depth in culture media. In silo filled with grass, oxygen passed through the water lock might be used up by yeast. As a result, a little damage was occurred during ensilage found in our experiment. The oxygen passing through the water lock did make small but not significant impact on laboratory silos and fitting a water lock on the top of silo lid was proved beneficial in our experimental work.

6 Conclusion

This thesis work shows that using water locks on laboratory silos can be used to indicate whether the silo is sealed or not. The weight loss observed in silos with water lock and without water lock was not significantly differed during ensilage. Data from this experiment showed that the use of removable rubber stoppers used in laboratory silo did not make any difference on the ensiling result. In addition, oxygen analysis in an empty silo proved that some oxygen passed through the water lock but it does not significantly affect the ensiling result unless the storage time is carried out for more than 6 months. Therefore, scientists who are going to work with laboratory silo with water lock for a longer period should take into account that fermentation losses may occur.

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