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Applying response surface methodology to optimise maize silage making for cattle feed

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KEYWORDS

Livestock Feed

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ABSTRACT

One of the important ingredients in cattle feed is protein. Yet, a high quality cattle feed is influenced by the balanced concentration of protein, carbohydrates, minerals and vitamins. Agricultural waste, such as maize crop waste, can be used as potential feedstock for making cattle feed. Silage is a product resulted from fermentation process, which was mostly used as a feed source to many livestock. Silage making is functioned to preserve the quality of the feeds. Especially during dry season. Maize silage has been highlighted to be alternative feed for livestock or cattle. This study was aimed to optimise the concentration of molasses and storage time in producing high quality maize silage. The parameters measured include pH, moisture content (MC), crude protein, and crude fibre. Response Surface Methodology (RSM) with two factors was used in this study, while the optimisation was formulated using statistical software Design Expert 7.0.0. The results showed that by adding molasses of 6.97% with storage time of 240 hours, the optimum quality of maize silage was achieved, giving the value of pH (3.88), water content (23.80%), crude protein (9.01%), and crude fibre (21.67%).

Introduction

The quality for livestock feed is often influenced by the nutritional values as well as by the balanced ingredients of protein, fat, carbohydrates, minerals and vitamins (Van Soest, 2018). Cattle feed can also be produced using agricultural waste, such as maize crop waste (Klopfenstein et al., 2012; Hellin et al., 2013; Khan et al., 2015). Maize crop residues consist of the stalk (50-56%), leaf (20-30%, cob (15%) and husk (<10%) (Singh et al., 2009).

Silage is one of the products from the application of forage preservation method, also known as ensiling process, based on natural fermentation involving the conversion of water-soluble carbohydrates into organic acids under anaerobic condition (Da Silva and Santos, 2016). Silage making is aimed to enhance the feed quality and the feed supply, especially during the dry season (Ferraretto et al., 2018; Muck et al., 2018). Furthermore, maize silage have been found to be widely used as dietary feeding for various livestock, for instance dairy cows and goats (Dunière et al., 2013; Keady et al., 2013; Rezaei et al., 2015). Therefore, in this study, it is necessary to investigate the concentration of additives (i.e. molasses) and the storage time to produce good quality maize silage.

Optimisation of silage making process is expected to increase the valorisation of maize crop waste as cattle feed, as well as to enhance the availability and variety of the cattle feed. Thus, it can contribute to overcome the problem of limited feed's supply, particularly during dry season. This study was aimed to optimise the concentration of molasses and storage time needed for producing high quality maize silage.

Research Methods

This research was carried out at Laboratory of Animal Nutrition and Food, Faculty of Animal Husbandry, Universitas Brawijaya, Malang, Indonesia.

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Materials

Maize crop waste was collected from *Balai Besar Inseminasi Buatan* (BBIB) in East Java Indonesia. The maize crop waste was then aerated for 1-2 days to reduce the moisture content (MC) up to 60% and cutted into 3-5 cm in length. Molasses was freshly collected from Krebet Sugar Factory Malang, East Java Indonesia.

Experimental set-up

Prior the experiment, the treatment combination was planned using Response Surface Methodology (RSM) with two factors of concentration of molasses (2.17, 3, 5, 7, and 7.83 % w/w) and storage time (140.59, 240, 480, 720, and 819.41 hours). From applying RSM, 13 treatments were obtained, as shown in Table 1.

The silage making procedures were modified based on Nussio (2005). A 500 g of dried maize crop waste was mixed with molasses at different concentration. The prepared maize samples were placed in a silo (or jar) covered with plastic sheets and then compacted and tightly closed. The plastic covers were function as insulation and to prevent air entering the jar, as well as to ensure that the anaerobic condition was achieved during the fermentation. These samples were stored at different storage time in a closed and lightproof room. The maize silage products were then analysed for the quality's characteristics include pH, MC, crude protein, and crude fibre content.

Analysis

pH and MC was analysed based on Standard Methods (APHA, 2005). Analysis of crude protein was performed according to Kjeldahl digestion for crude protein method, while crude fibre was measured based on fritted glass crucible method (AOAC, 2005).

Statistical analysis

The responses for the maize quality were analysed using RSM and a statistical software of Design Expert 7.0.0. In the statistical analysis, the $P<0.05$ were applied, indicating the relationship between the variables tested was statistically significant.

Results and Discussions

Table 1 shows the quality parameters of maize silage after optimisation using RSM; include pH, MC, crude protein and crude fibre. These characteristics of maize silage are important to determine its quality. The details are discussed in the following section.

pH

The results showed that the pH response was well fitted to a quadratic model, as indicated by a p value of 0.0906 ($p <0.05$). The value demonstrated that variables of concentration of molasses and storage time and their interactions present no significant effects on pH response. Similarly, when the quadratic model was tested for the model uncertainty, the p value was 0.9014. This value indicated that the interaction between the two variables has no significant effect on pH by 90.14%.

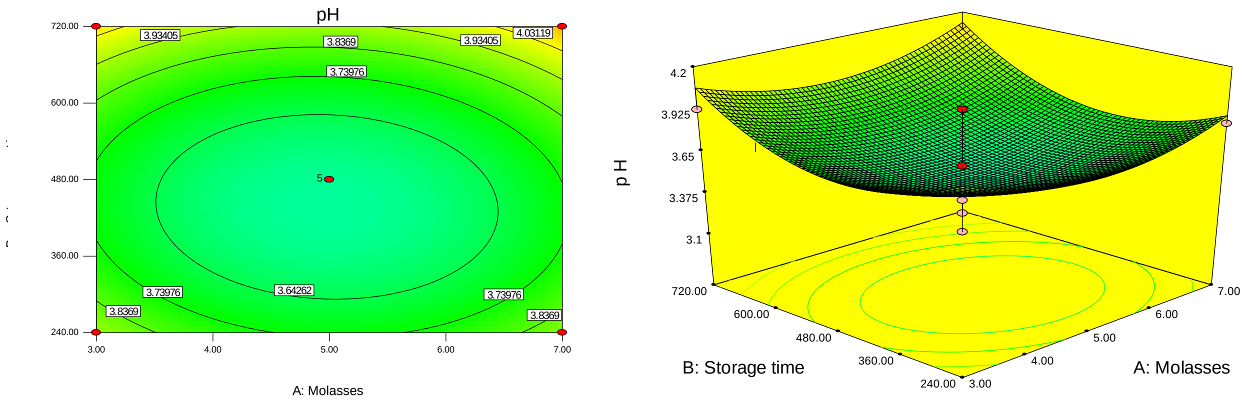
Table 1. The characteristics of maize silage based on pH, MC, crude protein and crude fibre responses

No	Code		Variables		Responses			
	X1	X2	Molasses (%)	Storage Time (hour)	pH	MC (%)	Crude Protein (%)	Crude Fibre (%)
1	1	1	7	720	4.02	69.13	9.87	22.49
2	0	0	5	480	3.93	29.39	8.67	22.35
3	-1.41421	0	2.17	480	4.02	31.41	9.07	23.98
4	0	0	5	480	3.93	29.39	8.67	22.35
5	0	0	5	480	3.24	29.36	8.49	22.28
6	-1	-1	3	240	3.84	74.26	7.68	28.03
7	0	1.41421	5	819.41	4.36	63.38	12.79	22.43
8	0	0	5	480	3.56	29.22	8.31	22.19
9	1.41421	0	7.83	480	4.00	31.17	9.79	23.25
10	0	-1.41421	5	140.59	4.01	33.74	10.45	21.25
11	-1	1	3	720	3.93	69.92	9.52	22.32
12	0	0	5	480	3.11	29.12	8.22	22.18
13	1	-1	7	240	3.84	29.51	8.07	27.97

The model was also contained the smallest value of Prediction Error of Squares (PRESS) (1.47), demonstrating that the quadratic model was indeed the best model obtained. The R^2 value was 0.9014, indicating the diversity of data related to the effect of addition of molasses and the storage time to maize silage pH can be described by the model was 90.14%. The remaining 9.86% was explained as errors and other inaccurate factors.

The highest pH values was 4.36 obtained from addition of 5% molasses and storage time of 819.41 hours, with the lowest pH was 3.11 from addition of 5% molasses and storage time of 480 hours (Table 1). Figure 1 shows the three-dimensional form and contour response surface plots which indicating the relationship between the molasses concentration and storage time and their interactions effects on pH of the maize silage. The findings also confirmed that the pH responses obtained were well fitted to the following mathematical equation:

$$Y = 3.55 – 0.4698\,X_1 – 4.30262.10^{-3}\,X_2 + 4.6875\,X_1X_2 + 0.18\,X_1^{\,2} + 0.27\,X_2^{\,2}$$



(a) (b)

Figure 1. Response surface contour plots (a) and three-dimensional form (b) on the effect of molasses concentration and storage time on pH of maize silage.

The results of this study demonstrated that storage time has no significant effects on pH. The changes on pH value may possibly be due to the breakdown of water soluble carbohydrates into organic acids by the lactic acid bacteria (LAB) during the fermentation. As explained by McDonald et al. (2002) that the organic acids (i.e. lactic acid, acetic acid, and butyric acid) formed during silage making process reduce the pH of the silage. Coblentz (2003) supported that a good fermentation process was indicated by a lower pH value. The number of LAB may also contribute to decrease the pH during the fermentation (Ni et al., 2017). They further added that addition of molasses additives could promote the pH drop during fermentation, because a direct increasement of molasses stimulates the sufficient growth of LAB to produce lactic acid and, thus, lowering the pH.

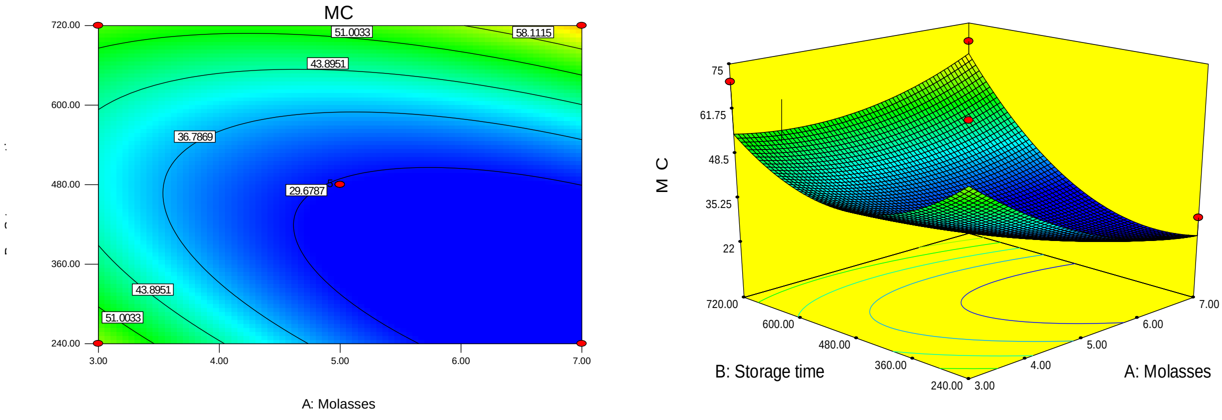
MC

The statistical model for the MC response was the quadratic model with the p value of 0.0456 ($p < 0.05$). This model shows a significant effect on the MC response. Similarly, the model inaccuracy test also results a p value of 0.0001, which indicating the model has a significant uncertainty on MC.

The lowest PRESS value was 8152.48, supporting the selection of the quadratic model as the best model. The R^2 value was 0.7298, demonstrating that the selected model was able to explain the diversity of data in relation with the effect of addition of molasses and the storage time on MC of maize silage by 72.98%.

Table 1 shows that the lowest MC (29.12%) was resulted from the molasses concentration of 5% and storage time of 480 hours. The highest MC (74.26%) was obtained from the addition of molasses at concentration of 3% and storage time of 240 hours, respectively. Figure 2 also indicates that a significant relationship between the two factors used on MC of maize silage. The mathematical model for MC responses was as follows:

$$Y = 179.739 – 29.962X_1 – 0.32317X_2 + 0.023417X_1X_2 + 1.579{X_1}^2 + 12.55243.10^{-4}\ {X_2}^2$$



(a) (b)

Figure 2. Response surface contour plots (a) and three-dimensional form (b) on the effect of molasses concentration and storage time on MC of maize silage.

The results in this study confirm that adding molasses into the forage can influence the MC of maize silage. Lado (2007) found that addition of additives (i.e. molasses, *putak*, and rice bran) at different concentration on silage fermentation proses has effect on MC, organic matter (OM) and dry matter (DM) of the silage. They also claimed that the initial MC of the forage may contribute to the MC of maize silage.

In addition, storage time has also found to have a significant effect on the MC of maize silage. McDonald (1981) stated that OM and DM was decreased during the ensiling process due to the respiratory stage which converts glucose into H_2O. Decreasing OM and DM have contributed to an increase in MC. He further clarified that two phases were occurred during ensiling, as follows:

1. Phase 1 is ongoing cell respiration where glucose is converted to CO_2, H_2O, and heat. During the process, a fraction of glucose (i.e. DM) was depleted as a result of oxidation process.
2. Phase 2 is anaerobic fermentation where the trapped oxygen was depleted. During this stage, the glucose was converted into lactic acid, ethanol and CO_2. The loss of DM and OM are greater when hetero-fermentative bacteria dominate the fermentation activity.

Crude protein

Similarly, the quadratic model was also selected for the crude protein response since the p value was 0.0430 ($p < 0.05$). This suggests that the probability of a model error was less than 5%, indicating that the model can show a significant effect of molasses addition and storage time on crude protein. The model inaccuracy test also indicated a lower p value of 0.0014, which means that the determination was significant towards the crude protein response by 0.14%.

The statistical model shows the lowest PRESS value was 43.49. Based on this test, the quadratic model was also chosen as the best model, with R^2 value of 0.7131. This states that the quadratic model was able to explain the data’s diversity on the effect of molasses addition and storage time on crude protein of maize silage by 71.31%.

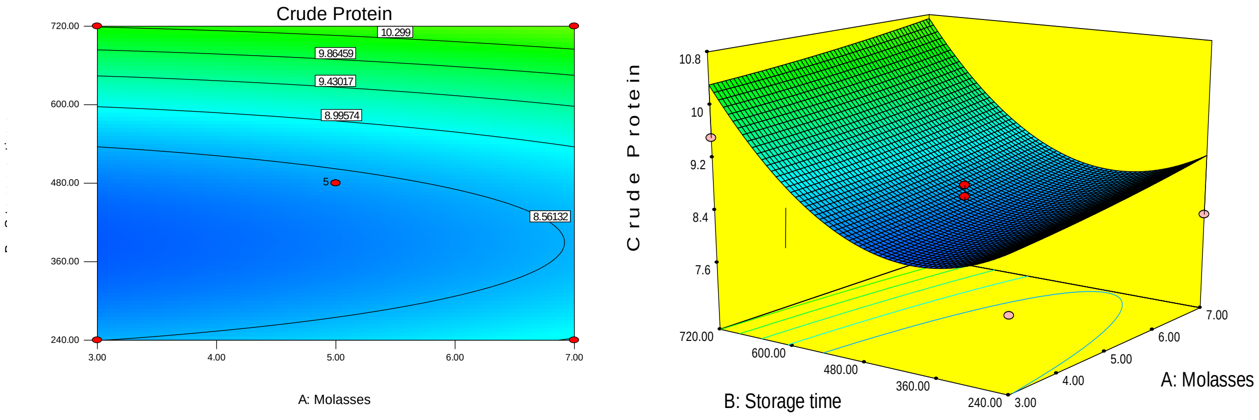
Table 3 shows that addition of molasses at concentration of 3% with storage time of 240 hours produced maize silage with the lowest crude protein content. While, at the addition of 5% molasses and storage time of 819.41 hours, the crude protein content increased to 12.79%. The mathematical equation of crude protein response was:

$$Y = 10.96624 + 9.88961.10^{-3}X_1 – 0.015260X_2 - 2.08333\ .\ 10^{-5}X_1X_2 + 0.011000{X_1}^2 + 1.97743\ .\ 10^{-5}{X_2}^2$$

Figure 3 indicates that both molasses concentration and storage time have a significant effect on crude protein content. This study shows that the duration of storage may likely to affect the nutritional of maize silage as indicated by the changes in the crude protein content. Concentration of crude protein increases with the length of storage (Man and Wiktorsson, 2002). However, when the storage time was increased from 140.59 hour to 480 hours, the crude protein was decreased, and then continued to increase at storage time of 819.41 hours. A decrease in crude protein content during the fermentation may be due to the proteolytic microbial activity, where the proteolytic microbes transformed protein into amino acids and NH_3 (Der Bedrosian et al., 2012).

According to Ranjit and Lung (2000), storage time during the fermentation may increase the *Lactobacillus plantarum*. *L. plantarum* was able to convert complex organic compounds into lactic acid. According to Buckle et al. (1987), production of lactic acid can lower the pH and create an acidic condition in fermentation. Such conditions inhibit both pathogenic and decomposing bacteria (Delgado et al., 2001); including the proteolytic microbes which interrupted the crude protein degradation.

Furthermore, the addition of molasses has impact on increasing the number of LAB, which eventually increasing crude protein concentration in silage making. This is because, LAB utilise high dissolved carbohydrates contained in maize forage as nutrients for growth, thus the growth of LAB can be further increased, particularly during the maturing period (Singh et al., 2009).



(a) (b)

Figure 3. Response surface contour plots (a) and three-dimensional form (b) on the effect of molasses concentration and storage time on crude protein of maize silage.

Crude Fibre

This study also demonstrated that the quadratic model was also better fitted to explain a significant effect on crude fibre response, with the p value of 0.0082 ($p < 0.05$). The model inaccuracy test showed a lower p value of 0.0009, suggesting that the quadratic models still have deficiencies if used as predictions.

Similar to other responses, the lowest PRESS value for crude fibre response was 74.45, confirming that the best model selected was the quadratic model. The R^2 value obtained was 0.8759, demonstrating that the model was able to explain the diversity of data related to molasses addition and storage time of the maize silage crude fibre by 87.59%. The remaining 12.41% was explained by errors and other factors not examined in the present study.

However, as shown in Table 1, the 6th and 13th data of crude fibre concentration were much higher (outlier) compared to the others. As a result, if this model is forced, it is likely to result an error in determining the actual optimal point. This is because the outlier data can influence the optimal point's determination, which contradict to the RSM objectives; therefore a surface model of a strong response to outliers is needed. Treating the outlier data can be carried out by deleting them with consideration that the data obtained have represented by most of the data available. Thus, such deletion has no impacts on the information generated. Furthermore, it is also possible that the outlier data was caused by errors in the data collection process or the data generated was not the actual data. Therefore, removing the outlier data is expected to eliminate the cause of the violation of the normality assumption.

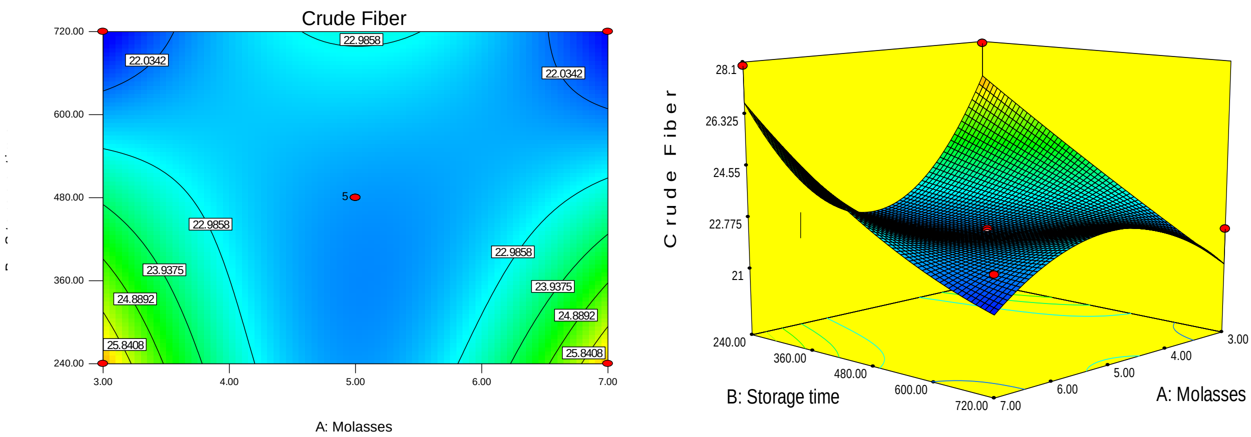
This study shows that the lowest crude fibre was resulted from the molasses addition of 5% and storage time of 140.59 hours, with the value of 21.25%. While, at the addition of molasses 2.17% and the storage time of 480 hours, the highest crude fibre concentration (23.98%) was obtained. The mathematical equation of the model was as follows:

$$Y = 26.561 - 1.92224X_1 + 2.51826.10^{-3}X_2 + 7.14779.10^{-4}X_1X_2 + 0.14501{X_1}^2 - 5.33788.10^{-6}{X_2}^2$$

Figure 4 shows the relationship between molasses concentration and the length of storage, as well as their interactions to crude fibre response. Storage time did not significantly affect the crude fibre concentration. However, the crude fibre of maize silage decreased with the increase storage time. McDonald (1981) reported that LAB may contribute to reduce the crude fibre and lignin concentration, as during the ensiling process, it was broken down into more accessible carbohydrate such as cellulose. This was possibly due to the rapid enzyme activity produced by LAB to break down fibre is proportional to the growth of LAB.

Optimisation Results

In this study, the optimisation using RSM was carried out using the predetermined limits for the molasses factor at the upper limit value of 7% and for the lower limit of 3%. The storage time has an upper limit of 720 hours and the lower limit of 240 hours. Based on a predetermined range, the optimal values for pH, MC, crude protein, and crude fibre were determined. The determination of upper and lower limits for all responses was obtained from the literature. According to Widodo (2014), a good quality maize silage has pH with upper limit of 4.8 and the lower limit of 3.67; MC upper limit of 56.76% and the lower limit of 38.81%; as well as the crude protein upper limit of 14.7% and lower limit of 6.52%, respectively. Kushartono and Iriani (2003) added that the best result on crude fibre of maize silage was with the upper limit of 23.30% and lower limit of 21%. The limits for the optimal solutions are presented in Table 2 and the optimal solution of the RSM calculation is presented in Table 3.



(a) (b)

Figure 4. Response Surface contour plots (a) and three-dimensional form (b) on the effect of molasses concentration and storage time on crude fibre of maize silage.

Table 2. The optimal limits for research response

Criteria	Parameters	Aim	Research Standard			
			Upper Limit		Lower Limit	
Factor	Molasses (%)	In Range	3	3	7	7
Factor	Storage Time (hour)	In Range	240	240	720	720
Response	pH	In Range	3.67**	3.11	4.8**	4.36
Response	MC (%)	Minimise	38.81*	29.12	56.76*	75.26
Response	Crude Protein (%)	Maximise	6.52*	7.68	14.7*	12.79
Response	Crude Fibrer (%)	Minimise	21**	21.25	23.3**	23.98

Source: *Widodo (2014), **Kushartono and Iriani (2003)

Table 3. Solution from computation results

Parameters	Prediction Standard
Molasses (%)	6.97
Storage Time (hour)	240
pH	3.88
MC (%)	23.80
Crude Protein (%)	9.01
Crude Fiber (%)	21.67

Table 3 shows that the optimum level of molasses was 6.97% and the storage time was 240 hours, with desirability value of 0.601. The treatment was predicted to produce maize silage with the pH value of 3.88, MC of 23.80%, crude protein of 9.01%, and crude fibre of 21.67%. According to Myers and Montgomery (2002) and Lee et al. (2018), the desirability function approach is a method widely used to determine multi-response optimisation by indicating the desirability or ideal response values. This value was based on how closely the upper and lower limits are set up to the actual optimum values. Higher value for the optimisation determination was indicated when the value was close to 1.0.

As seen in Table 4, the optimal solution prediction results from all maize silage responses were in accordance with the existing standard quality. Therefore, the optimal result solution verified was using the computational results of the data based on the standard with the specified limits. The optimal point values can be seen as the value of deviations that may occur.

Table 4. Prediction results for optimal molasses concentration and storage time

Parameters	Prediction	SE Pred.	95% PI low	95% PI High
pH	3.88	0.24	2.97	4.81
MC (%)	23.80	10.01	14.61	62.22
Crude Protein (%)	9.01	0.74	6.18	11.85
Crude Fiber (%)	21.70	0.47	20.20	23.20

Comparison of maize silage responses between the optimal and control treatment

Table 5 shows the response parameters (i.e. pH, MC, crude protein, and crude fibre) of maize silage from the optimal and control treatment. In this study, the control treatment used was the parameters values of maize silage samples produced by Balai *Besar Inseminasi Buatan* (BBIB) in Singosari, Malang, East Java, Indonesia. For the optimal treatment, the maize silage sample was resulted from the treatment with the molasses concentration of 6.97% and storage time of 240 hours.

The pH value of the optimal treatment (3.88) was lower than that of the control treatment (4.20), indicating that both treatments has different pH value. According to Santoso et al. (2009; 2012), the addition of LAB can accelerate the rate of fermentation and the production of organic acids (i.e. lactic acid), thus improving the quality of silage. The result of aerobic reaction that occurs at

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the beginning of ensiling, silage produces volatile fatty acids which dropped the pH values.

MC from the control treatment of 67.23% was significantly different from the optimal treatment of 23.80%. Widodo (2014) explained that during ensiling process, an increase in the MC of the silage was due to conversion of DM and OM into CO_2, H_2O and heat, especially during the respiration stage.

The study showed that storage time of 688.89 hours with the addition of molasses as a mixed inoculum was capable to enhance the quality of maize silage, in which giving the optimal crude protein concentration of 9.01%. While the crude protein from the control treatment was slightly lower at concentration of 8.9%. Der Bedrosian et al. (2012), explained that during the ensiling process LAB in forages would use the containing organic matter forage as energy source and produce organic acids including lactic acid. The protein was degraded by the proteolytic microbes to amino acids and NH_3

The optimum treatment was carried out at a storage time of 688.89 hours and the addition of molasses as a mixed inoculum. This resulted the lowest crude fibre percentage of 21.70%. However, the crude fibre from the control treatment was higher, giving the value of 36.64%. Based on Widodo's research (2014), the use of LAB in silage making process can bind cellulose in forages containing crude fibre and reduce lignin bonds, thus increasing the forage digestibility.

Table 5. Value of silage response on optimal treatment and control treatment

Parameters	Optimum Treatment	Control Treatment *
pH	3.88	4.20
MC (%)	23.80	67.23
Crude Protein (%)	9.01	8.90
Crude Fiber (%)	21.70	36.64

Source: * *Balai Besar Inseminasi Buatan (BBIB) Singosari* (2015)

Conclusions

The findings confirmed that the molasses concentration of 6.97% with a storage time of 240 hours can optimise the quality of maize silage, with the pH value of 3.88, MC of 23.80%, crude protein of 9.01%, and crude fibre of 21.67%, respectively. The addition of molasses additives was found to enhance the maize silage quality. Maize crop waste can be valorised as cattle feed by transforming it into silage products.

Conflict of interest

The authors declare that there is no conflict of interest in this publication.

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