Effect of Different Additives and Varying Fermentation Days on pH And Lactic Acid of Wheat Straw Silage

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Abstract

This research was conducted to evaluate the effects of different additives and varying fermentation days on pH and lactic acid of wheat straw silage. The experiment was laid out in a factorial arrangement in a Randomized Complete Block Design (RCBD) with five treatments and 3 replications in each treatment at varying fermentation days (0, 6, 12, 18, 24 and 30). Urea at 2.5% (UWS), poultry litter at 25% (PLWS) and watermelon peels at 25% (PPWS) as additives were ensiled with wheat straw at varying fermentation days while wheat straw (SWS) was ensiled without additives as control, all silages were made in an open mouthed Kilner® jars. Silages were opened following the fermentation 0, 6, 12, 18, 24 and 30 days for sample collection to determine pH and lactic acid. The results show significant differences ($P<0.05$) on pH (7.36 to 4.37) and lactic acid (0.11 to 3.07) across varying fermentation (0 to 30) days and using different additives. The most stable pH was observed in PPWS at 30 fermentation day which produce the highest quantity of lactic acid and stable pH. While highest pH was observed in UWS at 0 FD which produces significantly lower quantity of lactic acid. The interaction between silage additives and the fermentation days also shows that within a specific additive, the increase in fermentation day increases lactic acid production and decreases pH value. It was concluded that ensiling wheat straw with pineapple peels for 30 fermentation days produce a palatable silage with stable pH.

Keywords: Additives; Lactic acid; Silage pH; Fermentation and Wheat straw

Effect De Differents Additifs Et Variation Des Jours De Fermentation Sur Le Ph Et L'acide Lactique Du Silage De Paille De Blé

Résumé

Cette recherche a été menée pour évaluer les effets de différents additifs et de la variation des jours de fermentation sur le pH et l'acide lactique du silage de paille de blé. L'expérience a été réalisée selon un arrangement factoriel dans un plan d'expérience en blocs complets randomisés (PBCR) avec cinq traitements et trois répétitions pour chaque traitement à différents jours de fermentation (0, 6, 12, 18, 24 et 30). L'urée à 2.5% (UWS), le fumier de volaille à 25% (PLWS) et les pelures de pastèque à 25% (PPWS) ont été ensilés avec de la paille de blé à des jours de fermentation variés, tandis que la paille de blé (SWS) a été ensilée sans additifs comme contrôle, tous les sillages ayant été réalisés dans des bocaux Kilner® à ouverture large. Les sillages ont été ouverts après fermentation aux jours 0, 6, 12, 18, 24 et 30 pour la collecte d'échantillons afin de déterminer le pH et l'acide lactique. Les résultats montrent des différences significatives ($P<0.05$) concernant le pH (de 7.36 à 4.37) et l'acide lactique (de 0.11 à 3.07) selon les jours de fermentation (de 0 à 30) et les différents additifs. Le pH le plus stable a été observé dans le PPWS au 30ème jour de fermentation, produisant la plus grande quantité d'acide lactique et un pH stable. En revanche, le pH le plus élevé a été observé dans l'UWS au jour 0 de fermentation, produisant une quantité d'acide lactique significativement plus faible. L'interaction entre les additifs de sillage et les jours de fermentation montre également que, pour un additif spécifique, l'augmentation des jours de fermentation accroît la production d'acide lactique et diminue la valeur du pH. Il a été conclu que l'ensilage de paille de blé avec des pelures d'ananas pendant 30 jours de fermentation produit un sillage appétissant avec un pH stable.

Mots-clés : Additifs ; Acide lactique ; pH du sillage ; Fermentation et paille de blé

Introduction

During ensiling, lactic acid produced by lactic acid bacteria (LAB) is usually the highest acid concentration found in silages (Sun *et al.,* 2022). Lactic acid contributes the most to the decline in pH during fermentation because it is about 10-12 times stronger than any other acid found in silages (Cheng *et al.,* 2021). Typical concentration of lactic acid in commonly fed silages ranges from 2-4% of the DM and at this range; the pH value of the ensiled material should be within the stable pH value 3.5-4.5 in most silage and higher in other legumes silage. Several factors can be responsible for silages that present a pH higher than normal. For example, an abnormally high buffering capacity (e.g., in legume silages with very high protein and ash contents) or a restricted fermentation (e.g., cold climatic conditions) may be the cause of a higher than expected pH. Various silage additives can change the pH value. Higher silage pH was reported in a urea and poultry litter silage used independently (Oladosu *et al.,* 2016 and Bo *et al.,* 2015) but fruit peels were said to regulate silage pH within the recommended range (Dahiru *et al.,* 2022). Silages inoculated with *Lactobacillus buchneri* often will be 0.1 to 0.2 pH units higher than untreated silage (Kleinschmit and Kung, 2006) because of the moderate conversion of lactic acid to acetic acid, 1,2-propanediol (1,2PD), and ethanol (Oude Elferink *et al.,* 1999). Aerobic spoilage initiated by lactate-assimilating yeasts can also be responsible for higher than normal. Two samples may have the same pH, but different concentrations of acids. In general, legume silage has a higher pH than corn, rice or other grass silages and takes longer to ensile because of their buffering capacity. Silages that are over mature or drought stricken have lower pH including crop residues.

Crop residues most abundant in the tropics like wheat straws contain higher DM and poor feeding value (Bhandari 2019). A scientific procedure such as ensiling was used to improve the feeding values of wheat straw. Production of silage from wheat straw is vital technique in animal feeding for conserving and improving the palatability and quality of wheat straw for ruminant feeding. Ensiling with additives improve the fermentation quality of the ensiled material (Morais *et al.,* 2017). Various additives were identified, but Kang *et al.* (2018) recommended urea for boosting low-quality crop residue though higher pH was recorded. Similarly, Araki *et al.* (2017) reported that urea restricts the growth of undesirable microorganisms such as yeasts. Other silage additives like poultry litter was reported by Bo *et al.* (2015) to moderate pH and produce significant quantity of lactic acid. Similarly, fruit peels such as pineapple peels and watermelon peels were reported by Upadhyay (2010) and Feumba *et al.* (2016), respectively to stabilize pH and produce resultant silage with moderate lactic acid and higher quality.

In this study, urea, poultry litter, watermelon and pineapple peels were used as silage additives to evaluate the pH and lactic acid levels in wheat straw silage ensiled at different fermentation days.

Materials and Methods

Study area

The study was conducted in the Department Animal Science, Faculty of Agriculture, Federal University Dutse, Jigawa State. The university is located at latitude 11.69174° N and longitude:

9.34525º E, with an average temperature ranging between 20℃ and 39.76℃, with the longest dry season last for about 7 month and shortest rainy season for about 4 months which makes animal feed more scares within the year (NIMET, 2022).

Collection and preparation of experimental materials

Wheat straw was obtained from a farm in Kiyawa Local Government Area, Jigawa State after mechanical threshing of wheat grains. This wheat straw was screened for other impurities and foreign particles and then transported to the study area. The screened wheat straw was weighed and mixed with silage additives adequately in the recommended quantities The additives was added as follows; urea was used as 2.5% and 97.5% of wheat straw as reported by (Morais *et al.*, 2017), 25% each of poultry litter, watermelon peels and pineapple peel was used plus 75% of wheat straw ensiled

Experimental design and treatments combinations

The experiment was laid in a 5x 6 factorial consisting of five different (5) treatments with 3 replications each arrangement in a Randomized Completely Block Design (RCBD) as shown in Table 1.

Table 1: Treatments combinations

Treatments	Combinations
T1 Control (SWS)	Sole wheat straw
T2 UWS	Wheat straw + urea
T3 PLWS	Wheat straw + poultry litters
T4 WPWS	Wheat straw + watermelon peels
T5 PPWS	Wheat straw + pineapple peels

Ensiling procedure

The treatments combinations were ensiled in open mouthed Kilner® jars (Cope BS 910-8, 1000 mL). However, treatments were varied in 0, 6, 12, 18, 24 and 30 fermentation days in triplicates, a total of 90 bottles were ensiled (5X3X6). The mouth was sealed tightly to prevent air from entering into the jar and was stored at the temperature of 28-30 ℃ in the laboratory.

Analytical methods

Samples were collected according to the days of fermentation for each treatment (day 0, 6, 12, 18, 24 and 30). The jar was opened and the upper layer of the material was scrubbed off and samples were taken from middle of the jar to prevent possible contamination

pH determination

pH was measured according to the recommended procedure described by Bernardes *et al.* (2019). 25 g of sample from each replication was soaked in 225 ml distilled water and blended for 30 seconds, then pH was determined with a portable electrode pH meter (Model: PHS-25).

Lactic acid determination

Lactic acid concentration was determined in silage extract by high performance liquid chromatography (HPLC) as described by Kostulak-Zielińska and Potkański (2001) and Gąsior (2002). The fresh silage samples were homogenized in a manual blender in an ice bath (for 2 min), pouring in five times more water than the weight of the given sample. The homogenate was filtered by straining through miller gauze; the filtrate was then passed through a soft filter (Filtrak No. 388), deproteinized with 24% (w/v) metaphosphoric acid (FLUKA) and centrifuged (7min., 10000x g at 4°C) in an MPW-350R centrifuge. The supernatant was filtered and analyzed by HPLC system, RP, column: METACARB 67H (Organic Acids Column, Varian), mobile phase: 0.002 M (v/v) sulphuric acid solution (95%, Sigma-Aldrich) in deionised water, flow rate $1cm^3$/min., loop 20 l, detector SDP-20A UV/Vis – 210 nm). The external-standard method was employed using the FLUKA lactic acid standard. A mixture of standard was prepared as: lactic acid 3 mg/cm^3. The peak areas from the chromatogram map of the sample were compared with the peak areas of the chromatogram maps obtained from the standards.

Data analysis

All data were subjected to analysis of variance (ANOVA) according to standard procedure of the Generalized Linear Model (GLM) procedures of GenStat version 17.1. Means was separated using fishers LSD

The yield equation is shown below;

Yijk = 𝓜 = Ai + Bj + Ck + Dl + ABCDijkl + £ijkl

Where,

Y = observation on the performance of wheat straw

𝓜 = universal mean

Ai = i^{th} effect of urea in wheat straw silage

Bj = jt^{h} effect of poultry litter on wheat straw silage

Ck= k^{th} effect of pineapple peel on wheat straw silage

Dl = l^{th} effect of watermelon peel on wheat straw silage

.

ABCDijkl = interaction effect of silage additives in wheat straw silage

£ = random and residual error

Yijkl = observations of silage additives on ensiled what straw

Results and Discussion

pH

The interaction effects of silage additives and fermentation days shown in the below table 2 revealed that the type of additive have a significant effect ($p < 0.05$) on the drop in pH values. Various additives were identified, but kang *et al.,* (2018) reported for ensiling low-quality crop residue, urea is recommended in boosting the quality though higher pH was recorded. PLWS decreased significantly up to 5.33 which is within the recommended range of pH values for poultry litter-treated silages (Shahowna *et al.,* 2013). Many researches showed that poultry litter treated silage pH values tend to be higher than the recommended range of pH because of the alkaline nature of the additive. While, WPWS showed a decline in pH (up to 4.81) values which indicated a smooth fermentation and adequate production of lactic acid and volatile fatty acids. Similarly, PPWS (4.37) had the lowest pH values at 30 days which indicated qualitative silage with good aroma and adequate lactic acid production

Table 2 Effect of silage additives and fermentation days on pH of the resultant silage

Treatments	Fermentation days						P-value
	0	6	12	18	24	30	
SWS	7.36[a]±0.14	6.21[e]±0.14	7.06[abc]±0.14	5.27[fg]±0.14	6.10[e]±0.14	6.01[e]±0.14	<0.001
UWS	7.20[ab]±0.14	6.88[bcd]±0.14	6.82[bcd]±0.14	6.76[cd]±0.14	6.71[cd]±0.14	6.47[de]±0.14	<0.001
PLWS	6.93[bc]±0.14	6.05[e]±0.14	6.05[e]±0.14	5.41[f]±0.14	5.08[fgh]±0.14	5.33[fg]±0.14	<0.001

WPWS	6.72^{cd}±0.14	6.27^{e}±0.14	6.24^{e}±0.14	5.19^{fgh}±0.14	5.05^{fgh}±0.14	4.81^{h}±0.14	<0.001
PPWS	6.03^{e}±0.14	5.33^{fg}±0.14	5.02^{fgh}±0.14	4.89^{gh}±0.14	4.43^{i}±0.14	4.37^{i}±0.14	<0.001

SWS = sole wheat straw, UWS = urea + wheat straw, PLWS = poultry litter + wheat straw, WPWS = watermelon peels + wheat straw and PPWS = pineapple peel + wheat straw.

Table 3 Effect of silage additives and fermentation days on lactic acid (%) of the resultant silage

Treatments	Fermentation days						P-value
	0	6	12	18	24	30	
SWS	0.11^{q}±0.07	2.10^{ghijk}±0.07	2.41^{de}±0.07	2.54^{cd}±0.07	2.58^{c}±0.07	3.07^{a}±0.07	<0.001
UWS	0.49^{p}±0.07	1.31^{n}±0.07	1.87^{lm}±0.07	2.18^{fghi}±0.07	2.22^{efgh}±0.07	2.10^{ghijk}±0.07	<0.001
PLWS	0.51^{p}±0.07	1.79^{m}±0.07	1.95^{jklm}±0.07	1.96^{jklm}±0.07	2.05^{hijkl}±0.07	2.17^{fghij}±0.07	<0.001
WPWS	0.07^{q}±0.07	1.08^{o}±0.07	1.85^{lm}±0.07	1.97^{ijklm}±0.07	2.03^{hijkl}±0.07	2.31^{efg}±0.07	<0.001
PPWS	0.11^{q}±0.07	2.29^{efg}±0.07	2.36^{ef}±0.07	2.69^{c}±0.07	2.91^{b}±0.07	2.88^{b}±0.07	<0.001

SWS = sole wheat straw, UWS = urea + wheat straw, PLWS = poultry litter + wheat straw, WPWS = watermelon peels + wheat straw and PPWS = pineapple peel + wheat straw.

Lactic acid

Lactic acid is the principal acid produced in silage, it is stronger than the remaining acids (acetic acid, propionic acid and butyric acid) produced and it is usually responsible for the drop in silage pH (kung *et al.*, 2018). Table 4 showed the levels of acids produced in the resultant silage. All additives and fermentation days showed significant difference (P<0.05) in the values of lactic acid. The highest proportion of lactic acid was obtained in PPWS (2.88%) in PPWS at 30 FD and (2.91 %) in PPWS at 24 FD which are within the recommended range of lactic acid in silage which is 1.8-3.2 for silages with < 30% DM except silages treated with homolactic acid bacteria (Kung *et al*, 2018). The values of acids decreased with increase in The increase in lactic acid in PPWS and at 30 FD also yields the lowest silage pH which indicates a better-quality silage.

pH

The pH of silage is the measure of the acidity and alkalinity of that silage and the determinant of more stable fermenting silage. The pH of the resultant silages varied significantly (P <0.05) with change in additives and increase in fermentation days. Silage additives affected in pH of the resultant silage as shown in table 4 below. Lowest pH is obtained in PPWS (5.01) and WPWS (5.71) though Filya, (2010) suggested pH of every ensiled material should be less than 5, but Gümüş, (2021) reported higher pH of around 6-6.5 when a sucrose additive is use to ensile alfalfa. Also, Zhang *et al*, 2022 also reported higher pH in mulberry silage with acetic acid as an additive. Higher silage pH obtained in UWS (6.81) is because of the nature of the additive used (urea). Urea is known to increase pH value because of the presence of ammonia (Adeosun and Jinadu 2021). Also, in PLWS (5.81), poultry litter tends to rise pH above the normal range 3.5-4.5 Nieman *et al*, 2023). The increase in fermentation shows decrease in pH because silage fermentation is a process that begins with the removal of oxygen from the silo followed by anaerobic fermentation which enhance lactic acid production and lactic acid lowers pH (kung *et al*, 2018) that is basically why the pH value drops drastically from 6.85 in 0 FD to 5.39 in 30 FD.

Conclusion

The results obtained from this study indicated that the increase in fermentation days within a specific additive increased the production of lactic acid and decrease pH level.

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