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Biogenic Amine Synthesis in Wheat Straw Silage with Different Additives and Varying Fermentation Days

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Target Audience; Animal production research institutes, Universities, and colleges of Agriculture, agricultural extension agents and livestock farmers.

Abstract

The concentration of biogenic amines in wheat straw silage ensiled with urea (UWS), poultry litter (PLWS), watermelon peels (WPWS) and pineapple peels (PPWS) for 0, 6, 12, 18, 24, and 30 fermentation days was determined In Federal University Dutse, Jigawa state. The experiment was conducted using a factorial randomized complete block design. Wheat straw was treated with the additives an ensiled in kilner-jar. A control group sole wheat straw was also ensiled, making it 5 treatments and each treatment in triplicate. Samples were collected from each replicate and fermentation day for the analysis of biogenic amines (Histamine, Putrescine, and tyramine). The results of obtained from this study shows that the synthesis of biogenic amines increases with increase in fermentation days (P<0.05). Higher significant values were obtained in WPWS (10.23) and UWS (9.74) for histamine, while putrescine was higher in UWS (98.32) and PLWS (85.63), and tyramine was higher in WPWS (125.95) and PLWS (122.92). Conclusively, biogenic amine synthesis has a direct relationship with increase in the days of fermentation of the resultant silage.

Keywords: Fermentation days, urea, poultry litter, watermelon peels and pineapple peels.

Description of problem

In silages, Biogenic amines occur naturally because of the presence of anaerobic fermentation , thus converting various amino acids into respective amines with the help of either plant enzymes or microbial enzymes .

Biogenic amines comprise a group of aliphatic, heterocyclic or aromatic bases derived from amino acids. Biogenic amines are present in all feeds that contain proteins or free amino acids, and they also exist in fermentative feeds . The concentration of biogenic amines (mono-, di- and polyamines) in silage and in the rumen, body tissues and body fluids mainly depend upon the crop at harvest, the ensiling process, the

silage and the digestion in the animal.

Various species of lactic acid bacteria (LAB) (*Lactobacillus, Pediococcus* and *Streptococcus*) and species of the genera *Clostridia, Bacillus, Klebsiella, Escherichia, Pseudomonas, Citrobacter, Proteus, Salmonella, Shigella* and *Photobacterium* are involve in the fermentation process (4), (5), (6), (7), (8). Since BAs occur in fermentation process, the levels at which they occur and their quantity strongly depend on the stage and days of the fermentation. When consumed by the animal, BAs take part in the cellular metabolism of microorganisms (9), but poor organoleptic characteristics increase the content of BAs (10) which causes acute and long-term toxicity depending on the range (11).

Crop residues are fibrous by-products that resulted from crop cultivation . These residues have been a major source of feed for animals for a very long time and will continue to be so for the predictable future . Even though most of the crop residues are poor in nutritional value to meet the requirement of ruminants . Crop residues like wheat straw are among the largest potential feed resources now in Nigeria, but its use and development have not received proper recognition due to their bulkiness, poor nutrient density and high transport costs .

Wheat straw is one of the most abundant crops residues in Nigeria as reported by the Flour Millers Association of Nigeria (FMAN) and Wheat Farmers Association of Nigeria (WFAN), wheat production has increased by 42% from 2021 meaning that wheat production is expected to rise from 110,000 metric tons (MT) in 2022-2023 to 156,000 MT in the 2023-24 market year, according to the USDA (15). The increase is due to the regular production of wheat across the country, for every ton of wheat produced more tons of wheat straw are generated which were usually used as animal bedding, and sometimes it is treated as waste or burned, releasing CO_2 into the atmosphere . The sad part is that these stalks still have value. With adequate processing techniques like silage it will be an absolute replacement for other expensive animal feeds.

Silage fermentation at the lag phase is where the depletion of oxygen and production of BAs occur by the decrease in pH and conversion of soluble carbohydrates to lactic acid (17). Ensiling a crop residue like wheat straw with sugar additive yields lower BAs synthesis because the rate of fermentation reduced due to higher DM content. Silage with additives improves the stability and acid productivity of the resultant silage (18). Additives such as watermelon peels and pineapple peels have proven to lower pH level in silage (19) Which improve BAs synthesis, while urea and poultry litter increase the CP content by which BAs are synthesized from. Silage additives should be cheap, and has the potential to increase nutritive value of the product and limit deterioration. . In this study, a sugar source (watermelon peels and pineapple peels) and non-protein nitrogen source (urea and poultry litters) was used as silage additive to improve silage quality. The objective of this study is to determine the effects of urea, poultry litter, watermelon peels and pineapple peels on the synthesis of biogenic amines in wheat straw silage ensiled in

various fermentation days.

Materials and Methods

Study area

The research was conducted at the laboratory, Department of Animal Science, Faculty of Agriculture, Federal University Dutse, Jigawa State (11.69174 N and 9.34525 E), with an average temperature ranging between 20 and 39.76 . The dry season lasts for almost 7 and half months from October to May while the rainy season last for about 4 and half months from May to October with august as peak of the raining season Some of the most common plant species grown in the area include maize, millet, rice, sesame, and cowpea and recently wheat (21).

Collection and preparation of experimental materials

Wheat in the study area is usually planted during the winter season, basically wheat is cultivated by the means of irrigation at the study area. Wheat Straw was obtained from a farm in Kiyawa Local Government Area, Jigawa State after mechanical threshing of wheat grains at harvest. It was screened for other impurities and foreign particles to prevent contamination; and was transported to the laboratory. The additives were added as follows; urea was used as 2.5% of the wheat straw as reported by . 25% of poultry litter (22) was used plus 75% of wheat straw ensiled, watermelon peels were used at rate of 25% and Pineapple peel at the rate of 25% (23).

Experimental design

The experiment was laid in a 2-factorial Randomized Completely Block Design (RCBD) (2: 5 different silage additive treatments and x6 different fermentation days) consisting of five different (5) treatments with 3 replications each, as shown in Table 1 below;

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

Wheat straw including additives was thoroughly mixed, homogenized and ensiled in an open mouthed Kilner jars (Cope BS 910-8, 1000 ml). Treatments were varied in 0, 6, 12, 18, 24 and 30 fermentation days in triplicates, a total of 90 jars were ensiled. The mouth was sealed tightly to prevent air from entering into the jar and was stored in the laboratory.

Analytical methods

Samples was 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

Table 2: Experimental Layout

	Fermentation Days					
Treatment	**0**	**6**	**12**	**18**	**24**	**30**
T1 (SWS) 100%	SWS	SWS	SWS	SWS	SWS	SWS
wheat straw	SWS	SWS	SWS	SWS	SWS	SWS
	SWS	SWS	SWS	SWS	SWS	SWS
T2 (UWS) 2.5%	UWS	UWS	UWS	UWS	UWS	UWS
U	UWS	UWS	UWS	UWS	UWS	UWS
	UWS	UWS	UWS	UWS	UWS	UWS
T3 (PLWS) 25%	PLWS	PLWS	PLWS	PLWS	PLWS	PLWS
PL	PLWS	PLWS	PLWS	PLWS	PLWS	PLWS
	PLWS	PLWS	PLWS	PLWS	PLWS	PLWS
T4 (WPWS) 25%	WPWS	WPWS	WPWS	WPWS	WPWS	WPWS
WP	WPWS	WPWS	WPWS	WPWS	WPWS	WPWS
	WPWS	WPWS	WPWS	WPWS	WPWS	WPWS
T5 (PPWS) 25%	PPWS	PPWS	PPWS	PPWS	PPWS	PPWS
PP	PPWS	PPWS	PPWS	PPWS	PPWS	PPWS
	PPWS	PPWS	PPWS	PPWS	PPWS	PPWS

SWS = sole wheat straw, PL WS = poultry litter + wheat straw, UWS = urea + wheat straw, WST = watermelon peel, PPWS = pineapple peel + wheat straw

Determination of Bas

Sample Preparation

A portion of sample (50 g) was freeze dried in a vacuum freeze-drying machine for 3 days before determining the biogenic amines (putrescine, histamine and tyramine. Lyophilized powder (2.5 g) was added to 10 mL of 50 g/L trichloroacetic acid and shaken on an orbital shaker for 30 min. The mixture was centrifuged for 10 min at 1800× g. The supernatant was filtered with filter paper. Trichloroacetic acid (50 g/L, 10 mL) was added to the remnant, and the whole mixture was shaken as described above. This mixture was then centrifuged at 1800× g for 10 min, and the supernatant was filtered. Finally, the volume of the filtrate was adjusted to 25 mL with 50 g/L trichloroacetic acid. One milliliter of the extract was placed in a 5-mL volumetric flask.

Then, sodium hydroxide (2 N, 200 µL), saturated sodium bicarbonate (300 µL) and dansyl chloride solution (10 mg/mL, 1 mL) was added to the sample extract. After incubation at 40 for 45 min in the dark, the mixture was treated with 100 µL of 25% ammonium hydroxide to remove the residual dansyl chloride. After 30 min at ambient temperature, the volume of the reaction mixture was adjusted to 5 mL with acetonitrile.

The mixture was centrifuged for 5 min at 5000× g. The supernatant was filtered through a 0.22-µm syringe filter and subjected to HPLC (high performance liquid chromatography). Separation was carried out on a C18 column (Reprosil-Pur Basic, 5 µm, 250 mm × 4.6 mm (Maisch GmbH) with a DAD. Gradient elution was performed with acetonitrile (solvent A) and 0.1 mol/L ammonium acetate (solvent B). The gradient program was run at a flow rate of 0.8 mL/min as follows: 50% A to 50% B in 0.01 min; 90% A to 10% B in 25 min; 90% A to 10% B in 35 min; 50% A to 50% B in 45 min. The column temperature was set at 30 ◦C, wavelength at 254 nm and injection volume of each sample was 20 µL.

Results and Discussion

Biogenic amines in the resultant silage

The interaction effects of silage additives and fermentation days on Histamine, Tyramine and Putrescine is shown above. With the addition of a silage additive, BAs shows increase with increase in FD. This means the synthesize of BAs requires other favorable silage conditions like stable pH, lactic acid and good dry matter. WPWS was observed to synthesis the highest quantity of BAs, this may be because of the organic and fermenting properties of watermelon peels but researches on the production of BAs using watermelon peels or other fruit peels as silage additives are recently not available. The significant increase in the quantity of BAs observed in PLWS and UWS was because the additives used contain a significant amount of NH_3-N which (1) and (3) described as chemical compound that enhance the synthesis of BAs in favorable condition.

Histamine

During the ensiling process, biogenic amines

Table 3. Effect of silage additives and fermentation days on histamine (mg/kg DM) of the resultant silage

Treatments	Fermentation Days						P-value
	0	6	12	18	24	30	
SWS	$4.48^{n}\pm0.11$	$7.63^{j}\pm0.11$	$8.59^{g}\pm0.11$	$8.63^{g}\pm0.11$	$8.56^{g}\pm0.11$	$8.21^{i}\pm0.11$	<0.001
UWS	$4.77^{m}\pm0.11$	$7.88^{i}\pm0.11$	$8.95^{f}\pm0.11$	$9.58^{d}\pm0.11$	$9.74^{c}\pm0.11$	$9.42^{cd}\pm0.11$	<0.001
PLWS	$4.05^{o}\pm0.11$	$7.11^{k}\pm0.11$	$9.21^{e}\pm0.11$	$9.33^{cd}\pm0.11$	$9.33^{cd}\pm0.11$	$9.40^{cd}\pm0.11$	<0.001
WPWS	$4.77^{m}\pm0.11$	$7.58^{j}\pm0.11$	$10.11^{b}\pm0.11$	$10.11^{b}\pm0.11$	$10.19^{a}\pm0.11$	$10.23^{a}\pm0.11$	<0.001
PPWS	$4.02^{o}\pm0.11$	$6.42^{l}\pm0.11$	$8.23^{i}\pm0.11$	$8.34^{h}\pm0.11$	$8.38^{h}\pm0.11$	$8.32^{h}\pm0.11$	<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 4. Effect of silage additives and fermentation days on tyramine (mg/kg DM) of the resultant silage

Treatments	Fermentation days						P-value
	0	6	12	18	24	30	
SWS	$51.42^{t}\pm0.54$	$65.51^{q}\pm0.54$	$75.42^{jk}\pm0.54$	$75.52^{jk}\pm0.54$	$74.64^{k}\pm0.54$	$71.84^{mn}\pm0.54$	<0.001
UWS	$56.39^{s}\pm0.54$	$68.81^{o}\pm0.54$	$76.37^{ij}\pm0.54$	$91.01^{c}\pm0.54$	$95.84^{b}\pm0.54$	$98.32^{a}\pm0.54$	<0.001
PLWS	$55.41^{s}\pm0.54$	$77.16^{i}\pm0.54$	$80.95^{h}\pm0.54$	$82.82^{g}\pm0.54$	$85.63^{e}\pm0.54$	$85.07^{ef}\pm0.54$	<0.001
WPWS	$51.75^{t}\pm0.54$	$66.75^{p}\pm0.54$	$71.28^{n}\pm0.54$	$84.13^{f}\pm0.54$	$84.25^{f}\pm0.54$	$87.00^{d}\pm0.54$	<0.001
PPWS	$50.06^{u}\pm0.54$	$63.73^{r}\pm0.54$	$72.09^{lmn}\pm0.54$	$73.26^{lm}\pm0.54$	$73.40^{l}\pm0.54$	$72.80^{lm}\pm0.54$	<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 5. Effect of silage additives and fermentation days on putrescine (mg/kg DM) of the resultant silage

Treatments	Fermentation days						P-value
	0	6	12	18	24	30	
SWS	54.68^{s}±1.04	76.88^{q}±1.04	98.59^{m}±1.04	109.40^{ij}±1.04	108.12^{jk}±1.04	104.07^{l}±1.04	<0.001
UWS	52.22^{r}±1.04	83.68^{p}±1.04	100.16^{m}±1.04	105.62^{kl}±1.04	110.87^{i}±1.04	115.13^{1}±1.04	<0.001
PLWS	60.95^{r}±1.04	95.13^{n}±1.04	117.21^{efg}±1.04	119.79^{cdef}±1.04	119.79^{cd}±1.04	122.92^{b}±1.04	<0.001
WPWS	61.93^{r}±1.04	95.23^{n}±1.04	118.81^{efg}±1.04	122.00^{bcd}±1.04	122.26^{bc}±1.04	125.95^{a}±1.04	<0.001
PPWS	61.10^{r}±1.04	86.80^{o}±1.04	104.43^{l}±1.04	106.12^{kl}±1.04	106.33^{kl}±1.04	105.45^{kl}±1.04	<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.

are formed through proteolysis; a decarboxylation process of amino acids by amination or transamination of aldehydes and ketones (2), (24), (25). The effect of additives and fermentation days on BAs shown in table 2 above shows significant difference (P<0.05). Histamine production is very slow with addition of treatments. Highest values were obtained in UWS (9.42), PLWS (9.40) and WPWS (8.83) which were in line with the findings of (1) and (26) for silages of mixed poacea family with chemical and biological additive respectively. Histamine along the fermentation days was increasing significantly (P<0.05) with increase in the days of the fermentation. This was explained as every BAs will increase as FD increases until a certain level was attained depending on the crop maturity and nature, by (3) when Lucerne was ensiled for 90 FD.

Tyramine

Tyramine during fermentation process is synthesize from the amino acid tyrosine. Tyramine synthesis in the resultant silage shows higher values in ammonia presence additives UWS and PLWS than the fruit peels additive WPWS, PPWS and the control SWS this is possibly because of the fermentation ability of non-protein nitrogen (NPN) source additives. Also, fermentation days affect the synthesis of tyramine significantly (P<0.05). The increase in FD causes significant increase in tyramine. The highest value of tyramine was obtained in 30 FD.

Putrescine

Putrescine is synthesized from ornithine. The addition of silage additive has a direct effect on putrescine synthesis in the resultant silage, compared to the control (SWS), highest values were observed in WPWS (125.95), PLWS (122.92), UWS (115.13) and PPWS (105.45). In a silage with an inoculant, putrescine tends to lower in production (27) basically because it requires an organic catalyst to enhance it synthesis. Increase in FD provide better condition for putrescine synthesis. The highest values were obtained at 30 FD similar findings were reported by (28) when oat was ensiled for 60 FD and (1) on ensiled grass for 90 FD.

Table 6. Effect of silage additives and fermentation days on Biogenic Amines

Treatments	Parameters		
Additives (A)	**Histamine (mg/kg)**	**Tyramine (mg/kg)**	**Putrescine (mg/kg)**
SWS	7.68^{d}±0.04	69.06^{d}±0.22	91.96^{d}±0.4
UWS	8.39^{b}±0.04	81.12^{a}±0.22	95.78^{c}±0.4
PLWS	8.07^{c}±0.04	77.84^{b}±0.22	105.97^{b}±0.4
WPWS	8.83^{a}±0.04	74.19^{c}±0.22	107.69^{a}±0.4
PPWS	7.28^{e}±0.04	67.56^{e}±0.22	95.04^{c}±0.4
P-value	**<0.001**	**<0.001**	**<0.001**
Fermentation days (FD)			
0	4.42^{e}±0.05	53.01^{e}±0.24	59.58^{e}±0.4
6	7.33^{d}±0.05	68.39^{d}±0.24	87.55^{d}±0.4
12	9.02^{c}±0.05	75.22^{c}±0.24	107.84^{c}±0.4
18	9.20^{ab}±0.05	81.35^{b}±0.24	112.59^{b}±0.4
24	9.24^{a}±0.05	82.75^{a}±0.24	113.47^{b}±0.4
30	9.11^{b}±0.05	83.01^{a}±0.24	114.70^{a}±0.4
P-value	**<0.001**	**<0.001**	**<0.001**
Interaction	*	*	*

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

Conclusion

1. In all the treatments and across the fermentation days the concentration of BAs is within the safe zone for ruminant production.
2. However, the concentration increases with increase in fermentation days but higher concentration was obtained in WPWS for tyramine and putrescine and in UWS for histamine.

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