# Innovative Use of Endogenous Enzymes to Enhance Silage Chemical Quality of Corn Straw for Buffalo (*Bubalusbubalis*) Feed in Samosir Island

Nurzainah Ginting<sup>1,2,\*</sup>, Raden Edhy Mirwandhono<sup>1</sup>, Muhammad Adanan Purba<sup>1</sup>, Muhammad Amran Nasution<sup>1</sup>, Winda Fransisca Saragih<sup>1</sup>, Nurjama'yah br. Ketaren<sup>1</sup> and Anjas Asmara Samsudin<sup>3</sup>

Abstract: On Samosir Island, there is an annual shortage of buffalo feed during the dry season. However, this feed shortage has caused farmers to scramble to find substitute feed. Still, it has also caused stress in the buffalo, which has led to the emergence of Haemorrhagic septicemia (HS), as Samosir is an endemic area (HS). Beginning in 2024, corn cultivation began to be widely practiced on the island of Samosir, which generates a significant amount of waste such as corn straw. This research focuses on preparing feed for buffalo (Bubalus bubalis) using fermented corn straw, with the fermentation process utilizing endogenous enzymes derived from rumen fermentation (EERF). It was obtained from fermented 100- day buffalo rumen, where the buffalo rumen comes from buffalo that consume corn straw. This study used a completely randomized design, a 3×3factorial with three replications. Factor I was various doses of EERF(2%, 4%,6%),and Factor II was different fermentation times(5d,10d, 15d). Parameters that were observed in this study were chemical quality: Dry Matter (DM), Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), Crude Protein (CP), and pH. Previously, isolation on EERF was carried out to identify the dominant fiber-degrading colonies, as they produce enzymes. Analysis of the potential of corn straw for buffalo feed was conducted by calculating corn straw production from the corn harvest area on Samosir Island. The result of this study is that corn straw fermentation using endogenous enzymes improves the chemical quality of silage, such as DM increasing from 38.59 to 46.17, NDF from 46.23 to 40.48, ADF from 30.53 to 24.21, CP from 9.25 to 9.86, and pH from 5.15 to 4.76. Through this improvement in nutritional quality, the dietary needs of buffaloes are met. This corn straw is sufficient for 9,565,101 buffaloes. Since corn cultivation has become intensive, there is a tendency of HS cases to decrease, with only 6 instances of HS in 2024 and 13 cases up to June 2025, while in 2023 there were 202 cases.

**Keywords:** ADF, Dry season, enzymes, fermented corn straw, NDF, rumen.

### INTRODUCTION

Buffalo farming in Samosir is carried out in an extensive-traditional way, i.e. during the day they are grazed on empty land, without rotation. There is no special drinking water and at night they are kept in pens or not in pens, also without feed and drinking water. Buffaloes serve multiple purposes, including as workers, milk and meat producers, savings that can be cashed when needed, and animals for traditional ceremonies and as a tourism attraction [1]. Buffalo power is used for processing agricultural land, driving water pumps, pressing sugarcane and sugarcane shoots, grinding grain, and pulling carts [2, 3].

Traditional extensive maintenance management has led to overgrazing of pastures, resulting in reduced consumption and low-quality feed. This can be seen in the grazing lands on Samosir Island, where the

condition of the pastures is overgrazing. In general, breeders have been unable to meet maintenance needs greater than 2.5% of body weight per day. Lack of feed, including drinking water, has an impact on decreasing production and reproductive performance of buffalo [4], which in the long run will affect reducing productivity and population. Meanwhile, in Samosir, a lack of feed causes buffalo stress and triggers Haemorrhagic septicemia (HS) disease, as Samosir is an endemic area of HS.

The primary step to improve buffalo feeding management is to increase daily feed consumption to 2.5-2.7% dry matter of female body weight and 2.7-3.5% dry matter of male body weight [5]. The use of grass as a source of fiber and energy in buffaloes can be combined with the use of agricultural plant waste. One of the wastes that has the potential to be used as a source of fiber is corn waste. Since 2024, corn has been intensively cultivated throughout Samosir. During the harvest season, the availability of corn plant waste is high, so it can be used as ruminant animal feed.

<sup>&</sup>lt;sup>1</sup>Animal Production Program Study, Faculty of Agriculture, Universitas Sumatera Utara, Medan, Indonesia

<sup>&</sup>lt;sup>2</sup>Head of Eco Enzyme Team of UI Greenmetric World University Ranking Network, Indonesia

<sup>&</sup>lt;sup>3</sup>Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia, Selangor Dahrul Ehsan, Malaysia

<sup>\*</sup>Address correspondence to this author at the Animal Production Program Study, Faculty of Agriculture, Universitas Sumatera Utara, Medan, Indonesia; E-mail: nurzainah@usu.ac.id

Corn plant waste that can be used as animal feed is part of the leaves and stems (corn straw) or cobs and cob skin.

Corn waste is a by-product of growing corn with a production level of 64.44 tonnes/h/y which includes corn straw, corn cobs, and corn husk. The nutritional content of corn straw contains 6.38% protein, 30.19% crude fiber, 2.81% crude fat, 7.28% ash, and 53.12% TDN [6]. The data shows that the main obstacle to the use of corn as feed is its low nutritional value, especially the high crude fiber content and low protein content. The high oil fiber content causes the low digestibility of corn plant waste. Efforts to overcome the limitations of corn plant waste involve treating it before feeding it to livestock, such as through fermentation to make silage, thereby increasing its nutritional content [7]. It is stated that good silage originates from forage or agricultural waste, which is preserved fresh (with a water content of 60-70%) through a fermentation process with the addition of a fermenter, such as indigenous enzymes.

Various products that are useful as fermenters to assist the silage process have been on the market. For breeders who live inland, such as in the mountains on Samosir Island, these products are difficult to obtain. Therefore, it is necessary to look for alternative fermenters. Making a fermenter involves a 100-day fermentation process using organic matter. [8] We found different enzyme activities when using different organic materials, such as protease, during papaya

fermentation. Hong-ya et al. (2015) found lignocellulose and hemicellulose enzymes in the rumen of cattle that consume corn [9]. The enzymes to be produced should be adjusted to the purpose of the fermentation. Suppose the goal is to make silage of agricultural waste rich in fiber. In that case, it is more effective to use materials that are also high in fiber during the enzyme production process. Therefore, this study aims to produce feed from fermented corn straw using indigenous enzymes. The feed should contain nutrition that fulfills the buffalo's needs.

## **MATERIALS AND METHODS**

The research was carried out *in vitro* in the Animal Production Laboratory, Animal Husbandry Study Program, Universitas Sumatera Utara.

#### **Materials**

The materials needed to make EERF were a 50-liter plastic container, 1 kg of molasses, 3 kg of buffalo rumen from Samosir slaughterhouse, and 10 liters of water. The mixture was then fermented anaerobically for 100 days. These materials were presented in Figure 1. Molasses was an energy source for microbes to grow.

Because this study also identified fiber-degrading bacteria in EERF, selective media were prepared by the Hungate method, using Carboxy Methyl Cellulose/CMC as the substrate, xylan, and tannic acid [10]. Other material were corn straw to make silage.

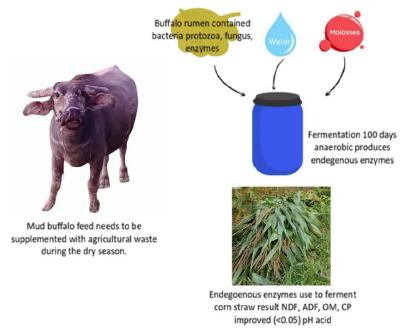


Figure 1: Buffalo rumen fermentation 100 days anaerobic.

### **METHODOLOGY**

Fermentation in making EEFF was carried out in this study for 100 days. After the time, stirring was carried out to homogenize the mixture, followed by separation of the liquid and solid components. The liquid component was collected, then centrifuged at 3000 rpm at a temperature of 4 °C for 10 minutes. This process was expected to yield an extract that was used in this study.

According to Srihardyastutie and Rosmawati (2024), during the fermentation of organic material for 100 days, there was a rapid increase in the population of bacteria, protozoa, and fungi. Over time, enzymes were produced that were compatible with the functions of these microorganisms [11]. Research conducted by [12, 13] found that fermenting fruit and vegetable peel for 100 days produces various enzymes such as protease, amylase, and lipase. This study introduced a novel approach, as the 100-day fermentation process in the rumen makes a population of lignocellulolytic bacteria, which are then followed by enzymes.

According to research by Mohamed and Chaudry (2008), there was a large population of bacteria, namely 1 billion bacteria and 1 million protozoa per 1 mm of rumen fluid [14, 15]. Isolated bacteria from the rumen of buffalo consuming corn stover revealed a bacterial consortium capable of utilizing complex carbohydrate food sources. Researchers [9, 16] successfully fermented stover by using corn lignocellulolytic bacteria, which resulted in the degradation of lignin and lignocellulose. Using complex microbial inoculants could improve the quality of corn straw fermentation [17]. In this study, rumen fluid containing complex microorganisms was found to produce complex enzymes.

A study on silage was conducted using an experiment with a completely randomized design, involving two factors. The first factor was the enzyme concentration, which consisted of 3 treatments: E1: 2%, E2: 4%, and E3: 6%. The second factor was the duration of fermentation, i.e., T1: 5 days, T2: 10 days, T3: 15 days. Three repetitions were carried out, resulting in 36 samples. Parameters that were observed in this study were dry matter, NDF, ADF, crude protein, and pH.

# Silage Study using EERF Fermenters

Corn straw was cut into 3 cm pieces and spread on plastic sheets. The corn straw to be fermented was mixed with other ingredients to make complete feed.

This made it easier for farmers to feed their buffaloes. 70% corn straw was mixed with 12% rice bran, 5% coconut husk, 7% corn flour, 1% minerals, 2% urea, 3% molasses, and the fermenter was mixed evenly. Next, fermenter was added to silage ingredients according to the treatment and stored it accordingly. The formula was divided into one kilogram, then sprayed with the fermenter according to the treatment and mixed to ensure even distribution. The formula was then placed in plastic, tightly sealed to maintain anaerobic conditions, and labeled. It was then stored according to the treatment duration of 5, 10, and 15 days. Fermentation was carried out in tightly sealed plastic bags to ensure anaerobic conditions were maintained.

### Isolation of Bacteria in EERF

This study isolated dominant bacteria from EERF using the Hungate isolation methods, as these methods can detect lignocellulolytic bacteria. The selective media used were Carboxy Methyl Cellulose (CMC) as a substrate for cellulolytic bacteria, xylan as a substrate for xylanolytic bacteria, and tannic acid as a substrate for lignolytic bacteria, and they grew on media inside roll tubes. Observations were made every day, and on the third day, bacterial growth began to appear in the form of white/cream or gray oval spots. Next, the most dominant bacteria were selected and purified by the streak method repeatedly until a single culture was obtained. Next, the morphological characteristics of bacteria were observed,

# Analysis of Dry Matter, NDF, ADF, Cude Protein, pH

This study analyzed dry matter content, NDF, ADF, crude protein, and pH. The methods used in this study were nutritional content testing using proximate analysis and fiber testing using Van Soest analysis. pH was measured using a Hana pH meter.

# Analysis of the Potential of Corn Straw for Buffalo

This analysis was conducted by calculating corn straw production from the corn harvest area on Samosir Island. This was then converted to dry matter. The potential of corn straw for buffalo feed was calculated based on the assumption that buffalo require 3% dry matter.

## **Statistical Analysis**

The data obtained were tabulated and analyzed using analysis of variance (ANOVA). Furthermore, if there were differences, Duncan's multiple range test was used to determine significant differences between treatment groups (p < 0.05), using Statistical Package version 29.0 (IBM Corp., NY, USA) [18]. In this study, the interaction between the first factor, enzyme concentration, and the second factor, fermentation time, was tested.

### **RESULTS**

# Morphological Characteristics of Isolates from EERF

From this study, 17 lignocellulolytic isolates, as seen in Table 1, were obtained, including 5 cellulolytic isolates, 3 xylanolytic isolates, and 9 lignolytic isolates.

### **DISCUSSION**

### Isolation of Bacteria in EERF

Morphological observations show that the cellulolytic bacteria isolated from buffalo rumen are

white in color, round in shape, and less than 1 mm in diameter (Table 1). However, some colonies are cream, grey-colored, round in shape, and between one and two millimeters in diameter (Figure 2). This is consistent with the findings of [19], who isolated bacteria from rumen and found dominant bacterial colonies that were circular in shape and white/cream in color.

The bacteria in EERF produce various enzymes after a 100-day fermentation period, namely lignocellulolytic enzymes, which, according to [20, 21], can degrade lignin, cellulose, and hemicellulose. [20] state that enzyme application is faster in degrading fibrous feed. The research findings of [9] revealed that the bacterium Bacillus amyloliquefaciens MN-8 is capable of producing lignin peroxidase, manganese peroxidase, cellulase, and hemicellulase enzymes. Hungate (2013) identified cellulose-digesting bacteria (Bacteroides succinogenes, Ruminococcus flavafaciens, Ruminococcus albus, Butyrivibrio fibrisolvens)

Table 1: Characteristics of Isolates from EERF

laciata	Characteristics						
Isolate –	Substrate	Form	Colour	Structure	Edge	Elevation	
1	Carboxy Methyl Cellulose/CMC	Rhizoid	White	Mucus	Slightly hairy	Flat rise	
2	Carboxy Methyl Cellulose/CMC	Rhizoid	White	Mucus	Slightly hairy	Flat rise	
3	Carboxy Methyl Cellulose/CMC	Circular	White	Mucus	Undulating	Flat	
4	Carboxy Methyl Cellulose/CMC	Ireguler	White	Mucus	Slightly hairy	Flat	
5	Carboxy Methyl Cellulose/CMC	Ireguler	White	Mucus	Slightly hairy	Flat rise	
6	Xylan	Circular	Cream	Mucus	Undulating	Flat rise	
7	Xylan	Circular	Cream	Mucus	Undulating	Flat	
8	Xylan	Circular	Cream	Mucus	Undulating	Flat	
9	Tannic acid	Rhizoid	Grey	Smooth	Undulating	Swollen	
10	Tannic acid	Circular	Grey	Smooth	Undulating	Swollen	
11	Tannic acid	Rhizoid	Grey	Smooth	Undulating	Swollen	
12	Tannic acid	Circular	Grey	Smooth	Undulating	Slightly swollen	
13	Tannic acid	Ireguler	Slightly grey	Smooth	Undulating	Swollen	
14	Tannic acid	Rhizoid	Slightly grey	Smooth	Undulating	Swollen	
15	Tannic acid	Circular	Slightly grey	Smooth	Undulating	Swollen	
16	Tannic acid	Ireguler	Slightly grey	Smooth	Undulating	Swollen	
17	Tannic acid	Rhizoid	Slightly grey	Smooth	Undulating	Swollen	

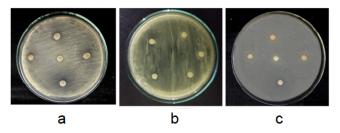


Figure 2: Lignocellulolytic isolates in this study consisted of a: cellulolytic isolate; b: xylanolytic isolate and c: lignolytic isolate.

Table 2: Dry Matter of Complete Feed Silage Based on Corn Straw Fermented using EERF

EERF		Average		
LEKI	L1(5)	L2(10)	L3(15)	Average
E1	38.59	38.61	40.58	39.26
E2	41.23	42.36	43.96	42.51
E3	42.46	44.12	46.17	44.25
Average	40.76°	41.69 <sup>a</sup>	43.57 <sup>b</sup>	

Note: Significant differences if there is different superscripts in the same column.

Table 3: NDF of Complete Feed Silage Based on Fermented Corn Straw using EERF

EERF	Le	Avorago		
EENF	L1(5)	L2(10)	L3(15)	Average
E1	52.33	46.27	40.11	46.23
E2	47.25	42.21	38.52	42.66
E3	43.21	41.14	37.10	40.48
Average	47.59°	43.21 <sup>b</sup>	38.58°	

Note: Significant differences if there is different superscripts in the same column.

Table 4: ADF of Complete Feed Silage Based on Fermented Corn Straw using EERF

EERF		Avenage		
EERF	L1(5)	L2(10)	L3(15)	Average
E1	30.25	32.24	29.11	30.53°
E2	27.25	26.21	26.22	26.56 <sup>a</sup>
E3	24.30	23.22	25.10	24.21 <sup>b</sup>
Average	27.25 <sup>a</sup>	27.22ª	26.81 <sup>b</sup>	

Note: Significant differences if there is different superscripts in the same column.

Table 5: Crude Protein of Complete Feed Protein Silage Based on Fermented Corn Straw using EERF

EERF	Length of fermentation (Days)			Average
EERF	L1(5)	L2(10)	L3(15)	Average
E1	7.73	9.77	10.24	9.25
E2	8.65	9.81	10.56	9.67
E3	8.90	9.96	10.73	9.86
Average	8.43 <sup>a</sup>	9.85 <sup>b</sup>	10.51°	

Note: Significant differences if there is different superscripts in the same column.

Table 6: pH of Complete Feed Silage Based on Fermented Corn Straw Using EERF

EERF	Len	gth of fermentation (Days	s)	Avorage
EERF	L1(5)	L2(10)	L3(15)	Average
E1	5.32	5.10	5.02	5.15
E2	5.21	4.95	4.90	5.02
E3	5.14	4.62	4.52	4.76
Average	5.22ª	4.89 <sup>b</sup>	4.81 <sup>b</sup>	

Note: Significant differences if there is different superscripts in the same column.

and hemicellulose-digesting bacteria (Butyrivibrio fibrisolvens, Bacteroides ruminocola, Ruminococcus sp) [10].

Guo et al. (2019) in their study of cattle fed fermented corn stover using lignisulolytic bacteria found that the dominant colonies were Bacteroidetes, Lentisphaerae, Firmicutes, and Fibrobacteres [22]. Guo et al. (2019) also stated that these dominant colonies accounted for 77% of the total bacteria, demonstrating the positive effect of microbial inoculants on the function of cellulose- and hemicellulose-degrading bacteria. This statement was also supported by the research of [23], who stated that rumen bacteria dominate and play a crucial role in degrading feed containing fiber.

# Dry Matter of Complete Feed Silage Based on Corn Straw Fermented using EERF

The increase in dry matter content with increasing doses of EERF enzymes and the length of fermentation time is due more to the activity of the lignocellulolytic enzymes. In this study, the 6% enzyme treatment is superior to the 2% and 4% treatments. Fermenting for 15 days is better than fermenting for 5 or 10 days. There is an interaction between the 6% fermenter with a 15-day fermentation period, which shows the highest DM. In their study on fiber degradation using bacteria [19], researchers found that livestock consuming fermented fiber feed with lignocellulolytic bacteria higher effective degradability of DM exhibited compared to those consuming unfermented feed, although the fermentation period was longer, specifically 21 days. According to [24], buffalo rumen microorganisms have the potential to make them more capable of degrading fibrous feed and are very well suited for use in silage production. According to Bacillus cereus, it was found to be a fiber-degrading bacterium [24]. Silage production using fiber-degrading bacteria reduces DM loss. However, the degradation process takes up to 60 days. Lignocellulolytic enzymes are capable of degrading more cellulose, hemicellulose, and lignin, resulting in lower dry matter loss.

Fiber-digesting enzymes were mainly synthesized by microorganisms, including bacteria, fungi, and protozoa [25], and these enzymes included Endoglucanases, Exoglucanases, and  $\beta$ -Glucosidases [25] proposed the use of enzymes to optimize the utilization of fibrous feed in the livestock industry. This is because fibrous feed derived from agricultural waste is abundantly available and does not need to be

purchased. For example, corn straw contains 36.89% cellulose, 20.42% hemicellulose, and 17.38% lignin [6], so if this material can be broken down, more nutrients will be available for livestock. Enzymes capable of breaking down lignin include lignin peroxidase, manganese peroxidase, and laccase [26, 27]. Enzymes that degrade cellulose include cellulase, glucanase, xylanase, and mannanase, which break down cellulose into oligosaccharides or monosaccharides [24]. Hemicellulose has a complex structure, so it requires a complex enzyme system for its breakdown. Hemicellulase enzymes break it down into monosaccharides and acetic acid [25] stated that non-starch polysaccharides such as cellulose, hemicellulose, and lignin were anti-nutritional components in plant-based feed. Glycoside hydrolase enzymes, including cellulase, glucanase, xylanase, and mannanase, are required because they can break down glycosidic bonds, thereby reducing these anti-nutritional properties while producing low-molecular-weight products such as oligosaccharides or monosaccharides.

### **NDF**

NDF is the part of feed that is insoluble in neutral detergent solution, such as cellulose, hemicellulose, and lignin. Meanwhile, ADF is the part of feed that is insoluble in acid detergent solution, consisting only of cellulose and lignin. The NDF and ADF content in animal feed can affect feed intake and feed utilization efficiency by livestock. The higher the NDF and ADF content in the feed, the slower the feed is digested by livestock and the more difficult it is to process by the digestive system.

In this study, the application of enzymes to corn stover significantly increase NDF digestibility (P < 0.05). 6% enzyme is superior to others, and 15 days of fermentation is preferable to 5 and 10 days. Additionally, an interaction between 6% enzymes and a 15-day fermentation period resulted in the best NDF. Similar results were also found by [28], where NDF, total volatile fatty acids, propionate, isobutyrate, isovalerate, and valerate were also degraded. NDF degradation was higher in livestock consuming corn stover fermented with cellulase, xylanase, pectinase, and glucanase enzymes [28] found the NDF value of unfermented feed to be approximately 40.30, while fermented feed had a value of 37.96 [29] found the NDF value of unfermented feed to be 64.39 and fermented feed to be 52.56 [29] used enzymes consisting of laccase, β-glucanase, xylanase, and

mannanase, and stated that fermented corn stover improves digestibility, which ultimately enhances nutrient absorption, optimizes rumen fermentation, and improves livestock growth performance. Enzymes work to improve NDF digestibility by breaking down the surface structure of corn straw cell walls, thereby increasing porosity. This facilitates enzyme penetration. With improved digestibility of cellulose hemicellulose, feed quality improves, and livestock absorb more nutrients. This study outperforms the results of studies by [28, 29]. This is possible because buffalo rumen enzymes are more diverse, originating from both cellulolytic and non-cellulolytic bacteria [30].

Innovative Use of Endogenous Enzymes to Enhance Silage

### **ADF**

ADF is the part of the feed that is insoluble in acid detergent solution, consisting only of cellulose and lignin. Similar to NDF, ADF content in animal feed can impact feed intake and efficiency of feed utilization by livestock. Higher ADF levels cause digestive tract distortion.

In this study, enzyme treatment of corn straw significantly improved ADF digestibility (P < 0.05). Notably, there was an interaction between 6% enzymes and a 15-day fermentation period, indicating that the longer the fermentation period, the better the enzyme results [28, 29] also found that ADF values fermented with enzymes were better than those without fermentation.

ADF without fermentation was 23.21, with fermentation 19.60 [28], ADF without fermentation was 33.94, with fermentation 31.01 [29]. The results of this study are better, likely due to the greater variety of enzymes originating from the large array of rumen microbes in buffaloes, which work in consortium [30].

### **Crude Protein**

The addition of EERF has been shown to increase the crude protein content of complete feed silage based on corn plant waste compared to a treatment without the addition of endogenous enzymes. Interaction between 6% **EERF** with dayfermentation period caused the best CP [31] stated that during the fermentation process, lignocellulolytic enzymes degrade cell wall components and synthesize microbial proteins. According to [32], the hydrolysis of carbohydrates into soluble carbohydrates by enzymes during fermentation increases the availability of nutrients for animals, including protein. Meanwhile,

from the degradation of lignin-cellulose, additional plant proteins are obtained and facilitate microbial protein synthesis through the utilization of fermentation substrates [33].

### Hq

This study shows that the higher the enzyme concentration, the more acidic the silage produced. Meanwhile, 15-dayfermentation period а influences it, as there is an interaction between 6% EERF and this fermentation period. This is because enzyme application makes more feed components digestible, as evidenced by lower NDF and ADF values. During decomposition, acetic acid is produced, causing the pH of the silage to decrease from 5.22 to 4.81. This was also found by [34, 35], who reported a pH of 4 to 4.8 from the fermentation of corn waste using xylanase enzyme [4, 5] stated that the acidic conditions in corn waste fermentation occurred due to various enzymes such as cellulose enzymes, xylanase enzymes, and lignin/lignocellulase, which synergize with each other in degrading fiber.

### The HS Case on Samosir Island

The buffalo population on Samosir Island in 2022 was 11,800 heads, whereas the previous data for 2017 was 24,207 heads [36]. The sharp population decline is due to intensive livestock sales outside the region, declining birth rates, and disease [1]. Samosir is an endemic area for Hemorrhagic septicemia (HS). HS is a highly infectious disease with high morbidity and mortality rates [37, 38]. HS recurs annually in Samosir, caused by factors such as low vaccination rates, buffalo gathering in grazing fields, and livestock suffering from stress due to feed shortages during the dry season [1]. HS causes significant losses to livestock farmers in South Asia and Southeast Asia, including Indonesia, Malaysia, the Philippines, and Thailand [39]. From Table 7, it is evident that, according to [1], HS cases were more prevalent on Samosir Island, likely because many farmers do not report cases due to communication difficulties. Many farmers live in mountainous areas with no communication signals.

The dry season on Samosir Island is longer than the rainy season, so pastures cannot provide sufficient feed for buffaloes, and farmers also face difficulties in finding additional feed. In 2023, an El Niño event in Indonesia caused extreme drought, resulting in a very high incidence of HS.

No.	Regency	2023	2024	2025 (until June)
1	Sianjur mula-mula	0	0	0
2	Harian	0	0	0
3	Sitio-tio	0	0	0
4	Onan runggu	52	0	0
5	Nainggolan	15	0	0
6	Palipi	31	1	2
7	Pangururan	17	2	3
8	Ronggur nihuta	79	1	3
9	Simanindo	8	2	5
	TOTAL	202	6	13

Table 7: HS Cases Per Subdistrict on Samosir Island 2023, 2024, 2025

Since 2024, corn cultivation has been carried out more intensively on Samosir Island, with a planting area of 13,876 ha and a harvest area of 10,716 ha [36]. Corn cultivation is evenly distributed across all subdistricts, especially in subdistricts with the highest number of buffaloes, such as Pangururan, Simanindo, and Palipi. Corn cultivation provides additional feed from corn waste. The availability of corn waste makes it easier for farmers to offer corn waste as buffalo feed. Additionally, communities no longer allow their buffalo to graze on grasslands because grasslands have been converted into corn fields. Communities now keep their buffalo in pens, reducing the spread of disease if any buffalo contract HS.

Unprocessed corn waste does not adequately meet the nutritional needs of buffaloes. In this study, fermentation using endogenous enzymes derived from the rumen fermentation of buffaloes consuming corn waste successfully improved the nutritional quality of corn waste, with DM increasing from 38.59 to 46.17, NDF from 46.23 to 40.48, ADF from 30.53 to 24.21, CP from 9.25 to 9.86, and pH from 5.15 to 4.76. These improvements in nutritional quality will support the nutritional needs of buffaloes. The acidic pH will make the silage more durable. The harvest area in 2024 is 10,716 ha. Each hectare of corn produces 64,443 kg/h/y of fresh corn straw, so with a harvest area of 10,716 ha, 690,571,188 kg is produced, yielding 318,836,717 kg of dry matter. The feed requirement for one buffalo is approximately 3% dry matter [5], so the potential supply of fermented corn straw is sufficient for 9,565,101 buffaloes. Currently, the buffalo population on Samosir Island is around 11,000 head, so the corn straw supply is more than enough to meet the buffalo's feed requirements. It is hoped that the support from fermented corn waste will reduce feed shortages during the dry season. Even though an abundant supply of corn waste is available now in Samosir Island, it is not the only factor that can reduce HS cases. However, data obtained from the Samosir Regency Agricultural Office shows that since corn cultivation has become more intensive, there has been a reduction in HS cases. In 2024, there were 6 reported HS cases, and by June 2025, there were 13 cases compared to 202 cases in 2023 [36].

### CONCLUSION

Corn straw fermentation using endogenous enzymes improves the chemical quality of silage, such as DM increasing from 38.59 to 46.17, NDF from 46.23 to 40.48, ADF from 30.53 to 24.21, CP from 9.25 to 9.86, and pH from 5.15 to 4.76. The acidic pH of the silage makes it more durable. Through this improvement in nutritional quality, the nutritional needs of buffaloes are met. This corn straw is sufficient for 9,565,101 buffaloes. Since corn cultivation has become more intensive, cases of HS have decreased, with only 6 cases in 2024 and 13 cases up to June 2025, compared to 202 cases in 2023.

# **AVAILABILITY OF DATA AND MATERIALS**

Data and materials used for this research are available from the main author upon request.

### **DECLARATION OF INTEREST**

The authors declare that no conflict of interest could be perceived as prejudicing the impartiality of the research reported.

# **AUTHORS CONTRIBUTION**

Nurzainah Ginting conceptualized and conducted the experiments, while Raden Edhy Mirwandhono and

Muhammad Adanan Purba analyzed the results and wrote the manuscript. Muhammad Amran, Winda Saragih, Nujama'yah br Ketaren, and Anjas Asmara Samsudin contributed to the conceptualization of the research study, as well as its finalization, review, revision, and proofreading for publication.

#### **ACKNOWLEDGEMENTS**

The funding of this research is supported by the Directorate General of Higher Education, Research and Technology, and the Lembaga Pengelola Dana Pendidikan (LPDP) Indonesia.

### **REFERENCES**

- Ginting N, Hasnudi H, Mirwandhono E, Hellyward J, [1] Sulistyowati E, Hafid H, et al. A Study of Infection Areas and Social Assessment Findings in Hs (haemorrhagic Septicaemia)-Affected Buffaloes on Samosir Indonesia. EEC J 2022; 28: 15-23. https://doi.org/10.53550/EEC.2022.v28i06s.003
- [2] Prihandini PW, Tribudi YA, Hariyono DNH, Sari APZNL, Praharani L, Handiwirawan E, et al. Biodiversity of Indonesian Indigenous Buffalo: First Review of the Status, Challenges, and Development Opportunities. Veterinary World 2023; 16(5): 2217. https://doi.org/10.14202/vetworld.2023.2217-2229
- El Debaky HA, Kutchy NA, Ul-Husna A, Indriastuti R, Akhter [3] S, Purwantara B, et al. Potential of Water Buffalo in World Agriculture: Challenges and Opportunities. Applied Animal Science 2019; 35(2): 255-268. https://doi.org/10.15232/aas.2018-01810
- Rohaeni ES, Santoso AD, Ariningsih E, Widaningsih N, [4] Hutahaean L, Priyanto D, et al. Analysing the Sustainability of Swamp Buffalo. Open Agriculture 2023; 8: 2022022. https://doi.org/10.1515/opag-2022-0224
- Mohd Azmi AF, Ahmad H, Mohd Nor N, Goh YM, Zamri-[5] Saad, M, Abu Bakar, MZ et al. The impact of feed supplementations on Asian buffaloes: A review. Animals 2021; 11(7): 2033. https://doi.org/10.1515/opag-2022-0224
- Noersidiq A, Yanuarianto O, Fahrullah F, Maslami V, Hasma [6] H. Nutrient Content of Corn Straw (Crude Fibre, Crude Fat and TDN) Caused by Different Doses of Urea. Jurnal Pijar Mipa 2024; 19(4): 673-677. https://doi.org/10.29303/jpm.v19i4.6643
- Ervinta E, Mirwandhono RE, Ginting N, Simanullang B. [7] Fermentation by Eco Enzyme on Nutritional Content of Rice Straw, Corn Straw, and Oil Palm Fronds. Jurnal Peternakan Integratif 2020; 8(3): 211-220. https://doi.org/10.32734/jpi.v8i3.12532
- [8] Ginting N, Damanik M. The Study of Sustainable Biodesinfectants for Pig Farms in Samosir Island, Indonesia. In IOP Conference Series: Earth and Environmental Science 2023; 1286(1): 012005. https://doi.org/10.1088/1755-1315/1286/1/012005
- Hong-ya LI, Shu-na LI, Shu-xiang WANG, Quan WANG, Yin-[9] yin XUE, Bao-cheng ZHU. Degradation of Lignocellulose in the Corn Straw by Bacillus Amyloliquefaciens MN-8. Chinese Journal of Applied Ecology/Yingyong Shengtai Xuebao 2015;
- [10] Hungate RE. The Rumen and Its Microbes 2013.
- Ginting N, Hasnudi H, Prayitno L. Dilution of Eco Enzyme [11] and Antimicrobial Activity Against Staphylococcus aureus.

- JITRO (Jurnal Ilmu dan Teknologi Peternakan Tropis) 2022; 9(1): 123-128.
- Arun C, Sivashanmugam P. Investigation of biocatalytic [12] potential of garbage enzyme and its influence on stabilization of industrial waste activated sludge. Process Saf Environ Prot 2015; 94: 471-478. https://doi.org/10.1016/i.psep.2014.10.008
- Rahman S, Haque I, Goswami RCD, Barooah P, Sood K, [13] Choudhury B. Characterization and FPLC analysis of garbage enzyme: biocatalytic and antimicrobial activity. Waste and Biomass Valorization 2021; 12: 1293-302. https://doi.org/10.1007/s12649-020-009
- Mohamed R, Chaudhry AS. Methods to Study Degradation of [14] Ruminant Feeds. Nutrition Research Reviews 2008; 21(1): 68-81. https://doi.org/10.1017/S0954422408960674
- [15] Prihantoro I, Toharmat T, Evvyernie D, Abdullah L. Ability of fiber bacteria isolated from buffalo rumen in digesting various sources of forage. Jurnal Ilmu Ternak dan Veteriner 2012; https://doi.org/10.14334/jitv.v17i3.699
- [16] Hu Y, He Y, Gao S, Liao Z, Lai T, Zhou H, Chen Q, Li L, Gao H, Lu W. The effect of a diet based on rice straw cofermented with probiotics and enzymes versus a fresh corn Stover-based diet on the rumen bacterial community and metabolites of beef cattle. Scientific Reports 2020; 10(1): 10721. https://doi.org/10.1038/s41598-020-67716-w
- Natsir A, Syahrir S, Nadir M, Mujnisa A, Purnomo N. [17] Biostarter Formulated from the Rumen Bacteria of Buffalo Effectively Changes the Chemical Composition of the Rice Straw. In IOP Conference Series: Earth and Environmental Science 2020; 492(1): 012003. IOP Publishing. https://doi.org/10.1088/1755-1315/492/1/012003
- IBM SPSS Statistics 29 Step by Step: A Simple Guide and [18] Reference. Routledge.
- Ghiasi SE, Gheibipour M, Motamedi H, Dar MA. Screening [19] and Evaluation of the Ruminal Cellulolytic Bacteria and their Potential Application as Probiotics. Iranian Journal of Microbiology 2024; 16: 3389. https://doi.org/10.18502/ijm.v16i3.15796
- [20] Kumar VP, Sridhar M. Study on Production of Microbial Lignolytic Enzymes for Deconstruction of Lignocellulosic Biomass for Feeding Ruminants: Current Trends and Future Research Aspects in Agriculture and Veterinary Science https://www.researchgate.net/publication/ 3. 355101906\_Study\_on\_Production\_of\_Microbial\_Lignolytic\_E nzymes\_for\_Deconstruction\_of\_Lignocellulosic\_Biomass\_for \_Feeding\_Ruminants\_Current\_Trends\_and\_Future\_Prospec ts?enrichId=rgreq-57596ad05e317ddb2c66ebcfc8cf34a4-XXX&enrichSource=Y292ZXJQYWdlOzM1NTEwMTkwNjtBU zoxMTM4ODYxNDMwMzE3MDU3QDE2NDg1MzcxMzIzMjc  $%3D\&el=1_x_2\&_esc=publicationCoverPdf.$
- [21] Rasool G, Irfan M. The Role of Microbial Diversity in Lignocellulosic Biomass Degradation: A Biotechnological Perspective. ChemBioEng Reviews 2024; 11(3): 613-635. https://doi.org/10.1002/cben.202300073
- Guo W, Guo XJ, Zhu BC, Guo YY, Zhou X. In situ [22] degradation, ruminal fermentation, and the rumen bacterial community of cattle fed corn stover fermented by lignocellulolytic microorganisms. Animal Feed Science and Technology 2019; 248: 10-19. https://doi.org/10.1016/j.anifeedsci.2018.07.007
- Welkie DG, Stevenson DM, Weimer PJ. ARISA Analysis of [23] Ruminal Bacterial Community Dynamics in Lactating Dairy Cows During the Feeding Cycle. Anaerobe 2010; 16(2): 94https://doi.org/10.1016/j.anaerobe.2009.07.002
- Zhong H, Zhou J, Wang F, Wu W, Xiong H, Yin H, et al. [24] Isolation and identification of Ligninolytic Bacterium (Bacillus

- cereus) from Buffalo (Bubalus bubalis) Rumen and its Effects on the Fermentation Quality, Nutrient Composition, and Bacterial Community of Rape Silage. Frontiers in Microbiology 2023; 14: 1103652. https://doi.org/10.3389/fmicb.2023.1103652
- [25] Xiaokang Lv, Qiao Z, Chen C, Hua J, Zhou C. Exploration of Multi-Source Lignocellulose-Degrading Microbial Resources and Bioaugmentation Strategies: Implications for Rumen Efficiency. Animals 2025; 15(13): 1920. https://doi.org/10.3390/ani15131920
- [26] Zhang S, Dong Z, Shi J, Yang C, Fang Y, Chen G, et al. Enzymatic Hydrolysis of Corn Stover Lignin by Laccase, Lignin Peroxidase, and Manganese Peroxidase. Bioresour Technol 2022; 361: 127699. https://doi.org/10.1016/j.biortech.2022.127699
- [27] Ríos-Ríos KL, Dejonghe W, Vanbroekhoven K, Rakotoarivonina H, Rémond C. Enzymatic Production of Xylo-oligosaccharides from Destarched Wheat Bran and the Impact of Their Degree of Polymerization and Substituents on Their Utilization as a Carbon Source by Probiotic Bacteria. J Agric Food Chem 2021; 69: 13217–13226. https://doi.org/10.1021/acs.jafc.1c02888
- [28] Xu J, Wang X, Niu H. Effects of corn straw-based fermented total mixed rations supplemented with exogenous cellulase on growth performance, digestibility, and rumen fermentation in growing beef cattle. Animal Bioscience 2024; 38(2): 293. https://doi.org/10.5713/ab.24.0246
- [29] Chen B, Liu J, Liu M, Zhang H, Li X, Tian C, et al. Synergistic Effect of Microorganisms and Enzymes on Nutritional Value of Corn Stover and Wheat Straw. Fermentation 2025; 11(4): 210. https://doi.org/10.3390/fermentation11040210
- [30] Kala A, Kamra DN, Kumar A, Agarwal N, Chaudhary LC, Joshi CG. Impact of Levels of Total Digestible Nutrients on Microbiome, Enzyme Profile, and Degradation of Feeds in Buffalo Rumen. PloS One 2017; 12(2): e0172051. https://doi.org/10.1371/journal.pone.0172051
- [31] Malik PK. Trivedi S, Kolte AP, Sejian V, Mech A, Biswas S, et al. Comparative Assessment of Rumen Microbial Diversity in Cattle and Buffaloes. In Climate Change and Livestock

- Production: Recent Advances and Future Perspectives 2022; 223-238.
- https://doi.org/10.1007/978-981-16-9836-1 19
- [32] Wang X, Song J, Liu Z, Zhang G, Zhang Y. Fermentation Quality and Microbial Community of Corn Stover or Rice Straw Silage Mixed with Soybean Curd Residue. Animals 2022; 12(7): 919. https://doi.org/10.3390/ani12070919
- [33] Yu P, Christensen DA, McKinnon JJ. In situ Rumen Degradation Kinetics of Timothy and Alfalfa as Affected by Cultivar and Stage of Maturity. Can J Anim Sci 2004; 84: 255-263. https://doi.org/10.4141/A03-116
- [34] Maftukhah S. Cellulase Enzyme Production Using Solid State Fermentation Method From Waste–A Review. Jurnal Keilmuan dan Aplikasi Teknik 2019; 6(2): 22. <a href="https://doi.org/10.33592/unistek.v6i2.197">https://doi.org/10.33592/unistek.v6i2.197</a>
- [35] Istiqomah L, Cahyanto MN, Zuprizal Z. Xylanase Production by Trichoderma Virens MLT2J2 Under Solid-State Fermentation Using Corn Cob as a Substrate. Biodiversitas Journal of Biological Diversity 2022; 23: 12. https://doi.org/10.13057/biodiv/d231251
- [36] Samosir Regency Agriculture Office. Interim Livestock Development Report Statistics of Samosir Regency 2025.
- [37] Abubakar MS, Zamri-Saad M. Clinico-Pathological Changes in Buffalo Calves Following Oral Exposure to Pasteurella Multocida B:2. Basic and Applied Pathology 2011; 4(4): 130-135. https://doi.org/10.1111/j.1755-9294.2011.01113.x
- [38] Khan A, Saleemi MK, Khan MZ, Gul ST, Irfan M, Qamar MS. Hemorrhagic Septicemia in Buffalo (Bubalus bubalis) Calves Under Sub-Tropical Conditions in Pakistan. Pakistan Journal of Zoology 2011; 43: 2. https://www.researchgate.net/ publication/241685040
- [39] Lestari TD, Khairullah AR, Damayanti R, Mulyati S, Rimayanti R, Hernawati T, et al. Hemorrhagic septicemia: A major threat to livestock health. Open Veterinary Journal 2025; 15(2): 519. <a href="https://doi.org/10.5455/OVJ.2025.v15.i2.3">https://doi.org/10.5455/OVJ.2025.v15.i2.3</a>

Received on 05-08-2025 Accepted on 02-09-2025 Published on 14-09-2025

https://doi.org/10.6000/1927-520X.2025.14.16

© 2025 Ginting et al.

This is an open-access article licensed under the terms of the Creative Commons Attribution License (<a href="http://creativecommons.org/licenses/by/4.0/">http://creativecommons.org/licenses/by/4.0/</a>), which permits unrestricted use, distribution, and reproduction in any medium, provided the work is properly cited.