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Effect of different additives on nutrient parameter and palatability of ensiled water hyacinth (*Eichhornia crassipes*)

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Abstract

Water hyacinth, *Eichhornia crassipes*, is an invasive aquatic weed that covers major water bodies in Nepal, and its silage has great potential to be used as ruminant's feed. An experiment was conducted with an aim to explore its ensiling attributes along with palatability test for cattle was conducted at Institute of Agriculture and Animal Science, Rupandehi in a completely randomized experimental design with three replications. The quality and palatability of water hyacinth silage prepared with additives such as rice straw, molasses, wheat flour, and rice bran were assessed. The treatments included: water hyacinth with rice straw(T1); water hyacinth with rice straw and rice bran(T2); water hyacinth with rice straw and wheat flour(T3); water hyacinth with rice straw and molasses(T4); water hyacinth with rice straw, wheat flour and molasses(T5); water hyacinth with rice straw, wheat flour and rice bran(T6) and water hyacinth with rice straw, wheat flour, molasses and rice bran(T7). The results showed, crude protein (CP), crude fiber (CF), ether extract (EE), total ash (TA), pH, and palatability had significant differences across all treatments ($p<0.05$). CP (15.13) and CF (23.73) were found to be highest under control whereas rice straw, wheat flour and rice bran had the highest EE (12.74), TA (15.13), pH (3.87), palatability (100%), and considerably high CP (12.40) and CF (19.75). Hence, silage of water hyacinth with rice straw, wheat flour, and rice bran has high nutrient content, palatability and can be used as a feed alternative to solve the problem of feed scarcity.

Keywords: Crude fiber, Crude protein, Ether extract, Feed alternatives, Nutrient content, Palatability, Silage, Water hyacinth.

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Contribution of this paper to the literature
The research provides information that differs in palatability and chemical quality of silage from other similar research and is a unique study against ensiled water hyacinth. The paper adds the knowledge of how different additives can be utilized as feed alternative to solve the problem of feed scarcity.

1. Introduction

Water hyacinth (*Eichhornia crassipes* Martius) is one of the most common weed species among floating water weeds in tropical and subtropical locations around the world due to its rapid development [1]. It originated in the Amazon Basin and quickly spread over Latin America, Africa, Southeast Asia, and the Pacific region by 1950 [2]. Water hyacinth appeared for the first time in Ethiopia in 1965, near Koka Reservoir as well as the Awash River Ayana [3]. Fessehaie [4] and Taye, et al. [5] reported water hyacinth infestations in Gambela Regional State, the Blue Nile from Lake Tana to Sudan, and Lake Ellen near Alem Tena. According to the International Union for Conservation of Nature, these plants are among the 100 most harmful invasive species and the top ten worst weeds worldwide [6]. Of the 182 foreign flowering plants found in Nepal, 27 are regarded as invasive [7].

There is an immediate rise in livestock production to fulfill the requirements for animal protein for the world's growing population [8]. The need for animal protein in the tropics has been escalating. There was a 31% deficit in dry matter content required for the well-being of existing cattle and poultry in Nepal. Scarce feed resources, particularly during the colder months from October to May, lack of understanding regarding feed nutrient composition, absence of expertise in feed formulation, and improper utilization of feed resources based on physiological requirements collectively impede the growth of Nepal's livestock sector [9]. This scarcity hampers the timely production of high-quality research due to inadequate research infrastructure and facilities. The aquatic nuisance known as water hyacinth (*Eichhornia crassipes*) poses a significant threat due to its tendency to obstruct water bodies such as rivers, lakes, marine routes, and sewage lagoons. This is particularly prominent in tropical and subtropical regions worldwide [10]. Water hyacinth has recently received a lot of interest because of its potential uses as animal feed, aqua feed, water filtration, fertilizer, biogas generation, and potentially human nourishment, among other things [11, 12]. Fresh water hyacinth contains 2.38% crude protein, 0.27% crude fat, 0.91% crude fiber, and 3.7% nitrogen-free extract. Water hyacinth's dry matter, which is mineral-rich and contains 10-20% crude protein, can be used to substitute some protein in feed and roughage Sharma [13]. Biswas and Mandal [14] discovered that water hyacinth comprised 15.58% crude protein, 19.97% crude fat, 1.33% crude fiber, and 1.85% crude ash after drying and chopping, whereas the leaves contained 16.04% crude protein and 14.97% crude fat. Abdelhamid and Gabr [15] demonstrated that water hyacinth boasts a crude protein value of 20% on a dry basis, thereby making it a suitable option for animal feed. Impressively, water hyacinth can produce 6–10 times more protein per unit area compared to soybeans, hinting at its potential as ruminant feed in regions abundant with water hyacinth. The research site has an abundance of rivers and ponds, which leads to the creation of enormous numbers of water hyacinths. A sizable section of the pond is covered with water hyacinth. Once the chemical makeup and nutritional value of water hyacinth are known, farmers may utilize it as an unusual feed for their animals, which will save feed costs and boost output. Water hyacinth has high quantities of cellulose and hemicellulose, which may be utilized as ruminant energy sources [16]. Because of the low amount of dry matter in water hyacinth, yellowing is often preferred to avoid silage losses [17]. Water hyacinth may be effectively ensiled using feed additives and organic acids, and ruminants have indicated that the silages are enjoyable. Dairy calves have been fed an ensiled combination of water hyacinth, grain straw, urea, and molasses. This has enhanced milk output [18]. Therefore, the purpose of this study was to determine the chemical makeup, nutritional value, and use of water hyacinth during a feed shortage.

2. Materials and Methods

2.1. Site Selection

The research was carried out at the horticulture farm of Paklihawa campus, Nepal, started in May 2023. The study area is located at 27.4829°N and 83.4457°E. Figure 1 illustrates study location which was chosen owing to the existence of suitable ponds, water reservoirs, and lowlands for the collection of *Eichhornia crassipes.*

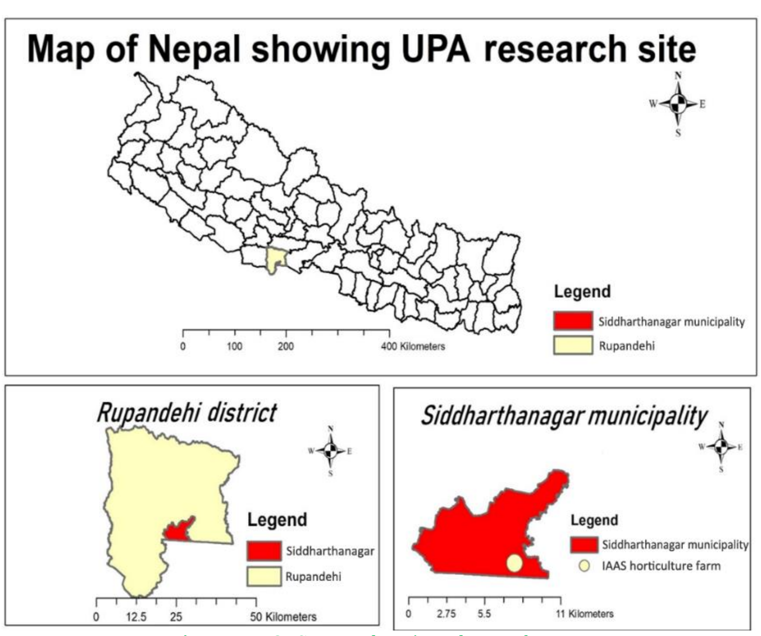

Figure 1. GIS map showing the study area.

2.2. Research Design and Layout

Research was carried out in a complete randomize design (CRD) with seven treatments and three replications. Wilted water hyacinth along with additives were used as a treatment. Table 1 presents the details of treatments used during experimentation.

Table 1. Details of treatments used in research.

Treatments	Treatment details
T_1	Wilted water hyacinth + Rice straw (10%) (Control)
T_2	Wilted water hyacinth + Rice straw (10%) + Rice bran (10%)
T_3	Wilted water hyacinth + Rice straw (10%) + Wheat flour (10%)
T_4	Wilted water hyacinth + Rice straw (10%) + Molasses (10%)
T_5	Wilted water hyacinth+ Rice straw (10%) + Wheat flour (10%) + Molasses (10%)
T_6	Wilted water hyacinth + Rice straw (10%) + Wheat flour (10%) + Rice bran (10%)
T_7	Wilted water hyacinth+ Rice straw (10%) + Wheat flour (10%) + Rice bran (10%) + Molasses (10%)

2.3. Silage Preparation

- Water hyacinth was harvested from the Dande River of Paklihawa. After removing the roots, it was thoroughly washed and allowed to wilt in the shade for about 2 days.
- Both the petiole and leaf fractions were cut into 4-5 cm pieces and mixed with additives according to the above-mentioned protocols in the polythene bags, each containing hyacinth with different additives of 3 kg, and vacuumed before it was left to ensile.
- Prepared bags were kept in an isolated place (free from rodents), maintaining temperature for 45 days.
- Regular monitoring was done to check for undesirable conditions.
- After 45 days of preparation, representative silage samples were taken for a laboratory test. A palatability test was also done on the farm.

2.4. Preparation of a Plant Sample

A sample from each treatment of silage was taken. The sample was dried immediately in an oven at 70˚C (60-80˚C) and kept for 24 hours. While drying, the samples should be thinly spread in the oven. The dried sample was ground in a mixture, and 0.20 g of plant sample was weighted, taken in a filter paper, and dropped as a package into a 100-ml digestion flask or a tube, adding 2 g of digestion mixture along with 10 ml of concentrated H_2So_4. The material was digested at low temperatures until foaming ceased. The temperature was raised to 400˚C, causing the acid to condense about one-third of the way into the center of the digesting flask. The flask was spun often, and digestion proceeded until carbonaceous particles formed and the color changed to green-blue. Before the solution solidified, the flask was refrigerated and 40 mL of filtered water was added. The solution was transferred to a 100-ml volumetric flask by rinsing the digestion flask with 3-4 batches of tiny volumes of distilled water and making up the volume. Four drops of mixed indicator solution were added to 20 milliliters of 4-percent boric acid solution in 125 milliliters, and the flask was then put under the condenser. A 20-ml aliquot of the digested solution was transferred to a distillation flask, and 100 ml of distilled water was added. Keeping the flask at a 45-degree angle, 20 milliliters of a solution containing 40 percent of sodium hydroxide were poured down the neck until it reached the bottom without mixing. The flask was quickly affixed to the distillation equipment and stirred to mix. The boiling temperature of the distillation flask prevented the boric acid from being drawn back. The distillate was created after about 75 milliliters of distillation. The nitrogen content was determined by titrating the distillation product with 0.05N HCl. At the end, the mixed indicator's hue simply changes from blue to reddish. For every batch of 21 samples, run the blank using all of the ingredients and procedures, excluding the plant sample.

$$\% \text{ N} = \frac{(S-B) \times n \times 7}{W}$$

Where,
S= Volume of standard acid (ml) used up by sample.
B= The volume of standard acid (ml) used up by blank.
n = Normality of the standard acid.
W = Oven dry weight of sample.
14= Equivalent weight of nitrogen.
20= Aliquot.

2.5. Ether Extract (Crude Fat)

First, a filter paper thimble was created, put on a weighing scale, and its weight was recorded. A silage sample was ground. 2 g of sample was taken into the thimble, and cotton was placed to cover the sample. A thimble was folded to enclose the sample. A cellulose thimble was taken and labeled with a sample number. A clean and dry flat bottom flask was taken. The weight of the flask was also taken. A Soxhlet extraction unit was setup placing, the sample was placed in it, and a sufficient amount of n-Hexane was added. Through the condenser of the Soxhlet extractor, water was run. The Soxhlet extractor was run for 6 hours. After 6 hours, thimble was taken out, and wastage n-Hexane was collected. A sample was taken out of the thimble, and wastage n-Hexane was collected from the thimble. By condensation, the rest of n-Hexane was collected from the flask. The flask was rotated to evaporate the excess n-Hexane from it. Inside the flask, extracted oil was seen. To remove moisture and hexane, a flask was placed inside the oven. The temperature was set at 110˚C and dried for 30 minutes. After 30 minutes, dried flask was taken out, and placed, and cooled in the desiccator. After cooling, the final weight of the flask with fat was taken.

Weight of Sample = Ws.
Weight of Flask = W1.
Weight of Flask with Fat = W2.
Now,
Crude Fat (%) = $\frac{(W2-W1)}{Ws} \times 100$

2.6. Crude Fiber

2.6.1. Boiling in Acid

A 2 g oven-dried sample was weighed before adding 200 ml (0.158M) of sulfuric acid to the cylinder and pouring it into a conical flask. The sample was added to the flask shown above. Following 30 minutes of boiling, a flask was put on a hot plate and shook by hand. The trash flask was placed inside a funnel lined with cotton fabric. The boiling sample was filtered to remove the acid solution. The flask was thoroughly cleaned with hot water to eliminate any remaining acid residue. A 200 mL 0.313 M NaOH solution was measured.

2.6.2. Boiling in Base

The NaOH solution was put into the conical flask to clean the filtrate. The flask was shaken to mix before being put on a heated plate. After 30 minutes of boiling, the flask was shook with one hand. The trash flask was placed next to a funnel lined with cotton fabric. The base solution was drained from the boiling sample using filtration. The flask was thoroughly cleaned with hot water to eliminate the base residue, and fiber was collected in the crucible.

2.7. Drying of Fiber

The crucible was put on a heated plate to evaporate any extra water. The obtained fiber was dried in an oven at 105°C for 2 hours. After drying, the crucible was weighed and the result was recorded.

2.8. Incineration of Fiber

The crucible was placed in a muffle furnace and fiber was burnt at 550°C for 2-4 hours. After burning, the crucible was cooled using a dessicator. After 10-20 minutes, the crucible was taken from a dessicator and weighed.
Wt. of sample = Ws.
Wt. of crucible with fiber = W1.
Wt. of crucible with ash = W2.
Now,
Crude Fiber = $\frac{(W1-W2)}{Ws} \times 100$

2.9. Ash Content

The crucible was weighted, and its weight was noted to 4 decimal places. 2 g of sample was weighted in a crucible, and the weight was recorded to 4 decimal places. The sample was placed in muffle furnance at 600°C for 2 hours. After that, it was cooled in the dessicator and weighed within 1 hour after reaching room temperature. The sample was now weighed and recorded at 4 decimal points.

$$\%\ \text{Ash} = \frac{\text{ashed wt. } - \text{crucible wt.}}{\text{crucible and sample wt.} - \text{crucible wt.}} \times 100$$

2.10. Palatability Test

Representative 12 cattle of the same breed were fed 2 kg of silage from each treatment for 3 consecutive days from each replication, and feed intake was noted. The first day of the trial was done by feeding 1.5 kg of silage from each treatment. The cattle were allowed to feed on a given silage weight (W_1). After 15 minutes, the left-over feed (W_2) was recorded.

$$\text{Percentage left over feed} = \frac{W2}{W1} \times 100\%$$

2.11. Ph Test

Representative samples were taken to the lab and grinded into fine dust, then weighed at 5 g each. Then each sample was dissolved in 20 ml of distilled water and left for 10 minutes. Data were collected by using a pH meter in the lab.

2.12. Data Analysis and Statistical Tools

The tabulation and processing of data were done using MS Excel 2016, and the data were statistically analyzed following an analysis of variance (ANOVA) using R-Studio (R 4.3.2). The significant differences between treatment means were compared by the least significant difference (LSD) at the 5% level of probability. Arc GIS was used for making a topographic map of the study area.

3. Results and Discussion

3.1. Physical Quality Parameter

After 45 days, the silage in each treatment was ready for use. pH is one of the primary quality parameters for silage. Silage is often categorized as very good (pH 3.8 to 4.2), excellent (pH 4.2 to 4.5), and fair (pH >4.5) depending on pH [19]. In the experiment, wilted water hyacinth + rice straw (10%) + rice bran (10%) and wilted water hyacinth + rice straw (10%) revealed very good quality with pHs of 4.51 and 4.21, respectively (Table 2). The odor of wilted water hyacinth + rice straw (10%) + molasses (10%) was rated excellent with a brownish green color. Nearly all treatments have a pH of less than 4, with wilted water hyacinth + rice straw (10%) + wheat flour (10%) + rice bran (10%) having the best pH.

Table 2. Effects of different feed additives on pH, color, and odor of silage after 45 days.

Treatments	pH	Color	odor
Wilted water hyacinth + Rice straw (10%) (Control)	4.51±0.06[a]	Dark brown	Bad
Wilted water hyacinth + Rice straw (10%) + Rice bran (10%)	4.21±0.09[b]	Brown	Bad
Wilted water hyacinth + Rice straw (10%) +Wheat flour (10%)	3.67±0.27[cd]	Brown	Good
Wilted water hyacinth + Rice straw (10%) + Molasses (10%)	3.16±0.05[e]	Brownish green	Very good
Wilted water hyacinth + Rice straw (10%) + Wheat flour (10%) + Molasses (10%)	3.39±0.04[de]	Golden brown	Good
Wilted water hyacinth + Rice straw (10%) + Wheat flour (10%) + Rice bran (10%)	3.87±0.03[bc]	Brownish green	Good
Wilted water hyacinth+ Rice straw (10%) + Wheat flour (10%) + Rice bran (10%) + Molasses (10%)	3.70±0.07[cd]	Golden brown	Good
LSD (P<0.05)	0.36***		
CV%	5.32		
Grand mean	3.80		

Note: CV: Coefficient of variation; LSD: Least significant difference followed by the same letter in a column are significantly different. ***= treatment means are significantly different at a 0.1% level of significance. In a column, the different letters represents data differs significantly at 5% level of probability.
a presented: Highest significant mean among treatments.
b presented: Second highest significant mean among treatments.
c presented: Significant mean after a and b.
d presented: Lowest significant means among treatments.

The water-soluble carbohydrates included in food additives influence silage fermentation [20]. Molasses has 700 grams of carbs per kilogram of dry matter, whereas rice bran has just 53 grams [21, 22]. Molasses increased the fermentation process more than rice bran. As pH is a valid metric of fermentation, increasing the pH of the rice bran-added compounds may imply a slower pace of fermentation [23]. Good silage tends to be a yellow-green to brownish green hue [24] having an agreeable sweet, sour, and smelling [19].

3.2. Chemical Quality of the Silage

The effect of different additives on the chemical quality of silage is presented in Table 4. Crude protein is the measure of approximate protein content in silage. Wilted water hyacinth + rice straw (10%) has the highest crude protein content (15.13%), followed by wilted water hyacinth + rice straw (10%) + wheat flour (10%) + rice bran (10%) + molasses (10%). The crude protein content of wilted water hyacinth, rice bran, and molasses was found to be 174, 53, and 33 gram per kg respectively [25]. Our findings contradicted the findings of Indulekha, et al. [19], who found a reduction in the nutrient composition of silage with the addition of straw.

The silages supplemented with rice straw had the greatest crude fiber content. Additionally, the quantity of crude fiber rose with the inclusion of rice bran. The combination of wilted water hyacinth + rice straw (10%) and wilted water hyacinth + rice straw (10%) + rice bran (10%) has the greatest fiber content. With a crude fiber content of 23.73 and 21.88 percent respectively (Table 3). The addition of straw and bran enhanced the crude fiber content of silage. When wheat bran is added to silage, the amount of crude fiber and crude fat increases, as stated by [26].

The maximum ether extract was recorded in wilted water hyacinth + rice straw (10%) + wheat flour (10%) + rice bran (10%), followed by wilted water hyacinth + rice straw (10%) + wheat flour (10%) + rice bran (10%) + molasses (10%). The wheat flour and rice bran have enhancing effects on the ether extract. The maximum total ash content was recorded in wilted water hyacinth + rice straw (10%) + wheat flour (10%) + rice bran (10%), followed by wilted water hyacinth + rice straw (10%) + rice bran (10%). Total ash content is an index for measuring total inorganic matter in food samples, which reflects the quantity of minerals present [27]. The higher ash content may be because the root sections used in the ensiling procedure have the ability to absorb different minerals from the water component. Although the effects of giving high ash diets to ruminant animals are not fully known, high ash levels in dairy cow diets or forages may operate as a covert antagonist against the effectiveness of dairy nutrition programs [28].

Table 3. Effects of different feed additives on crude protein, crude fiber, ether extract, and the total ash content of silage after 45 days.

Treatments	Crude protein (%)	Crude fiber (%)	Ether extract (%)	Total ash (%)
Wilted water hyacinth + Rice straw (10%) (Control)	15.13±0.20[a]	23.73±0.84[a]	11.83±0.09[cd]	12.11±0.05[c]
Wilted water hyacinth + Rice straw (10%) + Rice bran (10%)	10.10±0.19[d]	21.88±0.76[b]	11.39±0.22[d]	14.22±0.16[b]
Wilted water hyacinth + Rice straw (10%) +Wheat flour (10%)	11.06±0.11[cd]	21.04±0.03[bc]	12.12±0.05[bc]	11.69±0.03[cd]
Wilted water hyacinth + Rice straw (10%) + Molasses (10%)	8.09±0.13[e]	21.01±0.01[bc]	12.38±0.23[ab]	11.52±0.29[d]
Wilted water hyacinth + Rice straw (10%) + Wheat flour (10%) + Molasses (10%)	9.83±0.37[d]	18.13±0.20[d]	11.87±0.08[cd]	14.17±0.20[b]
Wilted water hyacinth + Rice straw (10%) + Wheat flour (10%) + Rice bran (10%)	12.41±0.50[bc]	19.75±0.44[c]	12.74±0.16[a]	15.13±0.13[a]
Wilted water hyacinth+ Rice straw (10%) + Wheat flour (10%) + Rice bran (10%) + Molasses (10%)	12.59±1.20[b]	17.33±0.66[d]	12.39±0.25[ab]	11.76±0.15[cd]
LSD (P<0.05)	1.49***	1.60***	0.51**	0.50***
CV%	7.42	4.40	2.36	2.18
Grand mean	11.32	20.41	12.10	12.94

Note: CV: Coefficient of variation; LSD: Least significant difference followed by the same letter in a column are significantly different. ***= treatment means are significantly different at a 0.1% level of significance. In a column, the different letters represents data differs significantly at 5% level of probability.
a presented: Highest significant mean among treatments.
b presented: Second highest significant mean among treatments.
c presented: Significant mean after a and b.
d presented: Lowest significant means among treatments.

3.3. Palatability of Silage

The palatability of silage is one of the major criteria when considering its quality. The estimated left-over silage after 3 days of feeding is presented in Table 4. The pH value of 3.87 has the highest palatability percentage, i.e. 100%, in wilted water hyacinth + rice straw (10%) + wheat flour (10%) + rice bran (10%). The addition of rice straw primarily decreases the palatability percentage, followed by the addition of wheat flour and rice bran. The preservative content, pH, and cattle acceptance of silage are positively correlated [29]. The pH of water hyacinth silage without additives was 7.33, indicating low quality. However, silage with 15% maize bran or molasses added had a pH of 4.1 and 4.2, respectively, and was well-liked by goats and young steers [30].

Table 4. Effects of different feed additives on the palatability percentage of silage.

Treatments	Left over feed after 3 days of feeding (%)
Wilted water hyacinth + Rice straw (10%) (Control)	48.23±6.00[a]
Wilted water hyacinth + Rice straw (10%) + Rice bran (10%)	4.00±2.65[c]
Wilted water hyacinth + Rice straw (10%) +Wheat flour (10%)	8.00±5.29[bc]
Wilted water hyacinth + Rice straw (10%) + Molasses (10%)	15.93±10.50[b]
Wilted water hyacinth + Rice straw (10%) + Wheat flour (10%) + Molasses (10%)	3.53±2.60[c]
Wilted water hyacinth + Rice straw (10%) + Wheat flour (10%) + Rice bran (10%)	0.00±0.00[c]
Wilted water hyacinth+ Rice straw (10%) + Wheat flour (10%) + Rice bran (10%) + Molasses (10%)	3.40±3.40[c]
LSD (P<0.05)	11.79***
CV%	55.83
Grand mean	11.87

Note: CV: Coefficient of variation; LSD: Least significant difference followed by the same letter in a column are significantly different; ***= treatment means are significantly different at a 0.1% level of significance. In a column, the different letters represents data differs significantly at 5% level of probability.
a presented: Highest significant mean among treatments.
b presented: Second highest significant mean among treatments.
c presented: Lowest significant mean among treatments.

4. Conclusion

From the investigation, it can be concluded that a higher palatability of water hyacinth silage was recorded with rice bran, wheat flour, and molasses by decreasing the pH value. The food additives supplemented with water hyacinth lower pH, enhance odor, and improve palatability thereby increasing the nutrient content of silage. Hence, it can be used as a feed alternative to solve the problem of feed scarcity. However, multiyear trials with other different feed additives were recommended to check their validity as well as enhance silage feed value.

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