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The Effect of Adding *Saccharomyces cerevisiae* and Sulfur Mineral in Ammoniated Citronella Waste Basal Ration to Consumptions, Nutrient Digestibility, Milk Production, and Milk Quality of Etawa Crossbreed Goat

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ABSTRACT

Citronella waste (*Cymbopogon nardus L. Rendle*) is potentially used as a fiber source in livestock feed but contains a high lignin content. Processing through ammoniation and supplementation with probiotics and minerals is required to enhance digestibility in livestock feed. *Saccharomyces cerevisiae* and mineral sulfur are essential probiotics and minerals for improving rumen microorganism balance and optimizing fiber-digesting microorganisms. This study aims to examine the effect of *S. cerevisiae* and mineral sulfur supplementation on an ammoniated *Citronella* waste (ACW) basal diet to optimize rumen bioprocesses, thereby enhancing intake, nutrient digestibility, milk production, and milk quality in Etawa crossbreed goats. The study employed experimental methods involving PE goats, grouped based on milk production into four groups, each receiving one of four treatments (P0, P1, P2, P3). Testing with ANOVA (Analysis of Variance) and the differences obtained from the analysis of variance for the treatments were followed by the DMRT (Duncan's Multiple Range Test). The results indicate that supplementing *S. cerevisiae* and mineral sulfur supplementation on ACW had no significant effect on nutrient intake, yet it significantly improved nutrient digestibility. This combination also exhibited no significant impact on milk production and quality (protein, and lactose) but substantially affected milk fat content. In conclusion, using a combination of 0.5% *S. cerevisiae* and 0.3% mineral sulfur, along with including *Citronella* waste in the diet, can enhance the efficiency of nutrient utilization in crossbred Etawa goats.

Key words: Etawa Crossbreed Goats; *Saccharomyces cerevisiae*; Mineral Sulfur; *Citronella* waste; Nutrient digestibility; Rumen bioprocess

INTRODUCTION

The Peranakan Etawa (PE) goat, a cross between Indonesian Kacang and Indian Jamnapari goats, serves the dual purpose of meat and milk production (Kusuma and Irmansyah 2009; Hapsari et al. 2022). Goat milk is digested easily, as it has a favorable composition, including fatty acids, proteins, and bioactive compounds suitable for treating or preventing health problems (Zenebe et al. 2014; Rai et al. 2022). The nutrient quality of feeds is a basis for the improvement in the milk production of PE goats. For example, dietary fiber is rumen microbes' major energy source for rumen influences both milk production and quality in PE goats. This is mainly through the production of volatile fatty acids (VFAs) like acetate upon the breakdown of crude fiber. Acetate is converted into short-chain fatty acids within the mammary epithelial cells and used in milk fat synthesis (Wibowo et al. 2013). Yang et al. (2023) directly correlate with milk quality (Chilliard et al. 2003; Matamoros et al. 2023). Therefore, higher milk fat content is indicative of superior milk quality.

Fiber sources fed to ruminants are usually in forage form, either from grass, agricultural waste, or legumes. In Indonesia, more than 2000 tons of citronella is supplied as raw material for essential oils. Citronella (*Cymbopogon nardus*) waste is locally abundant as an agricultural by-product. In 2017, about 19,370 hectares of citronella cultivation yielded 2,340 tons of biomass per year, with an

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increasing trend (Sulaswatty et al. 2019). Citronella waste is commonly burned or discarded, whereas it can be used as a fiber feed source for ruminants. It also still contains various bioactive compounds that positively impact livestock productivity such as citronellal, geraniol and citronellol (Burt 2004), as well as cellulose (Fabiani et al. 2023).

However, it is high in crude fiber, especially lignin, which is negatively associated with digestion. Hence, the feed processing methods, such as ammoniation and probiotic fungal supplementation, are required to reduce the fraction of crude fiber and to increase feed digestibility (Wan Zahari et al. 2003; Zain et al. 2011; Chanjula et al. 2019; Fariani et al. 2021). Ammoniation results in increased nutrient digestibility but is still ineffective in further enhancing citronella waste utilization and optimizing strategies for maximal rumen microbial growth (Zain et al. 2011; Herawaty et al. 2013).

Incorporating *Saccharomyces cerevisiae* (*S. cerevisiae*) and sulfur minerals as microbial supplements may be one way of overcoming such limitations. *S. cerevisiae* acts as a Direct-Fed Microbial (DFM) in ruminants to alter the rumen ecosystem and diminish acidity by preventing the colonization of toxic entities through the enhancement of gut microbiota balance (Fuller 1992; Roberfroid 2000; Shim et al. 2010; Ban and Guan 2021; Kalita et al. 2023; Magnoli et al. 2024; Srifani et al. 2024). Besides, supplementation with sulfur minerals enhances microbial synthesis and cellulose digestibility (Zain et al. 2011; Zhao et al. 2020). Sulfur contributes to forming sulfhydryl amino acids, such as cysteine and methionine, and synthesizing microbial proteins important for fiber digestion. Additionally, it plays a major role in synthesizing vitamins such as thiamine, biotin, and coenzymes, including COASH, which enhance nutrient digestibility and *in vitro* microbial protein synthesis (Shafura et al. 2024).

Hence, this study evaluated the effects of adding *S. cerevisiae* and mineral sulfur to an Ammoniated *Citronella* Waste (ACW) diet on feed consumption, digestibility, milk production, and quality in vivo in PE goats.

MATERIALS AND METHODS

Animal Ethics

The collection of samples of the goats was conducted through randomized block design research, following the recommendations of animal ethics No. B/026/UN.16.06.3.8/TA.00.03-2024.

Description of the study area

This study was conducted at a commercial farm in Payakumbuh City, West Sumatra, Indonesia. The area has a tropical climate. The city is situated at an elevation ranging from 514 to 957 meters above sea level, with most of its region located in highlands. The average air temperature ranges between 22 and 28°C. Humidity levels are also relatively high, given its location in the highlands and proximity to mountainous areas.

Experimental design

This study involves 16 Peranakan Etawa dairy goats, aged 2 to 2.5 years, in their second and third lactation, each weighing approximately 60kg. Based on their milk production, the goats were grouped into four categories, each receiving one of the treatments (P0, P1, P2, P3). An experiment was carried out under the principles of Randomized Block Design. Four treatments and four replicates were considered. For all treatment groups, the diets were formulated to maintain a forage-to-concentrate ratio of 60:40 (National Research Council 2007). The treatments allocated during the period of the experiment are:

P0: 60% Roughage + 40% Concentrate
P1: 30% Ammoniated Citronella Waste (ACW) + 30% Roughage + 40% Concentrate
P2: P1 + 0.5% Saccharomyces cerevisiae
P3: P1 + 0.5% Saccharomyces cerevisiae + 0.3% Mineral Sulfur

Feeding management

Feeding sources

The materials employed in this research comprise Peranakan Etawa goats from an Indonesian farm named Toni Farm and ACW sourced from citronella distillation at Penta Lab in West Sumatra, Indonesia. Commercial *Saccharomyces cerevisiae*, pure sulfur minerals, and various pharmaceuticals are also used. Other dietary components include field grass collected in Payakumbuh City, tofu residue purchased from a factory, and finely ground corn and rice bran from a livestock feed store.

Feeding preparation

The preparation of citronella waste with ammoniation treatment was first chopped/cut citronella waste with a size of 5-10cm, then dissolved urea with water in a ratio of 1:1 with the amount of urea used, which was 4% of the dry matter of citronella. Then, the citronella waste was watered with urea solution, put into a plastic bag, and compacted until full. Then vacuumed to create an anaerobic atmosphere in the plastic containing the citronella waste and tied. Then, the plastic is stored at room temperature in a safe and shady place for 3 weeks. After 3 weeks, the plastic was opened, and the ACW was taken out and air-dried to remove excess ammonia. After air drying, the ACW was ready to feed livestock.

Rations

The experimental ration was formulated from feed ingredients consisting of field grass, ACW, finely ground corn, tofu residue, and rice bran. Supplements added to the ration included 0.5% commercial *S. cerevisiae* and 0.3% sulfur in its pure form, calculated based on the dry matter (DM) requirements relative to body weight (BW). The nutrient content analysis of the feed components used in the ration is shown in Table 1-3.

Feeding stage

Ration feeding was done 3 times daily with ration requirements calculated at 4% of body weight.

Adaptation Period

During this period, livestock were given the ingredients used to prepare the treatment ration. This process lasted for 8 days. It was intended that livestock get used to the treatment ration and experimental environment.

Preliminary Period

The preliminary period aimed to eliminate the influence of the previous feed (carry ever effect). In this period, which was carried out for 25 days, the treatment ration was given according to the needs of 4% dry matter based on body weight. Feeding in the preliminary period was adjusted to the research design, and the remaining feed was weighed and recorded. In the preliminary period, daily milk production was recorded. At the end of the preliminary period, body weight was weighed again to calculate the needs of the collection period.

Table 1: Formulation and nutritional content of rations (in %)

Feed Nutrients	Field grass	ACW	Refined corn	Tofu dregs	Rice bran	Mineral
DM	23.29	61.86	90.00	21.29	87.50	100
OM	91.43	84.45	95.08	91.72	85.00	-
CP	7.00	7.72	10.98	24.56	13.60	-
EE	2.00	2.03	3.67	5.43	6.00	-
CF	25.44	27.67	1.12	7.43	22.00	-
ASH	8.57	15.55	4.92	8.28	15.00	
NFE	48.84	41.66	70.90	54.32	30.91	-
TDN	55.00	53.81	80.00	84.54	67.00	-
NDF	58.61	69.93	68.24	39.73	75.19	-
ADF	37.79	44.45	3.21	21.65	43.98	-
Celullose	31.54	30.39	1.66	3.11	21.62	-
Hemicelullose	25.06	25.48	-	-	26.25	-
Lignin	4.20	10.38	1.63	1.80	10.55	-

DM=Dry Matter, OM=Organic Matter, CP=Crude Protein, EE=Ether Extract, CF=Crude Fiber, NFE=Nitrogen-Free Extract, TDN=Total Digestible Nutrients, NDF=Neutral Detergent Fiber, ADF=Acid Detergent Fiber.

Table 2: Formulation and nutritionak content 100% concentrate

Feed Ingredients	%
Tofu dregs	62.5
Refined corn	25
Rice bran	10
Mineral	2.5
Total	100
Nutrients (%)	
DM	47.06
OM	89.60
CP	19.46
EE	4.91
CP	7.12
ASH	7.90
NFE	54.77
TDN	79.54
NDF	49.41
ADF	18.73
Hemicelullose	4.52
Celullose	2.63
Lignin	2.59

Footnote is the same as Table 1.

Collection Period

In this period, feces samples were collected using the total collection method for 5 days. The number of feces that came out for 24 hours was collected, then weighed and mixed homogeneously, and 10% was taken as a sample. Furthermore, the feces samples were mashed and composited, then analyzed for nutrient content. For milk samples, milk quality measurements were made by taking 5% of milk production during the collection period; the milk samples were stored in the refrigerator and then analyzed with Lactoscan machine.

Variables

This study measures the effect of the additional *S. cerevisiae* and sulfur mineral on rations from citronella waste on: i) Feed Consumption, consisting of Dry Matter Intake (DMI), Organic Matter Intake (OMI), Crude Protein (CP), and Crude Fiber (CF), ii) Feed Digestibility, consisting of DMI, OMI, CP, and Fiber Fraction (consisting of Neutral Digestible Fiber (NDF), Acid Digestible Fiber (ADF), Cellulose, and Hemicellulose), and iii) Milk production and Milk quality (consisting of fat, protein, and lactose).

Table 3: Formulation and nutritional content of rations (%)

Feed Ingredients	Ration Treatments			
	P0	P1	P2	P3
Field grass	60	30	30	30
ACW	0	30	30	30
Refined corn	10	10	10	10
Tofu dregs	25	25	25	25
Rice bran	4	4	4	4
Mineral mix	1	1	1	1
Total	100	100	100	100
Saccharomyces cerevisiae	-	-	0.5	0.5
Sulfur	-	-	-	0.3
Nutrients				
DM	32.80	44.37	44.37	44.37
OM	90.70	88.60	88.60	88.60
CP	11.98	12.20	12.20	12.20
EE	3.16	3.17	3.17	3.17
CF	18.11	18.78	18.78	18.78
ASH	8.30	10.40	10.40	10.40
NFE	51.21	49.06	49.06	49.06
TDN	64.82	64.46	64.46	64.46
NDF	54.93	58.33	58.33	58.33
ADF	30.17	32.16	32.16	32.16
Celullose	20.73	20.39	20.39	20.39
Hemicelullose	16.09	16.21	16.21	16.21
Lignin	3.56	5.41	5.41	5.41

Footnote is the same as Table 1.

Data Analysis

DMRT (Duncan's Multiple Range Test) tested differences between treatment means. Table 4 shows the analysis of variance. Steel and Torrie (1980) provided the structure for the model equation for the design**.**

$$Yij = \mu + \tau i + \beta j + \sum ij$$

Description:

Yij = Observation result of i-th treatment and j-th replication
μ = Generalized mean value
τi = Effect of i-th treatment
βj = Effect of jth group
∑ij = Residual effect of i-th treatment and j-th replication
I = Number of treatments (1,2,3 and 4)
J = Group (1,2,3 and 4).

The differences obtained from the analysis of variance for the treatments were followed by the DMRT (Duncan's Multiple Range Test) (Steel and Torrie 1980).

Table 4: analyzing variance (ANOVA)

SoV	DF	SS	KT	F Calculated	F Table	
					0.05	0.01
Treatment (P)	(t-1) = 3	JKP	JKP/3	KTP/KTS		
Group (K)	(n-1) = 3	JKK	JKK/3	KTK/KTS		
Residues (S)	t (n-1)=9	JKS	JKS/3			

SoV=Souce of Variation; DF=Degrees of Freedom; SS=Sum of Squares; KT=Mean Square.

RESULTS AND DISCUSSION

Feed consumption

The overall effect of the treatment on consumption is presented in Table 5. Treatments have a significant impact on organic matter consumption compared to other consumption.

Table 5: Effect of treatment on consumption

Parameter	Treatments				
	P0	P1	P2	P3	SE
Dry matter (Kg)	2.424	2.494	2.496	2.504	0.020
Organic matter (Kg)	2.200a	1.927b	1.928b	1.933b	0.016
Crude protein (%)	28.752	28.182	28.304	28.182	0.260
Crude fiber (%)	43.866	43.382	43.570	43.382	0.476

Values with different alphabets in a row differ significantly (P<0.01).

Dry Matter Intake

This study observed that the dry matter intake demonstrated no significant difference (P>0.05), with average consumption ranging from 2,424 to 2,504kg daily. Despite the statistically insignificant effect, a positive increase in dry matter consumption was noted. Treatment P0 had lower intake than treatments P1, P2, and P3. This may be attributed to the absence of additional Ammoniated Citroenella Waste (ACW), *S. cerevisiae* and mineral sulfur, which enhance the feed's palatability and nutritional value. Lower palatability in feed leads to restricted consumption, which only meets the basic nutritional needs of the livestock (Simanihuruk and Sirait 2010).

HSD (Honestly Significant Difference)

HSD= r × SE × q_α (k, df)

Where:

- **r**: Number of replications of the mean in each group.
- **SE**: Standard Error, calculated as:

$$SE = \sqrt{\frac{MSE}{r}}$$

- **MSE**: Mean Square Error from the analysis of variance (ANOVA).
- **q**: Quantile value from the Studentized Range distribution or **q** distribution (the q-value is determined based on the confidence level and the degrees of freedom of the error term).
- **k**: Number of treatments being compared.
- **df**: Degrees of freedom from the MSE in ANOVA.

For treatment P1, ACW sustained dry matter intake because the ammoniation process improves the citronella waste's nutrient quality. Rahadi (2008) also highlighted the benefits of ammoniation, such as extending shelf life, enhancing digestibility, improving nutritional value and reducing lignin content (Sari et al. 2023).

In treatment P2, adding *S. cerevisiae* to the ACW also maintained dry matter intake in this study. The utilization of *S. cerevisiae* likely impacts nutrient digestibility, particularly fiber fractions, more than nutrient consumption itself. According to Cai et al. (2023), *S. cerevisiae* creates favourable conditions for the growth of cellulolytic bacteria, thereby increasing their population and enhancing digestion in the rumen. Further research by Tavares et al. (2021) indicates that *S. cerevisiae* helps increase the population of beneficial microbes in the rumen, which play a role in the fermentation of fiber and other organic materials in the feed.

Treatment P3, which combined ACW, *S. cerevisiae*, and mineral sulfur, also successfully maintained dry matter intake. Sulfur is critical in protein synthesis and enzymatic functions, supporting increased consumption. Research by Smock and Taylor (2020) states that combining probiotics and minerals can synergistically optimize dry matter consumption and livestock performance. This combination enhances the rumen environment, thus improving the palatability and digestibility of the ration.

Organic Matter Intake

In this study, organic matter intake showed highly significant differences (P<0.01) with a marked decrease in groups fed ACW supplemented with *S. cerevisiae* and sulfur (P1, P2, P3) compared to the control group (P0). The findings revealed that treatment P0 (60% Roughage + 40% Concentrate) resulted in the highest organic matter consumption at 2.20kg, surpassing other treatments. The elevated organic matter intake in treatment P0 can be attributed to several factors related to the ration composition, particularly the highest organic matter content of 90.70% in P0, which drove the high consumption of organic matter in this study. Additionally, the lignin content in P0 was the lowest among the treatments. High levels of lignin in feed can reduce the digestibility of other organic matter because lignin binds to other fibers, such as cellulose and hemicellulose, making it difficult for rumen microbes to digest (Ginting 2021). As a result, animals select less lignin-rich feed, reducing overall organic matter intake.

Treatment P1, involving the addition of ACW, may alter the taste and texture of the feed, making it less palatable to goats and thereby reducing organic matter intake. Factors that can influence intake include the nutritional quality and palatability of the feed (Li et al. 2022).

Treatment P2, with ACW and *S. cerevisiae*, also reduced organic matter intake. Although *S. cerevisiae* can enhance fermentation and digestion, its impact on organic matter consumption was not as favorable as P0 due to changes in feed characteristics. Ogbuewu and Mbajiorgu (2022) reported that the effect of *S. cerevisiae* supplementation on organic matter intake also varies depending on the dose, duration of supplementation, and the lactation phase.

Similarly, treatment P3, including ACW, *S. cerevisiae*, and mineral sulfur, also decreased organic matter intake. Adding mineral sulfur did not significantly enhance organic matter consumption, likely because its effects are more on digestion and microbial protein synthesis rather than feed palatability. Adding organic minerals to feed does not affect palatability, which is an important factor that determines feed intake (Imsya et al. 2020).

Crude Protein Intake

In this study, crude protein consumption did not show a significant effect (P>0.05), with average consumption levels ranging between 28.182 and 28.752%. The absence of a significant effect on crude protein consumption could be due to the almost equal protein content in the rations of each treatment, which ranged from 11.99 to 12.20%, thus not affecting crude protein intake in this study. These values indicate that although crude protein intake was slightly different in all treatments, there was no significant difference in intake. The National Research Council (2007) has reported that the crude protein requirement for goats generally ranges from 9 to 12% of the total feed fed, depending on the level of milk production. The average consumption of crude protein for goats in Peranakan Ettawa (PE) is 239.16± 0.32 g/head of cattle/day (Siska and Anggrayni 2021).

Supplements such as ACW, *S. cerevisiae*, and mineral sulfur can affect nutrient consumption differently depending on the dose and the specific condition of the ruminant. The combination of ACW, yeast and sulfur can work synergistically to increase crude protein digestibility, although it does not significantly increase protein consumption. Astuti et al. (2022) reported that this combination can improve nutrient utilization efficiency without increasing total feed intake.

Crude Fiber Intake

In this study, the crude fiber intake was found to have no significant effect (P>0.05), with average consumption rates ranging from 43.382 to 43.866%. This indicates that the use of ACW maintained crude fiber intake. Ammoniation in feed optimizes rumen pH, feed degradability, and microbial protein synthesis, potentially improving their health and productivity in intensive production systems (Belanche et al. 2021). Moreover, Rahadi (2008) noted that the benefits of ammoniation include extending shelf life, enhancing digestibility, improving nutritional value, and reducing lignin content (Sari et al. 2023). Livestock is more likely to consume feed with a less hard and more digestible crude fiber content. Therefore, the ammoniation of citronella waste gives a significant effect on reducing the crude fiber and lignin content of citronella waste (Solehudin et al. 2024). By adding ammonia (NH3) as a chemical agent will improve digestibility and increase the nitrogen (protein) content of the feed material (Hanafi and Nevy 2008).

The role of *S. cerevisiae* in this context may be more apparent in the digestion of crude fiber rather than its consumption. *S. cerevisiae* can enhance the cellulolytic bacteria population, increasing cellulolytic activity to digest fiber (Zain et al. 2011; Phesatcha et al. 2021a). This yeast's capability to boost microbial populations facilitates more efficient fiber breakdown, thus contributing to overall fiber digestibility in the rumen.

Digestibility

Table 6 shows the effect of all treatments on the digestibility of dry matter, organic matter, crude protein, and crude fiber (ADF, NDF, cellulose, and hemicellulose). The most significant effect was produced by treatment P2 and P3 on almost all measurements. In more detail, we explain as follows.

Dry Matter Digestibility

In the dry matter digestibility study, the results were highly significant (P<0.01), with treatment P3 showing the highest rate at 65.609% following the addition of ACW, *S. cerevisiae*, and mineral sulfur. The lowest digestibility observed in the control treatment (P0) at 63.171% can be attributed to the suboptimal availability of nutrients in treatment P0 without any supplementation, which did not sufficiently enhance microbial activity or rumen fermentation, thereby reducing digestibility. Tilman et al. (1998) noted that factors influencing dry matter digestibility include the amount of feed consumed, the rate of digest passage, and the type of nutritional content in the ration.

Table 6: Effect of treatment on digestibility

Parameters	Treatments				
	P0	P1	P2	P3	SE
Dry matter (%)	63.171^{c}	63.389^{c}	64.743^{b}	65.609^{a}	0.161
Organic matter (%)	64.360^{b}	64.429^{b}	65.469^{ab}	66.221^{a}	0.241
Crude protein (%)	64.511^{c}	66.983^{b}	69.472^{a}	70.939^{a}	0.492
NDF (%)	59.942^{bc}	61.684^{b}	66.524^{ab}	67.740^{a}	1.090
ADF (%)	51.899^{b}	52.323^{b}	56.371^{a}	57.161^{a}	0.971
Cellulose (%)	54.418^{b}	55.432^{b}	61.687^{a}	62.639^{a}	1.192
Hemicellulose (%)	62.458^{b}	64.037^{b}	70.537^{ab}	73.274^{a}	1.834

Different superscript alphabet in the same row shows differ significantly (P<0.01), while ^{a} and ^{ab} not significantly (P>0.05), ^{ab} with ^{b} and ^{bc} with ^{c} showed significantly different effect (P<0.05). ^{ab} with ^{c} dan ^{bc} showed highly significantly different effect (P<0.01).

Treatment P1, with the addition of ACW, slightly increased the dry matter digestibility to 63.389%. This may be due to active compounds like citronellal in the citronella waste, which possess antibacterial properties capable of reducing the population of pathogenic bacteria in the livestock's digestive tract. Fermented concentrate can positively impact the growth and health of ruminant livestock by modifying rumen fermentation patterns and increasing beneficial microorganisms (Lee et al. 2023). With a decrease in pathogenic bacteria, beneficial rumen microflora can flourish, thereby enhancing the efficiency of feed fermentation. Increased fermentation efficiency can improve feed digestibility (Busquet et al. 2006). Moreover, ammoniation can enhance plant waste's nutritional value and palatability by increasing its nitrogen content, though its effects are limited without microbial supplements such as yeast.

Treatment P2, with the addition of ACW and *S. cerevisiae*, significantly increased dry matter digestibility to 64.743%. This shows a more positive role of *S. cerevisiae* in this treatment, as it is supposed to enhance microbial activities within the rumen, increase the pH of the rumen, and ferment fibers more effectively (Robinson and Erasmus 2009; Lee et al. 2023). The result of all these would be to stimulate the population of fiber-digesting microbes and thus increase the digestibility of dry matter (Zain et al. 2011, 2024).

Adding mineral sulfur, *S. cerevisiae*, and ACW in P3 treatment further increased dry matter digestibility to 65.609%, This is supported by previous research (Noersidiq et al. 2023) which reported that *S. Cerevisiae* can improve dry matter digestibility. The mineral sulfur is crucial for synthesizing microbial proteins in the rumen, which aids in maximizing fermentation and fiber degradation. An adequate supply of mineral sulfur can optimize cellulose digestibility through the specific stimulation of cellulolytic bacteria (Bal and Ozturk 2006).

Organic Matter Digestibility

In the study on organic matter digestibility, treatment P3 showed a significantly different effect (P<0.05) compared to treatment P2 and a highly significant difference (P<0.01) compared to treatments P0 and P1. The control treatment (P0), without adding ACW, *S. Cerevisiae*, and mineral sulfur, exhibited the lowest organic matter digestibility (64.360%) due to rumen microbial activity relying solely on natural conditions without additional stimulants. Similar to dry matter digestibility in treatment P0, organic matter (OM) is closely linked with dry matter (DM) since OM constitutes the largest part of DM (Noersidiq et al. 2023), resulting in the lowest OM digestibility in treatment P0.

Treatment P1, with the addition of ACW, slightly improved organic matter digestibility (65.429%). ACW alone does not provide sufficient additional nutrients to enhance microbial activity significantly. The remaining active compounds, such as citronellol, geraniol, and citronellal in ACW, also fail to optimize the digestibility of nutrients. The small quantity of active compounds in citronella waste does not significantly impact nutrient digestibility. Furthermore, the ammoniation process is still not optimal in transforming the structure of hard fiber fractions into softer ones without the assistance of *S. cerevisiae* and mineral sulfur supplementation.

Treatment P2, with a basal feed containing 30% ACW and adding *S. cerevisiae*, exhibited an organic matter digestibility of 65.469%. It can enhance microbial activity and change the ecology in favour of better digestibility of nutrients by rumen microbes (Shim et al. 2010; Lee et al. 2023). However, others, such as Chaucheyras-Durand et al. (2008) and Cai et al. (2023) showed that *S. cerevisiae* boosts the fermentative activity of rumen microbes to optimize fermentation.

In treatment P3, the basal feed with 30% ACW plus the addition of *S. cerevisiae* and mineral sulfur is underlined; sulfur is an important element in the diet that must be provided to the rumen microbes for the synthesis of sulfur-containing amino acids, such as methionine and cystine. Methionine is formed as much as sulfur is available, making the presence of mineral sulfur crucial in livestock nutrition. Rumen fiber-digesting microbes also require sulfur for their growth (Zain and Jamarun 2010; Zain et al. 2024). According to Bal and Ozturk (2006), adding mineral sulfur to low-quality fiber feed materials can enhance fiber degradation in the rumen, reflected by increased organic matter digestibility.

Crude Protein Digestibility

The results of the study showed that crude protein digestibility in treatments P2 and P3 exhibited a highly significant difference (P<0.01) compared to treatments P0 and P1. Although the protein content in the experimental rations was relatively similar (11-12%), treatment P0 displayed the lowest crude protein digestibility (64.511%) due to the absence of supplements that could enhance microbial activity or rumen fermentation. Feed digestibility is the amount of food that can be digested, measured by the difference between consumed and excreted food substances in the feces (Prajayati et al. 2021).

In P1, with the addition of ACW, crude protein digestibility increased to 66.983%. The active compounds in ACW, such as limonene, are enzymatic stimulants in the digestive system. These compounds can stimulate the secretion of digestive enzymes, which improves fiber and organic matter digestibility in the rumen, thereby increasing crude protein digestibility in this study (Burt 2004).Additionally, ammoniation increases nitrogen content and reduces lignin in citronella waste, enhancing the digestible quality of the protein, though the effect remains limited without additional supplementation.

In treatment P2, adding ACW and *S. cerevisiae* further improved crude protein digestibility to 69.472%. *S. cerevisiae* enhances microbial activity in the rumen, aiding in the breakdown of dietary protein and increasing microbial protein synthesis because it boosted nutrient digestibility and microbial protein synthesis while lowering protozoal population (Phesatcha et al. 2021b).

Furthermore, in treatment P3, the combination of ACW, *S. cerevisiae*, and mineral sulfur increased crude protein digestibility to 70.939%. Sulfur is crucial in improving nitrogen utilization efficiency and microbial protein synthesis in the rumen (Supapong et al. 2019). Additionally, sulfur is essential for forming sulfur-containing amino acids, such as methionine, cysteine, and cystine, which are necessary for microbial protein synthesis (Elihasridas 2012).

Crude Fiber Digestibility

Neutral Detergent Fiber (NDF) and Acid Detergen Fiber (ADF)

The results showed that the P2 and P3 treatments produced highly significant different effects (P<0.01) from the P0 and P1 treatments. The digestibility of NDF and ADF in P0 was the lowest (59.942% and 51.899%) because the field grass and concentrates given were not enough to increase the activity of microbes that digest fiber. In the lack of additives that can improve fiber fermentation, NDF and ADF digestibility efficiency remains low. Ghorbani et al. (2002) and Król et al. (2022) stated that adding supplements such as direct feed microbial (DFM) could increase the activity of rumen microbes that are degrading fiber, thereby increasing fiber digestibility and overall feed utilization.

In treatment, although not significantly, P1 with 30% ACW basal feed slightly increased NDF and ADF digestibility to 61.684% and 52.323%. Ammoniation increases nitrogen content and reduces lignin in ACW, making the fiber more digestible. The ammoniation process serves to stretch fiber bonds and decrease lignin by 4-9% (Pamungkas et al. 2024), which will then be further degraded in the fermentation process.

In treatment P2, with a 30% ACW basal diet supplemented with *S. cerevisiae*, the percentage digestibility of NDF and ADF became 66.524 and 56.371%, respectively. This occurred because *S. cerevisiae* is responsible for increasing the activity of ruminal bacteria that produce cellulases, which hydrolyze the fibrous feed. *S. cerevisiae* supplementation improves the digestibility of NDF and ADF by maintaining ruminal pH at or above 6.0 and reducing methane production (Phesatcha et al. 2021c).Jouany and Morgavi (2007) elaborated that natural additives, such as *S. cerevisiae*, may be a response to antibiotic feed additives in ruminant diets by increasing fermentative activity in the rumen. In P3, which also had a 30% ACW basal diet but with the addition of *S. cerevisiae* and mineral sulfur, NDF and

ADF digestibility increased even further to 67.740 and 57.161%. Sulfur is essential in produce sulfur amino acids (Roche 2016) which are important in synthesizing the most beneficial microbes.

Cellulosa and Hemiselulosa

The study's results on the digestibility of cellulose and hemicellulose showed that the treatment of P2 and P3 showed a highly significantly different effect (P<0.01) from the treatment of P0 and P1. Cellulose and hemicellulose digestibility in the P0 treatment was the lowest at 54.418 and 62.458%. The P0 treatment with field grass and concentrate did not meet the high fiber fermentation efficiency demand, so cellulose and hemicellulose digestibility was low. The addition of enzymes that hydrolyze cellulose and hemicellulose increases digestibility (Van Soest 1994; Iannaccone et al. 2022). The digestibility of cellulose and hemicellulose in the P1 treatment, where ACW substituted 30% of the basal diet, was marginally higher at 55.432 and 64.037%, respectively, but not significantly. Ammoniation in treatment P1 increased nitrogen content and reduced lignin in ACW, making the fiber more digestible by rumen microbes. Ammonia also serves to break down lignin and cellulose bonds in plant cell walls so that fiber becomes more easily broken down by fiber-breaking rumen microbes and increases fiber digestibility (Suningsih and Ibrahim 2019).

In the P2 treatment, 30% ACW basal feed and adding *S. cerevisiae* increased cellulose and hemicellulose's digestibility to 61.687 and 70.537. Dai et al. (2023) stated *S. cerevisiae* can use oxygen for the glycolysis process, producing ethanol and CO2, resulting in anaerobic environmental conditions required for fermentation. The anaerobic environmental conditions can lead to anaerobic bacteria that digest fiber to grow well and increase cellulose and hemicellulose digestibility.

Furthermore, the P3 treatment with 30% ACW and the addition of *S. cerevisiae* and mineral sulfur improved the digestibility of cellulose and hemicellulose to 62.639 and 73.274%. Mineral sulfur is necessary to avoid a decrease in feed digestibility and increase nitrogen retention (Silva et al. 2014). A sufficient sulfur mineral supply can optimize cellulose digestibility by stimulating cellulolytic bacteria, thus increasing cellulose and hemicellulose digestibility (Bal and Ozturk 2006).

Furthermore, Table 7 illustrates the Effect of treatment on Milk production and Milk Quality, which in general showed a significant effect on the milk fat percentage in Etawa goats. The following is a further detailed explanation of the effect.

Milk production

It was found that milk production in this study showed a non significantly different effect (P>0.05); the average milk production was 0.737- 0.935kg. Although the effect is insignificant, it can be seen as a positive increase in milk production, this result is supported the previous study by Ogbuewu and Mbajiorgu (2023) that *S. cerevisiae* had small to moderate effect to milk yield in ruminant. This shows that using 30% ACW basal feed with the addition of *S. cerevisiae* and mineral sulfur can optimize the milk production of PE goats. The slight increased in milk production is caused by the increased nutrient digestibility, hence, it would enhance the milk nutrients supply. The remain bioactive compounds in ACW, such as geraniol, also helps increase milk production. The effect of geraniol on livestock is not only to improve digestive health through microflora modulation but also to increase immune response and decrease oxidative stress. Oxidative stress is one of the factors that can hinder livestock productivity, including milk production (Lian et al. 2024). *S. cerevisiae* also increases rumen microbial activity for fiber fermentation. With increased fiber digestibility, cattle get more energy from the same feed, which can then be diverted to milk production. The mineral sulfur is important in synthesizing essential amino acids such as methionine and cysteine, which are important for milk production. This is supported by research conducted by Stella et al. (2007), which showed a significant increase in milk production in Saanen goats with *S. cerevisiae* supplementation.

Table 7: Effect of treatment on Milk production and Milk quality

Parameter	Treatment				
	P0	P1	P2	P3	SE
Milk Production (Kg)	0.737	0.742	0.769	0.935	0.066
Milk fat (%)	6.607b	6.038b	7.679a	8.450a	0.297
Milk protein (%)	3.271	3.253	3.315	3.346	0.092
Milk lactose (%)	3.064	2.990	3.109	3.131	0.081

Values with different alphabets in the same row differ significantly (P<0.01).

Milk quality

Milk fat

Fat is the primary component determining goat milk's texture, flavor, and content. The main structure of goat milk fat consists of triglycerides, phospholipids, and free fatty acids. In this study, the treatment showed a very significant difference (P<0.01) in the P2 and P3 treatments compared to others. According to Yalçın et al. (2011), this is due to the addition of *S. cerevisiae*, which produces metabolites (B vitamins, amino acids, and enzymes).

The synthesis of amino acids such as methionine and cystine requires sulfur, which is important to form microbial proteins for the rumen to support milk fat production. *S. cerevisiae* combined with mineral sulfur increases the efficiency of rumen fermentation, acetate production, and nutrient absorption, contributing to increased milk fat content. Nasiri et al. (2019) also reported that milk fat concentration and total solids in milk were also higher in yeast-fed cows. Furthermore, the content of active compounds in ACW, such as Geraniol acetate and citronellol acetate, contribute to improving digestive health and lipid metabolism in cattle.

This positively impacts milk composition, especially regarding a better fatty acid balance. Better fatty acid content in milk improves livestock productivity and the nutritional quality of milk produced (Patra and Saxena 2009). However, the P1 treatment, which only used ACW, could not provide an optimal effect in increasing milk fat. The P0 treatment without additives such as *S. cerevisiae* and mineral sulfur has also not been able to increase milk fat.

Milk protein

This study showed a significantly different effect (P>0.05) on milk protein in each treatment. However, the

30% ACW basal diet with the addition of *S. cerevisiae* and mineral sulfur in the P2 and P3 treatments showed higher effect than the P0 and P1 treatments. Increased nitrogen availability in the rumen through deamination and microbial protein synthesis by adding *S. cerevisiae* can increase milk protein content (Sutardi et al. 2003). This directly impacts increasing milk production and quality, including milk protein content. Sumarmono (2022) stated that the average composition of fresh goat milk in Indonesia contains 3.2% protein, and this study obtained the same results.

Milk lactose

Milk lactose in this study also had no significant effect (P>0.05). However, the treatment using ACW basal diet with *S. cerevisiae* and mineral sulfur supplements (P3) had a positive increase than the other treatments. The results of this study contrasted with Yalçın et al. (2011) who in their study showed a slight decrease. This suggests that lactose yield may vary depending on factors such as dosage and experimental conditions. *S. cerevisiae* can also produce compounds that favor the growth of lactic acid bacteria that break down lactose, by improving the fermentation environment, and contributing to the development of better dairy products in terms of texture, flavor, and stability (Kurtzman and Fell 1998).

The results of Promkot and Wanapat (2009) showed no significant change in lactose levels between diets supplemented with 0.15% S and 0.4% S. The availability of sulfur in feed can affect the general health of goats. Goats that are healthy and free from mineral deficiencies will have optimal milk production. Although sulfur does not directly increase lactose, good goat health and productivity contribute to overall milk production, which includes lactose (McDowell 2003). The standard lactose content of goat milk in the tropics according to Devendra and Burns (1994) ranges from 3 - 6%, this study confirms these results.The lactose content in milk in this study did not show a significant effect (P>0.05). However, the treatment involving the use of ACW basal feed supplemented with *S. cerevisiae* and sulfur minerals (P3) demonstrated a positive increase compared to other treatments. These findings contrast with the results of Yalçın et al. (2011), who observed a slight decrease in their study. This suggests that lactose outcomes can vary depending on factors such as dosage and experimental conditions.

Conclusion

This study showed that the combination of ration treatment with ACW adding *S. cerevisiae* and mineral sulfur in P3 was similar to the control diet P0 in the consumption of dry matter and crude protein and decreased organic matter. The P3 combination increased the digestibility of dry matter, organic matter, crude protein, NDF, ADF, cellulose, and hemicellulose to the highest level compared to other treatments. This proves that ACW could be a fiber source that is easier to digest and improves palatability. *S. cerevisiae* enhances the activity of rumen microbes in digesting fiber and protein, while mineral sulfur promotes the synthesis of essential microbial proteins for fiber fermentation. The P3 combination also had a positive effect on milk production and milk quality. A milk production increased in the treatment group with ACW adding *S. cerevisiae* and mineral sulfur supplementation. Milk composition, such as fat, protein, and lactose increased in the treatment group.

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Data availability statement: Raw data were generated at Andalas University. The derived data supporting this study's findings are available from the corresponding author on request.

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