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# Effect of Using Yeast, Fibrolytic Enzymes and Their Mixture on I*n Vitro* Ruminal Fermentation Characteristics

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**Abstract**

*IN VITRO* studies were carried out to investigate the effect of using yeast (Y), fibrolytic enzymes (FEN) and their mixture (Y+FEN ratio 1:1) on rumen fermentation using rumen fluid. Three levels of each additive were used (1, 2, and 3 g/kg diet). At each level, a sample (300 ±5 mg) of the contained clover hay (40%) and concentrate mixture (60%) was weighed into 125 mL glass bottles (six bottles per treatment) and two blank bottles. Each of these bottles was filled up with 40 ml of a mixture of rumen fluid and buffer solution (1:3 v/v). After 24 hours of incubation at 39°C, *in vitro,* total gas production (GP), dry-matter disappearance (IVDMD), organic-matter disappearance (IVOMD), and $CO_2$ were recorded. The results showed that by adding 2g of FEN, 3 g of Y, and 3g of Y+FEN, the concentration of Short chain fatty acids (SCFA), Ammonia ($NH_3$-N), $CO_2$, and GP levels increased significantly ($P<0.05$). Treatment 3g (Y+FEN) recorded the highest values of SCFA (1.46 mmol/g DM), $NH_3$-N (6.96 mg/dl), and gas production (123 ml/g). The highest concentration of $CO_2$ was detected at Y(3g), FEN(2g), and Y+FEN (1g) (67.46, 67.85, and 68.16), respectively. Significant ($P<0.05$) increase in the digestibility of NDF, ADF, ADL, hemicellulose, cellulose, DM, OM, CP, and CF of treatments FEN (2g) and Y (3g) and Y+FEN (2g). It is recommended to utilize yeast (3 g/kg diet) and fibrolytic enzyme (2 g/kg diet) or their mixture (1:1) at 2 g/kg diet in ruminant animal feed to create favorable rumen conditions.

**Keywords:** *in vitro*, yeast, fibrolytic enzymes, ruminants, fiber.

## Introduction

Ruminant production and feeding depend mainly on fodder fiber, which is a crucial component of ruminant diets. Among these components is cellulose, which is indigestible by internal enzymes except for microorganisms in rumen. Livestock producers have looked into alternative methods of improving animal performance [1]. There are a variety of feed additives to improve feed utilization [2]. The diets supplemented with yeast culture and fibrolytic enzymes improved rumen fermentation in buffalo, which was reflected in an increase in feed utilization [1]. Bennett et al., [3] found that the increase in bacteria utilizing lactate-stabilizing pH increases volatile fatty acid (VFA) concentration. Also, it has been found that adding yeast culture and fibrolytic enzymes to bovine diets improves feed intake, performance, cellulose decomposition, and nutritional digestibility [4]. Exogenous enzymes accelerate feed digestion and boost ruminal enzymatic activity and capacity once they reach the rumen [3].

Exogenous fibrolytic enzymes received more attention as ruminant nutrition additives to enhance the digestion of fibrous diets. Adding fibrolytic enzymatic supplements increased gas production

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and butyrate concentration, lowered ruminal pH, and enhanced DM and fiber degradation in sheep [5].

Researches have stated that the improvement in feed intake might be related to greater ruminal fiber digestion, appears to be the cause of the advantageous effects of fibrolytic enzymes in ruminant diets [4].

The mode of action of Yeast *Saccharomyces cerevisiae* is the development of an anaerobic and stable environment that speeds up the growth of two important types of ruminal bacteria (fibrolytic) [3] Yeast products may have an impact on alternating ruminal fermentation, as they promotes the development and activity of fibrolytic bacteria, which enhances fiber decomposition. Additionally, it increased total volatile fatty acids (VFA) in cow rumen. Meanwhile, it increased propionate levels and caused a fall in the proportion of acetate to propionate (A:P) ratio in bovine rumen [6].

For ruminants, yeast culture (*Saccharomyces cerevisiae*) has been extensively used as a dietary supplement. More DM and NDF digestion, as well as higher DMI and milk production, are among the advantages of utilizing *S. cerevisiae* [4].

The purpose of the present study was to determine the effects of using yeast, fibrolytic enzymes, and their mixture (1:1) as feed additives in a balanced diet (*in vitro)* on rumen fermentation and rumen parameters.

## Material and Methods

*Experimental feeds*

In this experiment, feed additives (Y and FEN) were assessed for their efficacy in improving the ruminal utilization of feed. Three levels of fibrolytic enzymes, or yeast or their mix (1:1) from yeast and fibrolytic enzymes, were added to the total mixed ratio (TMR), which served as a substrate. Rumen fluid was taken from the rumens of slaughtered buffalo that had been fed clover hay to get the rumen microorganisms The treatments were: control group (C) received TMR without enzymes or yeast; treatment 1 (T1) received TMR with enzyme (1,2,3 g/kg diet); treatment 2 (T2) received TMR with yeast (1,2,3 g/kg diet); and treatment 3 (T3) received TMR with a mixture of enzyme and yeast in a ratio of 1:1 at levels (1,2,3 g/kg diet). The chemical analyses of basal rations and those of formulated diets were presented in Tables 1 and 2, respectively. Saccharomyces cerevisiae dry live yeast 1 x 1010 cell/gram (Pro-Bio-Fair) and the multi-enzyme feed additive Polyzym®, commercially available in powder form each gram of multi-enzyme contains 50 standard phytase units, 750 protease units, 400 cellulase units, 4000 xylanase units, 200 beta-glucanase units, 150 amylase units, 50 lipase units, 200 mannanase units, 400 glucosidase units, and 240 pectinase units.

*Chemical analysis*

Dry matter (DM) was measured by drying the samples at 105 °C for 24 hours, and ash content was obtained by the combustion of dried samples in a muffle furnace at 550 °C for a period of eight hours [7]. The nitrogen (N) level was measured using the Kjeldahl technique [7]. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined using an ANKOM fiber analyzer [8]. The feed was analyzed for proximate analyses by AOAC [7], and the nitrogen-free extract was computed using the difference. Non-fiber carbohydrate (NFC) was estimated using the following equation: NFC (%) = [100− [NDF(%) +CP (%)+CF (%)+ ash(%)]] [9]; where NDF is Neutral detergent fiber, crude fat (CF) and crude protein (CP).

*In vitro ruminal fermentation*

Two days before starting the experiment, for every level (roughage + concentrate at a ratio of 40 to 60%), 300 ±5 mg of feed sample (TMR) was precisely weighed and placed into six identical 125 mL glass bottles, accompanied by 2 blank bottles. A buffer solution was made according to McDougall [10] and was prepared before the addition of rumen fluid; then bottles were filled with 40 ml of a mixture of rumen fluids: buffer solution 1:3 (v/v) and constantly purged with $CO_2$ at 39 °C during sample inoculation. The rumen fluid was collected from a slaughterhouse for buffalo. The collected rumen fluid has been mixed into a bottle of 1 L with an O2-free headspace and immediately transported to the laboratory at 39 °C within 30-45 min. Upon arrival at the laboratory, the rumen fluid has been filtered through six layers of cheesecloth to eliminate large feed particles. The buffer solution was added to the rumen fluid at a ratio of 4:1. Forty mL of this inoculum was used for in vitro fermentation [11], and then the headspace of each bottle was flushed with $CO_2$ and closed. The initial pH of the inoculums ranged from 6.8 to 6.9, according to Ismail et al. [ 12].

*Dry matter degradability measurement*

Dry matter degradability (% DMD) was measured as the difference in DM amount before

and after 48 hours of incubation (DM content x 100). The residuals of NDF and ADF remaining after fermentation were analyzed using the same procedures as feed component analysis. The degradation of NDF and ADF was estimated by multiplying the difference between the sample's concentration before and after incubation by 100.

*Gas production estimation*

Following 24 hours of sample incubation, the displacement of the syringe piston linked to the serum flasks was used to quantify total gas production (GP). The gas generated was estimated by deducting the gas produced in blank vessels from the total gas produced in the bottles at the end. Where GP is the net GP in mL from 200 mg of dry sample after 24 hours of incubation, 2.2 mg/mL is a stoichiometric factor that represents the mg of C, H, and O necessary to produce 1 mL of short-chain fatty acid (SCFA) gas [13].

*Rumen pH, ammonia and total volatile fatty acid*

After 24 hours of incubation, the filtrated rumen liquid from each sample was examined further. The pH of the rumen fluid was measured using a pH meter, and the quantitative measurement of ammonia concentration was carried out using the Nessler technique adapted by Szumacher-Strabel and Cieslak [14]. In contrast, the total volatile fatty acids (TVFAs) were quantified according to Barnett and Reid [15].

*Calculations and Statistical analysis*

The in vitro organic matter degradability (OMD, g/kg OM) and other nutrients were calculated based on [16]. Short-chain fatty acid (SCFA) concentrations were estimated according to the following equations [17]: OMD = 14.88 + 0. 889 GP + 4.5 CP (%) + 0.0651 ash (%), SCFA (mmol/200 mg DM) = -0.00425 + 0.0222 * GPMCP (mg/g DM) = mg d DM- GP*2.2.

Data were statistically analyzed using the general linear model procedure of [18]. SPSS software for Windows was used. The differences among means were separated according to Duncan's New Multiple Range tests [19].

## Results and Discussion

*Fermentation characteristics*

Results in **Table 3** showed that at the addition of 2g (FE) and 3 g (YE) and all levels of their mixture, the concentration of SCFA, $NH_3$-N, $CO_2$, and gas production increased significantly ($P<0.05$), while the pH value and $CH_4$ of ruminal liquor was reduced insignificantly ($P > 0.05$) compared with the control group. Groups receiving 3g of Y+FEN recorded the highest values of SCFA (1.46 mmol/g DM), NH3-N (6.96 mg/dl), and gas production (123 ml/g). The higher ($P<0.01$) concentrations of $CO_2$ at 3g of (Y) and 2g of (FEN) and 1g of their mixture were (67.46, 67.85, and 68.16), respectively, while the lowest ($P>0.05$) pH and CH4 at 3g of (Y) and 2g of (FEN) and 1g of (Y+FEN) were detected.

Similar results were obtained by Abou-Seri et al., [1] who found that the concentrations of TVFA and $NH_3$-N were higher ($P<0.05$) in daily buffalo diets supplemented with Y and FEN than in the control group ($P > 0.05$), and the pH value of rumen liquor dropped insignificantly ($P > 0.05$). The same results were noticed by [20], who found that buffalo bulls fed TMRs with fibrolytic enzyme supplementation had lower ($P > 0.01$) rumen pH values and higher ($P< 0.01$) concentrations of TVFA and $NH_3$-N in rumen liquor.

Increasing molar proportions of acetate with FEN supplementation is in line with Beauchemin et al. [21] who reported that cows fed a modest dosage of FEN had greater ($P<0.05$) proportions of acetate compared to the control group. The observation of increased overall digestion with a low degree of FEN supplementation is supported by the higher fraction of acetate.

Additionally, it was shown that adding exogenous enzymes to dairy cow diets improved fiber digestibility throughout the entire gastrointestinal system and the rumen [22].

Chaucheyras-Durand et al. [23] suggested that yeast stimulates rumen bacteria and increases the use of lactic acid and ammonia, resulting in a moderate rumen pH and an increase in microbial population activity, which improves rumen carbohydrate digestion and protein microbial synthesis.

Vallejo-Hernández et al. [24] found that the main gases produced during fermentation in the rumen are $CH_4$ and $CO_2$. As a result, the additives' inability to affect gas production and reduced proportional $CH_4$ output are evidence that they were successful in lowering $CH_4$ production.

*Nutrients degradability*

Results in **Table 4** showed a significant ($P<0.05$) increase in the degradability of DM, OM, CP, and CF with the addition of 2 g of FEN or 3 g of Y. It could be noticed that the addition of yeast was more effective than FEN compared with

the control treatment. Meanwhile, the addition of Y+FEN had a notable effect. On the other hand, the degradability of EE was not affected by any addition.

The present results coincide with those obtained by Yang et al., [25], who found that yeast addition to buffaloes' diets enhanced ruminal microbial enzyme activity and consequently increased digestibility of CP and CF through the beneficial effect of lactic acid bacteria in the gastrointestinal tract of buffaloes.

Similarly, Rajamma et al., [20] found that feeding male buffaloes calves a mixture of yeast and enzymes significantly increased digestibility of OM. Also comparable results were obtained by Yang et al., [22]. Comparing the supplemented (EFN) and yeast culture to the control group, the study showed no discernible difference in the digestion of nutrients [4].

The digestion of CP, EE, and CF was improved ($P<0.05$) when FEN was added to TMR fed to buffalo bulls. Increased microbial colonization was linked to the increases in digestibility and dry matter disappearance brought about by enzymatic treatment [1]. According to Beauchemin et al., [21] exogenous fibrolytic enzymes could help allow for a more thorough digestion of the feed by exposing more cell wall sites for bacterial adhesion.

According to Abou-Seri et al.[1], yeast culture increases gut health, CP, and CF digestibility. Rumen maturation and the beneficial activities of lactic acid bacteria in the gastrointestinal tract modify microbial enzyme activities in buffaloes.

*Degradability of fiber fractions*

Results in **Table 5** showed that the addition of 2g (FEN) or 3 g (Y) increased significantly ($P<0.05$) the degradation of NDF, ADF, ADL, hemicellulose, and cellulose compared with the control. Yeast addition was more effective than FEN. Meanwhile, an improvement in degradability was noticed with the mixture (Y+FEN) at 2g, which was higher than with Y or FEN individually.

Similar results were obtained by Kung et al., [26], who found that after 12 hours of *in vitro* incubation, the NDF was digested significantly more with enzyme-treated food compared to the control.

Rajamma et al., [20] found that feeding male buffalo calves a yeast and enzyme mixture significantly increased the digestibility of OM, NDF, and ADF. The same results were obtained by Yang et al., [22] using an *in vitro* study taking PH values into consideration (**Table 3**). The improvement in digestibility was noticed with the low PH values. In this connection, Gashe et al [27] found that most commercial fibrolytic enzymes have optimum PH values (4.5–5.5). Exogenous enzymes may directly hydrolyze ingested feed in the rumen [28].

Beauchemin et al.[29] and Yang et al. [30] showed that ruminal starch digestibility was reduced when ruminal PH was depressed as a result of smaller forage particle sizes. Calsamiglia et al. [31] noticed that when ruminal pH fell below 6.28, the ruminal fermentation pattern most likely altered from the digestion of structural carbohydrates to the digestion of non-structural carbohydrates, primarily starch.

## Conclusion

From the results obtained during this study, it could be advised to use yeast (3g) and fibrolytic enzyme (2g) or their mixture (1:1) at 2g in the feed of ruminant animals for getting good rumen conditions. In conclusion, the current study's observations of the effects of supplementing with yeast and fibrolytic enzymes, as well as their interactions, on in vitro gas production and the disappearance of DM and OM, showed that doing so may enhance the fermentation process for in vitro gas production of low-quality roughages.

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*Conflict of Interest:*

The authors do not have any conflicts of interest to declare

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*Author`s contribution :*

E-H. A.F. E. did the experimental work as part of his master thesis; M. A. H.; G. M. A.; A.M. E-S. and H.A.F. R. supervised the work, contributed to writing the manuscript, and revised it. All authors contributed to the present work.

**TABLE 1. Chemical composition of ingredients feed.**

| Chemical analysis % | Feed Ingredients | | | | |
|---|---|---|---|---|---|
| | Concentrate mixture | | | Roughage | |
| | Yellow corn | Soya bean meal | Wheat Bran | Wheat Straw | Clover |
| DM | 90.97 | 91.08 | 86.92 | 94.72 | 91.01 |
| Crude protein(CP) | 7.8 | 44.8 | 11.84 | 1.67 | 14.3 |
| Crude Fiber(CF) | 2.4 | 5.8 | 10.80 | 36.98 | 29.9 |
| Ether extract(EE) | 4.2 | 1.34 | 2.67 | 0.43 | 2.34 |
| ADF% | 11.66 | 27.88 | 18.78 | 55.01 | 44.48 |
| NDF % | 40.79 | 45.17 | 42.36 | 74.95 | 65.22 |

**TABLE 2. formulation and chemical analysis of basal ration DM:**

| Feed ingredients % | | Chemical analysis ration | |
|---|---|---|---|
| Clover | 20 | Dry matter, DM | 91.3 |
| Wheat straw | 20 | | |
| Yellow corn | 36 | Crude protein, CP | 12.8 |
| Soya bean meal | 12 | Ether extract, EE | 2.5 |
| Wheat bran | 12 | Crude fiber, CF | 16.2 |
| | | Neutral detergent fiber, NDF | 53.2 |
| | | Acid detergent fiber, ADF | 29.7 |

**TABLE 3. Effect of using yeast or/and fibrolytic enzymes ration on rumen parameters**

| | | Rumen Parameters | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Treatment** | **Level (g)** | **pH** | **$NH_3N$**,mg/dl | **SCFA mmol/g DM** | **GP/DM** | **GP/1g** | **$CH_4$** | **$CO_2$** |
| **C** | **0** | $6.28^{a}$ | $3.68^{f}$ | $1.22^{f}$ | $101.5^{g}$ | $275.6^{f}$ | $22.11^{a}$ | $65.05^{f}$ |
| | **1** | $6.13^{b}$ | $4.08^{e}$ | $1.28^{e}$ | $107^{f}$ | $288.2^{e}$ | $21.88^{ab}$ | $66.25^{e}$ |
| **T1 (FEN)** | **2** | $6.11^{b}$ | $4.46^{d}$ | $1.32^{d}$ | $111^{e}$ | $299.4^{e}$ | $21.83^{ab}$ | $66.34^{e}$ |
| | **3** | $6..09^{b}$ | $4.20^{de}$ | $1.28^{de}$ | $109^{ef}$ | $289.4^{d}$ | $21.63^{ab}$ | $67.46^{bc}$ |
| | **1** | $5.99^{c}$ | $4.39^{de}$ | $1.30^{de}$ | $109.5^{e}$ | $293.9^{de}$ | $21.60^{ab}$ | $67.44^{bc}$ |
| **T2 (Y)** | **2** | $5.96^{c}$ | $5.64^{c}$ | $1.39^{c}$ | $114^{d}$ | $313.8^{c}$ | $21.43^{b}$ | $67.85^{ab}$ |
| | **3** | $6.00^{c}$ | $6.35^{b}$ | $1.40^{bc}$ | $117^{c}$ | $316.1^{bc}$ | $21.85^{ab}$ | $66.99^{d}$ |
| **T3 (FEN+Y)** | **1** | $5.92^{c}$ | $6.96^{a}$ | $1.43^{abc}$ | $118^{bc}$ | $322.8^{ab}$ | $21.39^{b}$ | $68.16^{a}$ |
| | **2** | $5.96^{c}$ | $6.83^{a}$ | $1.44^{ab}$ | $120^{a}$ | $326.1^{a}$ | $21.67^{ab}$ | $67.59^{bc}$ |
| | **3** | $5.99^{c}$ | $6.81^{a}$ | $1.46^{a}$ | $123^{a}$ | $330.1^{a}$ | $21.89^{ab}$ | $67.35^{cd}$ |

C: Control received TMR, T1: TMR with enzyme; T2: TMR with yeast; T3: TMR with the mix between enzyme and yeast; PH; NH3: Ammonia; SCFA: Short chain fatty acids; and GP/DM Gas production/dry matter.

**TABLE 4. Effect of using yeast or/and fibrolytic enzymes on *in vitro* nutrients' degradability.**

| | | Nutrients degradability | | | | |
|---|---|---|---|---|---|---|
| **Treatment** | **Level (g)** | **DMD** | **DOM** | **CP** | **CF** | **EE** |
| **C** | **0** | 30.4$^{d}$ | 40.43$^{f}$ | 59.22$^{e}$ | 58.33$^{c}$ | 67.67$^{a}$ |
| | **1** | 37.72$^{bc}$ | 45.47$^{cd}$ | 61.58$^{d}$ | 60.05$^{c}$ | 68.21$^{a}$ |
| **T1 (FEN)** | **2** | 39.68$^{b}$ | 47.12$^{c}$ | 65.73$^{a}$ | 63.07$^{ab}$ | 70.25$^{a}$ |
| | **3** | 33.92$^{cd}$ | 42.67$^{e}$ | 63.24$^{bcd}$ | 61.10$^{bc}$ | 70.00$^{a}$ |
| | **1** | 36.15$^{bc}$ | 44.92$^{d}$ | 62.40$^{cd}$ | 60.73$^{bc}$ | 69.80$^{a}$ |
| **T2 (Y)** | **2** | 52.31$^{a}$ | 59.52$^{ab}$ | 64.81$^{ab}$ | 64.35$^{a}$ | 68.78$^{a}$ |
| | **3** | 54.52$^{a}$ | 61.27$^{a}$ | 64.45$^{abc}$ | 64.81$^{a}$ | 69.95$^{a}$ |
| | **1** | 54.37$^{a}$ | 60.33$^{ab}$ | 65.56$^{a}$ | 64.82$^{a}$ | 69.39$^{a}$ |
| **T3 (FEN+Y)** | **2** | 53.92$^{a}$ | 59.06$^{b}$ | 65.15$^{ab}$ | 65.82$^{a}$ | 69.33$^{a}$ |
| | **3** | 54.15$^{a}$ | 61.07$^{ab}$ | 65.03$^{ab}$ | 66.03$^{a}$ | 69.74$^{a}$ |

C: control received TMR, T1: TMR with enzyme; T2: TMR with yeast; T3: TMR with mix between enzyme and yeast; DOM: Digestible Organic matter; DMD: Dry matter disappearance; CP crude protein; CF crude fiber; EE Ether extract.

**TABLE 5. Effect of using yeast or/and fibrolytic enzymes on degradability of fiber fractions.**

| **Treatment** | | **Fiber fractions %** | | | | |
|---|---|---|---|---|---|---|
| | **Level(g)** | **NDF** | **ADF** | **ADL** | **Hemicell** | **Cell** |
| **C** | **0** | 29.65$^{d}$ | 21.08$^{e}$ | 6.32$^{e}$ | 8.57$^{d}$ | 14.76$^{e}$ |
| | **1** | 36.35$^{c}$ | 25.29$^{d}$ | 8.84$^{cde}$ | 11.05$^{bcd}$ | 16.45$^{de}$ |
| **T1 (FEN)** | **2** | 40.75$^{b}$ | 28.98$^{bc}$ | 11.34$^{abc}$ | 11.78$^{bcd}$ | 17.63$^{cd}$ |
| | **3** | 37.66$^{c}$ | 26.72$^{cd}$ | 9.80$^{bcde}$ | 10.94$^{bcd}$ | 16.92$^{cde}$ |
| | **1** | 40.27$^{b}$ | 31.07$^{ab}$ | 10.77$^{abcd}$ | 9.20$^{cd}$ | 20.30$^{b}$ |
| **T2(Y)** | **2** | 41.71$^{b}$ | 31.85$^{ab}$ | 7.55$^{de}$ | 9.86$^{cd}$ | 24.30$^{a}$ |
| | **3** | 46.33$^{a}$ | 33.51$^{a}$ | 9.58$^{cde}$ | 12.82$^{bcd}$ | 23.93$^{a}$ |
| | **1** | 45.72$^{a}$ | 32.34$^{a}$ | 13.44$^{ab}$ | 13.37$^{abc}$ | 18.90$^{bc}$ |
| **T3 (FEN+Y)** | **2** | 48.02$^{a}$ | 30.85$^{ab}$ | 13.72$^{ab}$ | 17.17$^{a}$ | 17.13$^{cd}$ |
| | **3** | 46.08$^{a}$ | 31.64$^{ab}$ | 13.55$^{a}$ | 14.45$^{ab}$ | 18.28$^{bcd}$ |

C: control received TMR, T1: TMR with enzyme; T2: TMR with yeast; T3: TMR with mix between enzyme and yeast; ADF: Acid detergent fiber; NDF: Neutral detergent fiber; ADL: Acid detergent lignin; Hemicell: Hemicellulose; Cell: Cellulose

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## تاثير اضافة الخميرة و الانزيمات المحللة للالياف على اداء الحيوانات الحلابة

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أجريت دراسات فى المختبر لمعرفة تأثير استخدام الخميرة (Y) والإنزيمات المحللة للالياف (FEN) وخليطهما بنسبة (1:1)(Y+FEN) على تخمر الكرش باستخدام سائل الكرش. تم استخدام ثلاثة مستويات من كل مادة مضافة (1، 2، 3 جم/كجم علف). في كل مستوى، تم وزن عينة (300 ± 5 مجم) من خليط تبن البرسيم والمركز (40:60٪) في زجاجات زجاجية سعة 125 مل (6 زجاجات / معالجة) وزجاجتين فارغتين. تمت تعبئة كل زجاجة بـ 40 مل من خليط من سوائل الكرش والمحلول المنظم (1:3 حجم / حجم). بعد 24 ساعة من الحضانة عند 39 درجة مئوية، تم تسجيل اختفاء المادة الجافة في المختبر (IVDMD)، واختفاء المادة العضوية (IVOMD)، وإجمالي إنتاج الغاز (GP)، وثاني أكسيد الكربون. أظهرت النتائج أن إضافة 2 جرام من 3 ،FEN جرام من Y، وفي جميع مستويات خليطهم، زاد تركيز CO2 ،NH3-N ،SCFA، و GP بشكل ملحوظ (P<0.05). سجلت المعالجة 3 جرام (Y+FEN) أعلى قيم (1.46 SCFA مليمول/جم 6.96) NH3-N ،(DM مجم/ديسيلتر)، وإنتاج الغاز (123 مل/جم). تم اكتشاف أعلى تركيز لثاني أكسيد الكربون عند (3 Y جم)، و(2 FEN جم)، و(1 Y + FEN جم) (67.46، 67.85، و68.16)، على التوالي. زيادة كبيرة (P <0.05) في قابلية هضم NDF وADF وADL والهيمسيلولوز والسليلوز DM وOM و CP وCF للمعالجات (2g FEN) و(3g Y) و(2g Y+FEN). يوصى باستخدام الخميرة (3 جم/كجم علف) والإنزيم الليفي (2 جم/كجم علف) أو خليطهما (1:1) بمعدل 2 جم/كجم علف في علف الحيوانات المجترة من أجل خلق ظروف مناسبة للكرش.

**الكلمات الدالة:** في المختبر؛ خميرة؛ الانزيمات المحللة للالياف،الكرش التخمير.