Research Article

The Effect of Natural Additives as Precursors in the Silage Process to Improve the Quality of Oil Palm Fronds as Ruminant Feed: An *In Vitro* Study

Dewi Febrina[1,2*], Rahmi Febriyanti[1], Jepri Juliantoni[1], Sadarman[1,2], Syukria Ikhsan Zam[3], Novia Rahim[4], Novia Qomariyah[2,5], Roni Pazla[6]

[1]Department of Animal Science, Faculty of Agriculture and Animal Sciences, Universitas Islam Negeri Sultan Syarif Kasim Riau, Indonesia. Jl. H. R Soebrantas KM 15 No 155 Tuahmadani Tampan-Pekanbaru 28293 Indonesia; [2]Animal Feed and Nutrition Modelling, Research Group, Faculty of Animal Science, IPB University, Bogor, Indonesia; [3]Department of Agriculture, Faculty of Agriculture and Animal Sciences, Universitas Islam Negeri Sultan Syarif Kasim Riau, Indonesia. Jl. H.R Soebrantas KM 15 No 155 Tuahmadani Tampan-Pekanbaru 28293 Indonesia; [4]Faculty of Education and Teacher Training Universitas Islam Negeri Sultan Syarif Kasim Riau, Indonesia. Jl. H. R Soebrantas KM 15 No 155 Tuahmadani Tampan-Pekanbaru 28293 Indonesia; [5]Research Center for Animal Husbandry, National Research and Innovation Agency (BRIN), Cibinong Science Center, Bogor District 16911, West Java, Indonesia; [6]Department of Nutritional Science and Feed Technology, Faculty of Animal Husbandry, Andalas University, Padang, West Sumatera.

Abstract | This study aimed to evaluate the nutritional quality of oil palm frond silage (OPFS) with natural additives as precursors, focusing on digestibility, rumen fermentation (*in vitro*), and bioactive compound content. A completely randomized design was employed, involving four treatments with five replications. The treatments included: PM = OPFS with poultry manure; BF = OPFS with buffalo faeces; EBAF = OPFS with empty bunch ash filtrate; and RHAF = OPFS with rice husk ash filtrate. Data were analyzed using analysis of variance (ANOVA), followed by Duncan's Multiple Range Test to determine significant differences among treatments. The parameters measured included fibre fractions, rumen fermentability, degradability, and bioactive compounds of the OPFS. The results demonstrated that the supplementation of natural additives had a significant effect ($P < 0.01$) on the fibre fraction content. OPFS treated with EBAF showed the lowest lignin content (18.562%), along with the highest cellulose (38.704%) and hemicellulose (18.138%) contents. The nutrient degradability of OPFS with natural additives ranged from 48.19% to 65.28%. Rumen fermentability parameters (pH, VFA, and NH_3) remained within normal ranges. Bioactive compounds identified included organic compounds, phenols, and their derivatives. In conclusion, the addition of natural additives during the fermentation of oil palm fronds can reduce fibre fractions, enhance digestibility, and contribute to the presence of secondary metabolites with potential antimicrobial, antioxidant, and anti-cholesterol properties.

Keywords | Bioactive compound, digestibility, oil palm frond, rumen fermentability, silage

Received | July 07, 2025; **Accepted** | October 08, 2025; **Published** | January 08, 2026
***Correspondence** | Dewi Febrina, Department of Animal Science, Faculty of Agriculture and Animal Sciences, Universitas Islam Negeri Sultan Syarif Kasim Riau, Indonesia. Jl. H. R Soebrantas KM 15 No 155 Tuahmadani Tampan-Pekanbaru 28293 Indonesia; **Email:** hanna_suska@yahoo.com
Citation | Febrina D, Febriyanti R, Juliantoni J, Sadarman, Zam SI, Rahim N, Qomariyah N, Pazla R (2026). The effect of natural additives as precursors in the silage process to improve the quality of oil palm fronds as ruminant feed: An *in vitro* study. J. Anim. Health Prod. 14(1): 170-182.
DOI | https://dx.doi.org/10.17582/journal.jahp/2026/14.1.170.182
ISSN (Online) | 2308-2801

INTRODUCTION

Oil palm frond (OPF) is the major by-product of the industry (Ayyildiz *et al*., 2023), with 47-70% resulting from palm oil production (Shamsuddin *et al*., 2021). However, this biomass waste product can still be utilised for many purposes, including soil mulch and fertiliser (Moradi *et al*., 2012); bioethanol production (Broda *et al*., 2022); composite board material (Kuittinen *et al*., 2021); absorption material (Imsya *et al*., 2023); and animal feedstuff (Febrina *et al*., 2025). Nevertheless, its use as animal feed is constrained by its high fibre (70%) and lignin (22.90%) content; low metabolisable energy and protein content; and low dry matter digestibility (Astuti *et al*., 2020; Hamdon *et al*., 2020; Rusli *et al*., 2021). The nutritional content, digestibility, and palatability of oil palm fronds can be increased through the silage process (anaerobic) which is influenced by the availability of substrates to support microbial activity (Febrina *et al*., 2020).

The silage process provides many benefits including reduced dry matter loss (McDonald *et al*., 2002), increased lactic acid synthesis, production of silage with a pleasant aroma (Karnatam *et al*., 2023), reduced proportion of fibre fraction and cellulose crystallinity, and increased palatability, digestibility, and fermentation products (Febrina *et al*., 2020, 2025), reduced feed costs (Balehegn *et al*., 2022), lowered pH, and increased NH_3 concentration and fermentation quality (Okoye *et al*., 2023). Improving feed quality will increase digestibility (Zhang *et al*., 2022) because it describes the nutrients that can be utilized by livestock to meet their needs, higher digestibility indicates better feed quality, because more feed can be utilized (Zewdie, 2019). These results are supported by previous researchers processing oil palm fronds into silage reduces the lignin content and increases the nutrient content and digestibility of OPF (Rusli *et al*., 2021; Saminathan *et al*., 2022); increases the production of volatile fatty acids (VFAs) and dry matter (DM) in vitro digestibility (Rahman *et al*., 2011; Rusli *et al*., 2019) reduces lignin content (Kumneadklang *et al*., 2019; Santoso *et al*., 2024).

In the silage process, the availability of precursors is a strategic factor to ensure its success, because it provides essential energy and protein for the growth and development of microbes and will affect the resulting silage product. Precursors that can be used in the silage process such as urea, poultry manure, buffalo faeces and empty oil palm bunch ash filtrate and rice husk ash filtrate. Urea provides nitrogen in the ammoniation process, which facilitates the cleavage of lignocellulose and lignohemicellulose bonds (New *et al*., 2021). This might be caused by the ability of urea to be quickly hydrolysed into ammonia, which, in turn, is synthesised by microbes into protein (Li *et al*., 2021). The use of urea in oil palm frond silage produces a crude protein content of 14.69% (Adli *et al*., 2022) and in oil palm stem pith silage produces a crude protein content of 13.27-15.67% (Febrina *et al*., 2025). However, the use of urea in OPF processing can pollute the environment (Chang *et al*., 2022). Therefore, the use of natural additives as a substitute for it is highly recommended. Filtrate of empty oil palm bunches ash and filtrate of rice husk ash are alkaline compounds, the mechanism is the same as urea (Rustamaji *et al*., 2022). Which can be used to loosen and break lignocellulose, lignohemicellulose and glycosidic bonds in the OPF fermentation process (Omar *et al*., 2021; Tahir *et al*., 2022). The inclusion of poultry manure (PM) in OPF fermentation provides urease to stimulate urea hydrolysis, resulting in fermented OPF with good physical quality) (Febrina *et al*., 2020) and lower lignin content (Febrina *et al*., 2018). In addition, buffalo rumen contains cellulolytic microbes which accelerate the decomposition of structural carbohydrates. Utilization of buffalo rumen as an additive in rape silage, produces a lignin degradation rate of 20.5% (Zhong *et al*., 2023).

In addition to being used as feed, oil palm frond silage also has the potential as an antimicrobial compound and its secondary metabolite content such as sterol and phenol in moderate amounts is needed by rumen microbes. This has been reported by previous researchers. Lactic acid bacteria from oil palm frond silage strain PDS 2 produced the highest inhibitory power, respectively, namely *Aspergillus parasiticus* FNCC 6033, *Penicilium citrinum* FNCC 6111 and *Aspergillus flavus* FNCC 6002 and can be used as an antifungal compound (Damayanti *et al*., 2015). Oil palm fronds contain bioactive steroids, tannins and phenolic compounds and can be developed as natural antimicrobials (Febrina *et al*., 2018, 2022); oil palm leaves have the potential as natural antibacterials and antibiofilms, economically and environmentally friendly (Nordin *et al*., 2023). In ruminants tannin and saponin function as defaunation agents and can protect feed protein from rumen microbial degradation (Kholif, 2023). The combination of tannin and saponin in feed can protect protein from rumen degradation, reduce NH_3 concentration, methanogenic and methane bacterial activity *in vitro* (Jayanegara *et al*., 2020) and increase the digestibility of crude protein, NDF and ADF (Zhang *et al*., 2021).

Many studies have been conducted to improve the quality of oil palm frond silage, through the addition of safe and environmentally friendly additives but the results have not been optimal. Therefore, this study aims to find natural additives as a substitute for urea with the same function in the silage process and the content of bioactive compounds in oil palm frond silage that can support the growth and activity of rumen microbes so as to reduce environmental pollution and improve the nutritional quality of oil palm fronds. By assessing the synergistic effects of these natural additives, our goal is to improve silage production techniques and promote sustainable agricultural practices. Through a comprehensive understanding of the complex dynamics of the studied processes, we seek to provide practical insights into improving oil palm frond utilization and increasing feed efficiency.

MATERIALS AND METHODS

Study design and analyses

Palm fronds were obtained from an oil palm plantation in Kampar Regency, Riau Province, Indonesia. Leaf blades covering 2/3 of the front part of the petioles were used. A total of around 150-200 leaf blades was collected and cut into 3-5 cm pieces using a leaf chopper. The leaf cuts were then dried in the sun before being ground into leaf meal. Rice husks and empty bunches were burned, and the resulting ashes weighed. Two hundred grams of ashes were soaked in 1 liter of water for 24 hours before being filtered. The filtrate was collected and used in the fermentation process. Poultry manure (PM) was obtained from a chicken layer farm in Pekanbaru City, and buffalo faeces (BF) was taken from the buffalo farm of the Department of Animal Science of Universitas Islam Negeri Sultan Syarif Kasim.

Oil palm fronds silage (OPFS) was produced in the Nutrition and Feed Technology Laboratory, Universitas Islam Negeri Sultan Syarif Kasim Riau. Fresh oil palm fronds (OPF) cuts (1 kg) were mixed homogenously with additives poultry manure (PM), buffalo faeces (BF), empty bunch ash filtrate (EBAF) or rice husk ash filtrate (RHAF) at a level of 10% (dry matter basis). The mixture was then placed and compacted in silo bottles (laboratory-scale silos with a capacity of 1 kg). The silo bottles were closed tightly to create an anaerobic condition and then stored at a room temperature of 29^{0}C. After 21 days, the OPFS was taken out of the bottles and dried. The fibre fractions of OPFS were determined using the method developed by Van Soest *et al*. (1991).

The rumen fermentability of OPFS was measured using cow rumen liquid, in line with the method developed by Tilley and Terry (1963). Rumen liquid was obtained from a slaughterhouse in Padang City, West Sumatra. The liquid was strained through sterile fabric and the obtained supernatant was analysed for its pH, NH_3 and VFA content. The precipitate was used for dry matter digestibility (DMD) and organic matter digestibility (OMD) analyses. A sample of dried OPFS (500 mg) was placed in a fermenter flask into which 10 ml rumen liquid and 40 ml McDougall buffer solution had been added. The flask was shaken by introducing a CO_2 gas flow into the flask for 30 seconds (pH 6.5-6.9). The flask was tightly sealed with ventilated rubber and incubated in a water bath shaker at 39 ^{o}C. After 4 hours, the incubated sample was removed from the flask for determination of rumen pH, NH_3, and total VFA content. Dry matter digestibility (DMD) and organic matter digestibility (OMD) determination was made on a sample incubated for 48 hours.

The identification of bioactive compounds was made on oil palm fronds silage (OPFS) extract which had been prepared based on the procedures used by Febrina *et al*. (2018). The OPFS was macerated in ethanol solution (96%) for 24 hours, with the maceration repeated eight times. Every 24 hours, the ethanol filtrate was separated, thickened using a vacuum evaporator, and weighed. Bioactive substances, including alkaloids, steroids, terpenoids, flavonoids, saponins, tannins and quinones, of the OPFS extract were identified using gas chromatography-mass spectrometry (GC-MS).

Statistical analysis

The study was conducted using a completely randomized design, with four treatments and five replicates. Treatments consisted of the use of natural additives including PM, BF, EBAF and RHAF. The data were subjected to an analysis of variance and a Duncan test (Petrie and Watson, 2013). Statistical analysis was performed using ANOVA at a significance level of 5%. Post-hoc comparisons between different treatments were conducted using Duncan's multiple range test, also at a significance level of 5%. The data of rumen fermentability and nutrient digestibility were analyzed descriptively.

RESULTS AND DISCUSSION

The fibre fraction contents of OPFS are shown in Figure 1. The supplementation of natural additives had significant effects ($P<0.01$) on the content of the fraction. The highest lignin content (20.83%) was found in OPFS with the addition of BF. The addition of PM and RHAF in the oil palm frond silage process resulted in lower lignin content compared to the addition of buffalo faeces, 19.58% and 19.26%, respectively. The lowest lignin content was found in the treatment with the addition of EBAF, namely 18.56%.

The fermentability and *in vitro* nutrient digestibility of OPFS were indicated by certain measured parameters, as shown in Table 1. The rumen fermentability of pH (6.70-6.82); VFA (112.00-128 mM) and NH_3 (12.40-15.50 mmol/L) were found to be in the normal range for microbial growth. Ammonia production in the rumen is influenced by the level of feed degradation and rumen pH. This shows that the level of feed degradation and rumen pH (Table 1) in OPFS with the addition of natural additives are not different so that the concentration of NH_3 and rumen pH are also normal. Oil palm frond silage with the addition of poultry manure resulted in higher nutrient digestibility (51.92-65.28%) compared to the addition of other additives (Table 1). This is related to the activity of rumen microbes and their products, namely VFA and NH_3 (Table 1). The high concentration of VFA (128 mM) and NH_3 (15.50 mmol/L) in oil palm frond silage with the addition of poultry manure was followed by high nutrient digestibility. The bioactive compounds of OPFS in this study are listed in Table 2 and Figure 2. The steroid compound content in oil palm fronds fermented with natural additives ranges from 2.26-6.21% with the types of compounds including: stigmast-5-en-3ol (3 beta), stigmasterol, squalene, methylcholesterol, and ethylcholest-5-3. beta-ol (Table 2). It is shown that the largest component of organic compounds and phenol compound. Palmitic, lauric, stearic and monostearic acids formed the largest part of the organic compounds produced from POFS in all treatment groups.

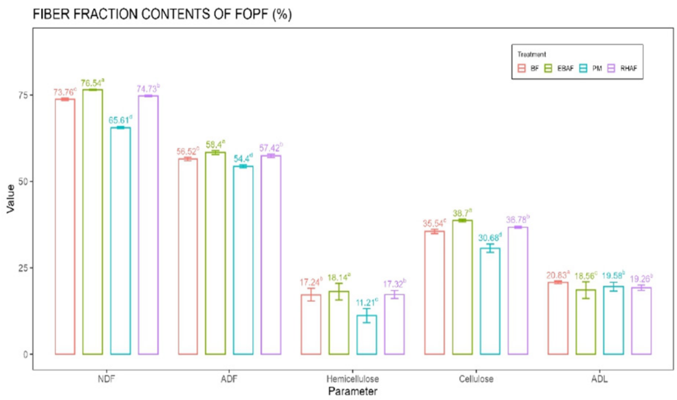

Figure 1: Fibre fraction content of OPFS (%).

Table 1: Rumen fermentability and *in vitro* nutrient digestibility of OPFS.

Parameters	Additives			
	BF	EBAF	PM	RHAF
Rumen fermentability				
VFA (mM)	112.00	117.50	128.00	120.00
NH_3 (mmol/L)	12.40	13.40	15.50	14.30
pH	6.82	6.71	6.74	6.70
Nutrient digestibility (%)				
Dry matter	58.91	58.30	62.47	60.32
Organic matter	61.52	60.25	63.83	61.39
Protein	62.27	61.09	65.28	62.52
Fibre	54.58	54.07	58.12	55.54
Ether extract	59.54	57.74	62.36	59.95
NFE	60.93	59.67	64.27	61.81
ADF	49.27	48.19	51.92	50.03
NDF	52.84	52.18	56.34	55.32
Cellulose	55.23	56.63	59.62	58.51
Hemicellulose	58.91	58.30	62.37	61.50

BF: buffalo faeces; EBAF: empty bunch ash filtrate; PM: poultry manure; RHAF: rice husk ash filtrate; VFA: volatile fatty acids; NFE: nitrogen-free extract; ADF: acid detergent fibre; NDF: neutral detergent fibre.

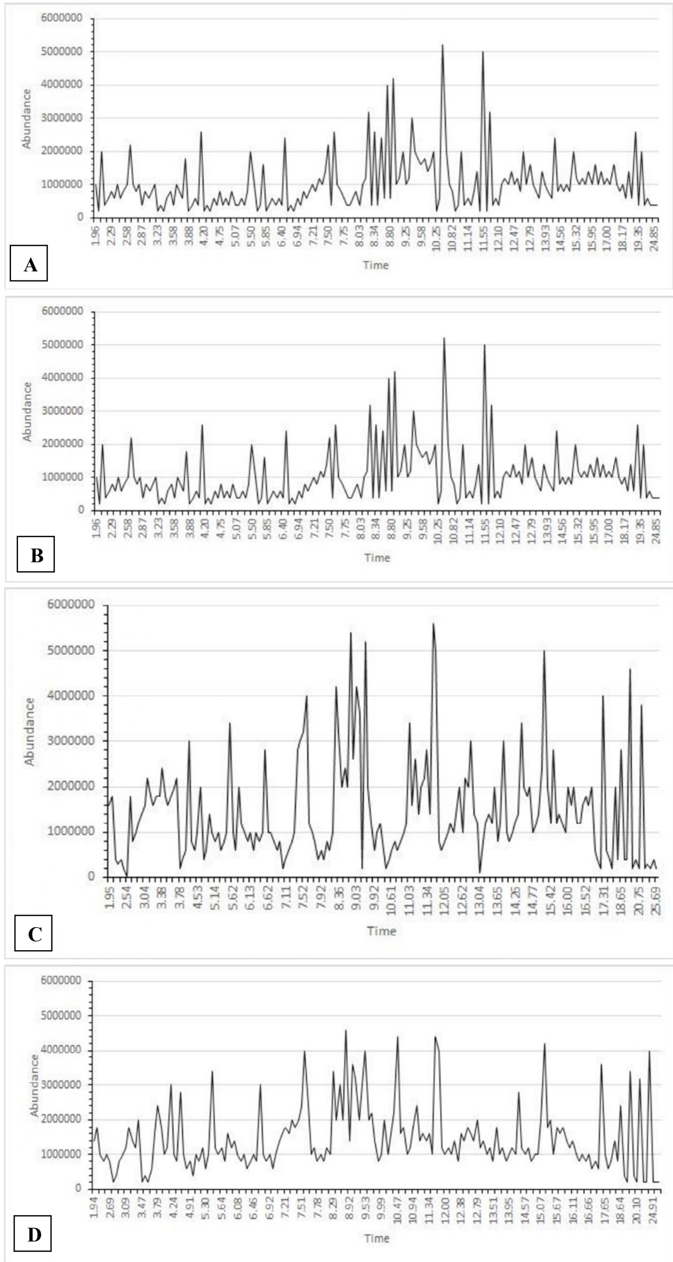

Figure 2: GC-MS analysis of bioactive compounds from methanol extract of OPFS with PM (A), BF (B), EBAF(C), and natural (D) additives.

The low lignin content in OPFS with the addition of EBAF this might be related to the mineral content, especially potassium, that may affect the fibre (lignin) content. Potassium is known to play an important role in lignin hydrolysis (Medvedev *et al.*, 2023). EBAF was found to have a higher potassium content (48.94%) (Novianti *et al.*, 2015) than RHAF (5.58%) (Flores *et al.*, 2021) or poultry manure (0.73-3.79%) (Palma, 2012; Tadele and Amha, 2015). The potassium content in RHAF (5.58%) which is almost the same as the potassium mineral content in PM (3.79%) causes the same lignin hydrolysis process so that the lignin content produced is also not different, 19.58% and 19.26%, respectively. The results of this study are almost the same as those reported by Febrina *et al.* (2020)

Table 2: Bioactive compounds of methanol extract of OPFS.

Poultry Manure Additives		Buffalo Faeces Additives		EBAF Additives		RHAF Additives	
%	Compounds	%	Compounds	%	Compounds	%	Compounds
	Organic Compounds (23.13%)		**Organic Compounds (21.70%)**		**Organic Compounds (17.52%)**		**Organic Compounds (22.35%)**
6.19	Octadecenoic acid	6.06	Hexadecoic acid (Palmitic acid)	4.53	Octadecenoic acid (Monostearat Acid)	5.41	Hexadecanoic acid (Palmitic acid)
6.02	Hexadecanoid acid/palmitic acid	5.90	Octadecenoic acid (Monostearat acid)	4.00	Hexadecanoic acid (Palmitic acid)	5.35	Octadecenoic acid (Oleic acid/Monostearic Acid)
2.46	Benzeneacetic acid	2.02	Benzeneacetic acid (Propionic acid)	2.91	Benzenepropanoic acid	3.35	Benzeneacetic acid
1.72	Benzene propanoic acid	1.97	Butanoic acid (Butyric acid)	2.58	Benzeneacetic acid (Propionic acid	1.84	Octadecanoic acid (Stearic acid)
1.59	Cyclohexanepropanoic acid/	1.67	Cyclohexanepropanoic acid	2.07	Propanoic acid (Lactic acid)	1.64	Cyclohexanepropanoic acid
1.47	Benzenepropanoic acid	1.59	Octadecanoic acid (Stearic acid)	1.43	Octadecanoic acid (Stearic acid)	1.32	Benzenepropanoic
1.39	Octadecanoic acid/stearic acid	1.26	Butanoic acid (Butyric acid)	-	-	1.22	Benzenepropanoic acid (Hydrocinnamic acid)
1.24	Dodecanoid acid (Lauric acid)	1.23	Dodecanoic acid (Lauric acid)	-	-	1.12	Dodecanoic acid (Lauric acid)
1.11	Fumaric acid	-	-	-	-	-	-
	Vitamin E Components (2%)		**Vitamin E Components (2.90%)**		**Vitamin E Components (3.67%)**		**Vitamin E Components (3.90%)**
2.00	dl-.alpha-tocopherol/vitamin E	2.90	alpha.-Tocopherol (Vitamin E)	1.90	alpha.-Tocopherol/Vitamin E	2.27	alpha.-Tocopherol (Vitamin E)
				1.77	alpha.-Tocopherol/Vitamin E	1.43	alpha.-Tocopherol (Vitamin E)
	Phenol Components (6.93%)		**Phenol Components (11.93%)**		**Phenol Components (6.99%)**		**Phenol Components (8.57%)**
2.38	3-Cyclohexene-1-methanol, 6-methyl	2.94	2-Cyclohexen-1-one, 4-(3-hydroxy-butenyl)-3,5,5-trimethyl-	3.28	Benzenamine, 2-methoxy/Anisidine	2.62	3-Cyclohexene-1-methanol, 6-methyl
1.78	Benzeneethanol 4 hydroxy/ 4-Methoxymethylphenol	2.36	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	1.33	2-Cyclohexen-1-one, 4-(3-hydroxy-1 -butenyl)-3,5,5-trimethyl-	1.78	1,3-Benzenediol, 4-hexyl-
1.53	Phenol (oxybenzene/monophenol)	2.23	3-Cyclohexene-1-methanol, 6-methyl	1.30	2-Cyclohexen-1-one, 3,5,5-trimethy 1-	1.78	Cyclohexanone, 5-methy l-2-(1-methylethylidene)
1.24	2-Cyclohexen-1-one, 3,5,5-trimethy l-4-(3-oxobutyl)	1.87	Benzeneethanol, 4-hydroxy	1.08	6-Acetyl-7-hydroxy-6-[2-(4-methylphenyl)ethyl]-9-phenoxy-1-azabicyclo	1.32	Benzeneethanol, 4-hydroxy
-	-	1.29	Benzenol	-	-	1.07	N-Phenyl-N'-(2-piperazin-1-yl-ethy l)-oxalamide
-	-	1.24	2-Cyclohexen-1-one, 3,5,5-trimethy l-4-(3-oxobutyl)	-	-	-	-
	Steroid Components (2.26%)		**Steroid Components (6.21%)**		**Steroid Components (3.47%)**		**Steroid Components (3.79%)**
2.26	Stigmast-5-en-3-ol, (3.beta)	3.69	Stigmast-5-en-3-ol, (3.beta.)	2.07	Stigmast-5-en-3-ol, (3.beta.)	2.41	ethylcholest-5-en-3.beta.-ol
-	-	1.41	Stigmasterol	1.40	Squalene	1.38	Squalene
-	-	1.11	Methylcholesterol	-	-	-	-
	Furan Componentss/pyrans (4.12%)		**Furan Componentss/pyrans (0%)**		**Furan Componentss/pyrans (1.31%))**		**Furans Components/pyrans (1.11%)**
3.04	3,7-Benzofurandiol, 2,3-dihydro-2, 2-dimethyl	-	-	1.31	3,5-O-Furylidene-d-xylofuranose	1.11	Loliolide/Calendin
1.08	1-Isobutyl-7,7-dimethyl-octahydro-isobenzofuran-3a-ol	-	-	-	-	-	-
	Sesquiterpen Components (3.54%)		**Sesquiterpen Components (3.18%)**		**Sesquiterpen Components (1.54%)**		**Vitamin 8 B Components (Inositol) (1.18%)**
3.54	Vomifoliol	3.18	vomifoliol	1.54	Vomifoliol	1.18	Inositol (Cyclohexanepentol
	Volatile Components (1.32%)		**Volatile Components (0%)**		**Volatile Components (0%)**		**Nucleic Acid Components (4.53%)**
1.32	1,2,3 propanetriol (gliserol)			1.00	2H-Pyran-2-one, 6-heptyltetrahydro/delta.-Dodecalactone	4.53	Pyrimidone
	Other Components (2.07%)				**Components of Quinones (1.09%)**		
2.07	Hydrazine, ethyl			1.09	2,6-Dimethyl-3-(methoxymethyl)-p-benzoquinone/		
					Aromatic Amine Components (2.11%)		
				2.11	3-(3-Fluoroanilino)-1-(3-nitrophenyl)-1-propanone		

PM: poultry manure, BF: buffalo faeces, EBAF: empty bunch ash filtrate, RHAF: rice husk ash filtrate.

the addition of PM on oil palm frond silage process produces a lignin content of 19.94%.

The highest NDF, ADF and cellulose content was found in the OPFS treatment with the addition of EBAF as an additive source. NDF is a part of the cell wall that contains ADF, hemicellulose, cellulose and pentose. The high NDF content will be followed by high ADF, cellulose and hemicellulose content but low lignin content (Figure 1). The same thing was reported by Wang *et al.* (2021) and (Zhong *et al.*, 2021), the NDF content is directly proportional to the ADF, cellulose and hemicellulose content but inversely proportional to the lignin content. In addition, the high content of potassium in EBAF loosens cellulose bonds, releasing lignin and hemicellulose, which eventually may reduce the fibre fraction content, resulting in the lowest lignin content (18.56%) which was significantly (P<0.01) lower than other treatments.

The released lignin will bind to alkalis, forming alkali-soluble lignin-alkalis (Ying *et al.*, 2018), while the loss of lignin and hemicellulose causes a loose cellulose bond that makes it easier for the cellulase enzyme to hydrolyse cellulose into simple sugars (Zeng *et al.*, 2014). The delignification process using alkaline compounds breaks hydrogen bonds (Chowdhury and Hamid, 2016) and stretches lignin away from cellulose and hemicellulose (Sukri *et al.*, 2014). Pretreating corncobs with potassium solution in the form of 2% (w/v) of potassium permanganate alkaline solution (APP) within 6 hours at 50 °C resulted in the regaining of cellulose and hemicellulose by 94.56 and 81.47%, respectively, and the elimination of lignin from the corncobs by 46.79% (Ma *et al.*, 2015).

Fermentability and nutrient digestibility of OPFS

pH plays a role in regulating various processes in the rumen, supporting microbial growth and producing products in the form of VFA and NH_3 (Shen *et al.*, 2023; Wang *et al.*, 2023). Rumen pH is influenced by the production of saliva containing sodium bicarbonate which functions as a buffer to maintain the stability of rumen pH (Castillo-Lopez *et al.*, 2021; Srivastava *et al.*, 2021); as well as the concentration of VFA which is the end product of carbohydrate metabolism and NH_3 is the end result of protein metabolism (Wang *et al.*, 2022; Zhang *et al.*, 2022). Carbohydrate fermentation was found to trigger a decrease in the pH of the rumen towards acid, while the fermentation of protein or NPN triggers an increase in the pH of the rumen towards the base (Hendawy *et al.*, 2022). These figures were in line with the rumen pH values (6.86 to 6.92) of Bali cattle found by Afzalani *et al.* (2022). The pH value of the rumen is influenced by the type of feed, animal health, microflora and fauna of the rumen (Ali *et al.*, 2020).

Treating OPFS with natural additives resulted in a rumen pH of 6.70–6.82. This shows that the addition of different additives to the oil palm frond silage process resulted in no different pH values. This showed that the rumen was in normal conditions, allowing rumen microbes to grow and develop suitably in order to perform the degradation process of OPFS. This is related to the availability of minerals, especially potassium minerals in EBAF and RHAF which are needed by rumen microbes for their growth and have a positive impact on the pH value and population of rumen microbes. Potassium plays an important role in cell metabolism as well as in the function of nerve and muscle cells (Wu, 2019). The addition of EBAF on oil palm pith silage produces a rumen pH of 6.78-6.80 (Febrina *et al*., 2025). Poultry manure contain the urease enzyme which can be utilized in the fermentation process, the addition of PM and urea to oil palm pith silage produces a rumen pH value of 6.76-6.80 (Febrina *et al*., 2025). Utilization of buffalo faeces as a source of inoculum in the fermentation of corn, soybean and corn straw produces a rumen pH (*in vitro*) of 6.5 (Cutrignelli *et al*., 2005). Thus, this confirms and supports previous researchers that the addition of natural additives to the oil palm frond silage process produces normal rumen pH values that can support microbial growth and activity.

The use of natural additives was found to produce OPFS with rumen NH_3 concentrations of 12.4–15.5 mmol/L, figures within the normal range of rumen NH_3 (6-21 mmol/L) for microbial growth (McDonald *et al*., 2002). Ammonia is a result of protein metabolism that is broken down into amino acids. Oil palm frond silage with the addition of buffalo faeces produces 12.40 mmol/L of NH_3, lower than the addition of other additives. The low concentration of NH_3 in this treatment is thought to be related to the number and activity of cellulolytic microbes in BF which have decreased and are unable to survive in aerobic conditions and the activity of microbes in buffalo faeces is lower than in buffalo rumen fluid. Microbial activity in faeces is lower than rumen microbial activity (Lourenco *et al*., 2020). Syapura *et al*. (2013) reported that straw fermented with the addition of buffalo faeces produced an N-NH_3 concentration of 4.66–6.86 mM.

Oil palm frond silage with the addition of PM produced an ammonia concentration of 15.50 mmol/L, higher than other treatments. The high concentration of ammonia in this treatment is thought to be related to the high crude protein content in PM. This is in accordance with what was conveyed by Lopes *et al*. (2020) that the rumen NH_3 concentration is influenced by the crude protein content. Kopeć *et al*. (2018) stated that PM contain 5% nitrogen; 3.9% phosphorus and 2.4% potassium. Potassium plays a role in carbohydrate metabolism and is a cofactor in protein synthesis; phosphorus is a component of adenosine triphosphate (ATP) and nucleic acids (Baj *et al*., 2020) which can be utilized by microbes for their growth and feed degradation processes. Poultry manure are also a source of urease enzymes so that they can accelerate the breakdown of urea into ammonia, resulting in higher NH_3 concentrations. This might be attributable to the notion that poultry manure contains urease enzyme (Yin *et al*., 2021), true protein and non-protein nitrogen in the form of rumen degradable uric acid (Tadele and Amha, 2015; Zurak *et al*., 2023).

VFA is a result of carbohydrate metabolism which functions as an energy source for microbes (Liu *et al*., 2021). In this study, the total production of VFA (112–128 mM) was within the normal range of rumen VFA of 70-150 mM (McDonald *et al*., 2002). These results suggest that the process of carbohydrate metabolism inside the rumen was progressing appropriately. Suitable rumen environmental conditions, as indicated by rumen pH, rumen NH_3 concentration, and rumen VFA content are important for supporting the optimal growth of rumen microbes (Xia *et al*., 2018). The highest VFA concentration was found in oil palm frond silage with the addition of poultry manure as an inoculum source in line with the high concentration of NH_3 (Table 1). This shows a positive correlation between VFA and NH_3. The availability of energy (VFA) will be used by rumen microbes as a carbon source for the formation of microbial proteins. This result is in line with Makmur *et al*. (2020) that increasing NH_3 concentration will increase VFA concentration.

The nutrient digestibility of oil palm frond silage with the addition of natural additives was 48.19-65.28% (Table 1), this value is in line with that reported by Safira *et al*. (2024) in vitro digestibility of dry matter and organic matter of oil palm frond silage were 61.96% and 60.91% respectively and Mulianda *et al*. (2018) the digestibility of dry matter and organic matter of oil palm frond silage fermented with local microorganisms (MOL) was 64.88% and 65.8%. This value is higher than that reported by Santoso *et al*. (2024) rations made from oil palm frond silage produced *in vitro* digestibility of dry matter of 44.7-57.7%; organic matter 43.7-54.7% and NDF 24.8-35.7%.

Volatile fatty acid is the result of carbohydrate metabolism that functions as an energy source for microbes and NH_3 is the result of protein metabolism needed by microbes for their growth. When energy and protein sources are available in optimal amounts, microbes will grow and develop well and produce enzymes to degrade feed, resulting in high nutrient digestibility. This shows the beneficial effects of protein-energy synchronization that can increase microbial activity and their ability to digest feed. High nutrient digestibility increases ruminant productivity, because nutrients can be utilized optimally (Sharif *et al*., 2019). The results of this study confirm Febrina *et al*. (2025) complete growth precursors (urea, molasses and EM) in oil palm stem pith silage, producing the highest concentration of

VFA, NH_3 and nutrient digestibility and Putri *et al.* (2021), a balance of 14% protein and 70% energy (TDN) produces the highest VFA, NH_3, microbial protein synthesis and nutrient digestibility. The addition of 10% poultry manure to baggage silage was found to lower NDF content and increase ash content and the digestibility of dry matter and organic matter *in vitro* (Shitandi *et al.*, 2009).

Oil palm frond silage with the addition of buffalo faeces and empty oil palm bunch ash filtrate resulted in lower nutrient digestibility (compared to the addition of other additives) (Table 1). This is thought to be related to the low microbial activity in buffalo faeces compared to buffalo rumen. The microbial population in buffalo faeces is lower and has not been able to compete with microbial activity in buffalo rumen (Agarwal *et al.*, 2020; Chiaravalli *et al.*, 2019; Marlina *et al.*, 2020). Buffalo faeces have been used as inoculum to increase the nutrient digestibility of rice straw (Syapura *et al.*, 2013) and tofu dregs (Bidura *et al.*, 2014). It is suggested that this was related to the dominant fibrolytic enzyme found in the buffalo rumen (Sujani and Seresinhe, 2015), an enzyme which is able to break down lignocellulose and lignohemicellulose bonds (Sio *et al.*, 2022).

The bioactive compounds of OPFS

Partial pyrolysis of cellulose and hemicellulose resulted in the production of organic compounds. Palmitic acid, together with oleic, linoleic and stearic acids, are also the most dominant organic compounds found in palm oil (Ping and Yusof, 2009; Simeh, 2004) and oil palm leaves (Islam *et al.,* 2021). Palmitic acid is a group of fatty acids with non-polar poles that are the same as gram-negative bacteria so that it can penetrate the cell walls of gram-negative bacteria (Gagat *et al.*, 2024). The antimicrobial properties of palmitic acid might be attributed to its ability to damage membrane permeability and ion partition on cell membranes, causing cell lysis (Agoramoorthy *et al.*, 2007; Al-Shammari *et al.*, 2012; Langner and Hui, 2000; Mikut *et al.*, 2016). The results of this study indicate that the organic acid content (palmitate, laurate, stearate) in oil palm fronds can be used as a natural antimicrobial while confirming previous researchers that the combination of palmitic acid and lauric acid effectively inhibits *S. aureus* and *E. coli*. and reduces dependence on chemical disinfectants (Auttajinda *et al.*, 2023).

Phenol is the second largest compound after organic compounds, which is produced from the fermentation of oil palm fronds with the addition of natural additives (6.93-11.93%). Phenol is a bioactive compound that functions as an antimicrobial through the process of denaturing bacterial cell proteins and precipitating proteins in bacterial cells so that bacterial cell metabolism is inhibited. Phenolic compounds can capture free radicals and inhibit the growth of pathogenic microbes (Ali *et al.*, 2020; Mohamad Said *et al.*, 2021). This is in accordance with what was reported by Febrina *et al.* (2018) that the tannin and steroid content in oil palm fronds can inhibit the growth of *Staphylococcus aureus* and *Escherichia coli.* The phenol content in oil palm fronds also functions as an antioxidant that can capture free radicals. The results of this study confirm the research of Imsya *et al.* (2013) phenolic compounds in oil palm fronds fermented with Phanerochaete chrysosporium showed antioxidant activity with an incubation time of 10 days; and Hussin *et al.* (2015) oil palm fronds can be used as a source of green antioxidants to inhibit mild steel corrosion.

Steroids in plants have activities as anticholesterol and anticancer (Mendoza *et al.*, 2025). Stigmasterol is a sterol compound that has anticholesterol properties, through the mechanism of inhibiting cholesterol absorption in the intestine, inhibiting the development of colon cancer and suppressing cholesterol in the liver (Li *et al.*, 2022). Under stressful conditions, stigmast-5-en-3ol (3. beta.) can boost the body's immune system (Madhubala and Santhi, 2019). Zain *et al.* (2022) reported that providing complete rations made from ammoniated oil palm fronds supplemented with direct-fed microbial (DFM) and virgin coconut oil (VCO) to Bali cattle had a very significant effect ($p<0.05$) in reducing cholesterol levels, LDL and increasing HDL in the blood of Bali cattle. The same thing was reported by Febrina *et al.* (2020) that providing fermented oil palm fronds in kacang goat rations significantly reduced blood cholesterol levels.

Inositol compounds, which possibly include inositol (vitamin B8) and B-complex vitamins (Bizzarri *et al.*, 2014), were found in FPOF treated with natural additives. Inositol plays a role in the transduction of hormone and neurotransmitter signals, regulating the insulin hormone and forming cell structures and antidiabetic compounds through the glycosylation inhibition process known as AGEs (Advanced Glycation End Products) (Nie and Cooper, 2021). Vitamin B complex functions as an enzyme cofactor for energy formation, protein and lipid metabolism (Hrubša *et al.*, 2022) so it is very necessary for rumen microbes for their growth and development (González-Montaña *et al.*, 2020). In ruminant livestock, Vitamin B complex does not need to be added to the ration because of the ability of rumen microbes to synthesize it, thus the use of oil palm frond silage can fulfill the need for Vitamin B complex and its inositol content can be developed as an antidiabetic compound. Febrina *et al.* (2021) reported that the provision of oil palm fronds fermented with *Phanerochaete chrysosporium* in Kacang goat rations significantly increased ration consumption, body weight gain, and ration efficiency, and Suyitman *et al.*

(2020) reported that the provision of ammoniated oil palm fronds supplemented with minerals in Simmental cattle rations significantly increased ration consumption, body weight gain, and ration efficiency.

Vitamin E (alpha tocopherols) was found in FPOF in all treatment groups, consisting of alpha tocopherol (2-3.9%) and sterols/steroids (2.26-6.21%). Vitamin E is a lipophilic antioxidant formed from four tocopherol molecules and four tocotrienol molecules (Munoz and Bosch, 2019). Vitamin E is able to suppress lipid peroxidation in cell membranes so that the integrity of the cell membrane is maintained to maintain the cell metabolism process (Rizvi *et al*., 2014). The antioxidant can react with free radicals, protect lipids from peroxidation, stabilise membrane structures, act as a body system, and avoid oxidative stress (Agarwal *et al*., 2014). Previous researchers reported the positive effects of adding vitamin E to rations in cattle which can increase immunity and antioxidative stress (Sordillo, 2016) and relieved inflammatory responses and prevent mastitis (Xiao *et al*., 2021). Jamarun *et al*. (2020) reported that providing rations made from ammoniated oil palm fronds, Titonia and Elephant Grass to Etawa crossbred dairy goats increased the concentration of Ca and P minerals in milk, but did not affect the levels of protein, fat and lactose in milk.

CONCLUSION AND RECOMENDATIONS

OPFS treated with natural additives was found to have nutrient digestibility (*in vitro*) and normal rumen fermentability conditions (pH, VFA and NH_3) to support microbial growth. The inclusion of natural additives in the fermentation of palm fronds resulted in OPFS possessing bioactive compounds including organic, phenols, steroids, furans, vomifoliol, palmitic acid, flavonoids and quinones. The results of the study have revealed new information on treatments that can be given to oil palm fronds to improve their digestibility. Secondary metabolites contained in OPFS deserve to be further studied for their utilisation as feed additive materials, as they contain certain antimicrobial and antioxidative properties.

ACKNOWLEDGEMENT

Funding assistance from the Institute for Research and Community Service of Universitas Islam Negeri Sultan Syarif Kasim Riau with a contract No. 0600/R/2020 in the Applied Cluster of National Strategic Studies for the fiscal year 2020 was acknowledged.

NOVELTY STATEMENT

The use of natural additives (poultry manure; buffalo faeces; empty bunch ash filtrate/EBAF; and rice husk ash filtrate/RHAF as a substitute for urea with the same function in the silage process and the content of bioactive compounds in oil palm frond silage that can support the growth and activity of rumen microbes so as to reduce environmental pollution and improve the nutritional quality of oil palm fronds.

AUTHOR'S CONTRIBUTIONS

DW, RF, RP, NQ format analysis; JJ, SIZ visualization; NQ, SS, SIZ writing-review & editing; DW conceptualization; DW, SS, NR supervision; RF, NR, JJ data curation, RR methodology; NQ, DW, RR writing-original draft, DW project-administration; SS, RP resources; SIZ software; JJ, RF, NR validation

Ethical approval

This research was conducted based on ethical clearance by Faculty of Agriculture and Animal Sciences, Universitas Islam Negeri Sultan Syarif Kasim Riau–Indonesia (No: KEP.FPP/10/09/2021).

Generative AI and AI-assisted technology statement

The authors used generative AI tools only for language refinement, and they take full responsibility for the accuracy and integrity of the manuscript's scientific content.

Conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Adli A, Febrina D, Zumarni Z, Khairi F, Sadarman S (2022). The effect of differences of adhesive and filtrates sources on fiber fraction and physical quality of complete ration wafer. J. Agripet. 22(1): 88–96. https://doi.org/10.17969/agripet.v22i1.21634

Afzalani A, Muthalib RA, Raguati R, Syahputri E, Suhaza L, Musnandar E (2022). Supplemental effect of condensed tannins from sengon leaves (*Albizia falcataria*) on *in vitro* gas and methane production. World J. Mens Health, 32(6): 1513–1520. https://doi.org/10.36899/JAPS.2022.6.0558

Agarwal A, Virk G, Ong C, du Plessis SS (2014). Effect of oxidative stress on male reproduction. World J. Men's Health, 32(1): 1–17. https://doi.org/10.5534/wjmh.2014.32.1.1

Agarwal P, Kala A, Kamra DN, Agarwal N, Chaudhary LC (2020). Spatial and temporal distribution of microbes and enzyme activity in the rumen of buffaloes. Indian J. Anim. Nutr., 37(2): 87–94. https://doi.org/10.5958/2231-6744.2020.00016.X

Agoramoorthy G, Chandrasekaran M, Venkatesalu V, Hsu MJ (2007). Antibacterial and antifungal activities of fatty acid methyl esters of the blind-your-eye mangrove from India. Braz. J. Microbiol., 38(4): 739–742. https://doi.org/10.1590/

S1517-83822007000400028
Ali AT, Mosa MN, Alshaheen ZG, Muhammad-Ali MA (2020). Synthesis, characterization and antibacterial evaluation of oxoazetidin- Benzene sulfonamide derivatives as a hybrid antimicrobial agents. Syst. Rev. Pharma. 11(2): 487–494.
Al-Shammari LA, Hassan WHB, Al-Youssef HM (2012). Chemical composition and antimicrobial activity of the essential oil and lipid content of *Carduus pycnocephalus* L. growing in Saudi Arabia. J. Chem. Pharm. Res., 4(2): 1281–1287.
Astuti WD, Widyastuti Y, Fidriyanto R, Rohmatussolihat, Ridwan R, Sari NF, Firsoni, Sugoro I (2020). *In vitro* gas production and digestibility of oil palm frond silage mixed with different levels of elephant grass. Di dalam: 2ISIBIO 2019 IOP Conf. Ser. Earth Environ. Sci., 439: 012022. https://doi.org/10.1088/1755-1315/439/1/012022
Auttajinda N, Raviyan P, Klangpetch Ueno W, Jirarattanarangsri W, Osiriphun S (2023). Antibacterial activities of palmitic and lauric acids from palm kernel oil for the development of food-grade disinfectants. Di dalam: The 1st KPRU International Student Research Conference on Science, Mathematics, and Technology. pp. 1–15.
Ayyildiz HF, Shoaib H, Kara H (2023). Bioactive phytochemicals from palm oil processing by-products. Di dalam: Ramadan Hassanien MF, editor. Bioactive Phytochemicals from Vegetable Oil and Oilseed Processing By-products. Reference Series in Phytochemistry. Springer Nature Reference. https://doi.org/10.1007/978-3-030-63961-7_11-1
Baj J, Flieger W, Teresiński G, Buszewicz G, Sitarz R, Forma A, Karakuła K, Maciejewski R (2020). Magnesium, calcium, potassium, sodium, phosphorus, selenium, zinc, and chromium levels in alcohol use disorder: A review. J. Clin. Med., 9(6): 1–24. https://doi.org/10.3390/jcm9061901
Balehegn M, Ayantunde A, Amole T, Njarui D, Nkosi BD, Müller FL, Meeske R, Tjelele TJ, Malebana IM, Madibela OR, Boitumelo WS, Lukuyu B, Weseh A, Minani E, Adesogan AT (2022). Forage conservation in sub-Saharan Africa: Review of experiences, challenges, and opportunities. Agronomy Journal, 114, 7599. https://doi.org/10.1002/agj2.20954.
Bidura ING, Siti NW, Utami IAP (2014). Isolation of cellulolytic bacteria from rumen liquit of buffalo both as a probiotics properties and has Cmc-Ase activity to improve nutrient quality of soybean distillery by-product as feed. Int. J. Pure App. Biosci., 2(5): 10–18.
Bizzarri M, Carlomagno G (2014). Inositol: History of an effective therapy for Polycystic Ovary Syndrome. Eur. Rev. Med. Pharmacol. Sci., 18(3): 1896–1903.
Broda M, Yelle DJ, Serwańska K (2022) Bioethanol Production from Lignocellulosic Biomass Challenges and Solutions. Molecules, 27, 8717. https://doi.org/:10.3390/molecules27248717.
Castillo-Lopez E, Petri RM, Ricci S, Rivera-Chacon R, Sener-Aydemir A, Sharma S, Reisinger N, Zebeli Q (2021). Dynamic changes in salivation, salivary composition, and rumen fermentation associated with duration of high-grain feeding in cows. J. Dairy Sci., 104(4): 4875–4892. https://doi.org/10.3168/jds.2020-19142
Cutrignelli MI, Calabrò S, Tudisco R, Zicarelli F, Gazaneo MP, Piccolo V (2005). Comparison of buffalo rumen liquor and buffalo faeces as inoculum for the *in vitro* gas production technique. Ital. J. Anim. Sci., 2017: 319–321. https://doi.org/10.4081/ijas.2005.2s.319
Damayanti E, Suryani AE, Sofyan A, Karimy MF, Julendra H (2015). Isolation of lactic acid bacteria for antifungal activity isolated from silage and animal digestives tract. J. Agritech., 35(2): 164–169. https://doi.org/10.22146/agritech.9402
Febrina D, Febriyanti R, Zam SI, Handoko J, Fatah A, Juliantoni J (2018). Antibacterial activity testing and ethanol extract characterization of oil palm fronds (*Elaeis guineensis* Jacq). Pak. J. Nutr., 17(9): 427–433. https://doi.org/10.3923/pjn.2018.427.433
Febrina D, Febriyanti R, Zumarni, Juliantoni J, Yendraliza, Elfawati, Irdha, Mirdhayati, Mochtisar Y, Rabani (2020). The effect of addition of fermented oil palm fronds extract on rumen fermentation and blood metabolites of kacang goat. J. Anim. Health Prod., 9(1): 58–64. https://doi.org/10.17582/journal.jahp/2021/9.1.58.64
Febrina D, Ode Hardiyanto L, Febriyanti R, Qomariyah N, Wahyono T, Nur Adli D (2022). Evaluation of nutritional content and physical quality of oil palm frond silage with different of additive and fermentation length. J. Ilmu Teknol. Petern. Trop., 9(3): 605–612.
Febrina D, Sadarman S, Mirdhayati I, Adelina T, Hidayat T, Al Afid A, Nasution RR, Qomariyah N, Pazla R (2025). Effect of microbial growth precursor supplementation on chemical composition and fermentation characteristics of palm stem pith silage. J. Anim. Feed Sci., 34(1): 98–112. https://doi.org/10.22358/jafs/189933/2024
Febrina D, Zumarni, Febriyanti R, Juliantoni J, Yendraliza, Mirdhayati I, Elfawati, Rifai M, Khan I, Prasiyo R (2021). Digestibility of nutrient and performance of Kacang Goats which are given fermented oil palm fronds extract. Adv. Anim. Vet. Sci., 9(3): 422–428. https://doi.org/10.17582/journal.aavs/2021/9.3.422.428
Flores CG, Schneider H, Dornelles JS, Gomes LB, Marcilio NR, Melo PJ (2021). Synthesis of potassium zeolite from rice husk ash as a silicon source. Clean Eng. Technol., pp. 100201. https://doi.org/10.1016/j.clet.2021.100201
Gagat P, Ostrówka M, Duda-Madej A, Mackiewicz P (2024). Enhancing antimicrobial peptide activity through modifications of charge, hydrophobicity, and structure. Int. J. Mol. Sci., 25(19): 10821. https://doi.org/10.3390/ijms251910821
González-Montaña JR, Escalera-Valente, F. Alonso AJ, Lomillos JM, Robles R, Alonso ME (2020). Relationship between vitamin B12 and cobalt metabolism in domestic ruminant: An update. Animals, 10(10): 1855. https://doi.org/10.3390/ani10101855
Hamdon HA, Kholif AE, Mahmoud GB, Khalifa AMA, Ati MNMA (2020). Enhancing the utilization of palm leaf hay using Bacillus subtilis and Phanerochaete chrysosporium in the diet of lambs under desert conditions. Ann. Anim. Sci., 20(4): 1395–1409. https://doi.org/10.2478/aoas-2020-0052
Hendawy AO, Sugimura S, Sato K, Mansour MM, Abd El-Aziz AH, Samir H, Islam MA, Rubayet Bostami ABM, Mandour AS, Elfadadny A, Ragab RF, Abdelmageed HA, Ali AM (2022). Effects of selenium supplementation on rumen microbiota, rumen fermentation and apparent nutrient digestibility of ruminant animals: A review. Fermentation, 8(4): 1–23. https://doi.org/10.3390/fermentation8010004
Hrubša M, Siatka T, Nejmanová, I. Vopršalová M, Kujovská Krčmová, L. Matoušová K, Javorská L, Macáková K, Mercolini L, Remião F, Máťuš M, Mladěnka P (2022). Biological properties of vitamins of the B-complex, Part 1:

Vitamins B1, B2, B3, and B5. Nutrients, 14(3): 484. https://doi.org/10.3390/nu14030484

Hussin MH, Shah AM, Rahim AA, Ibrahim MNM, Perrin D, Brosse N (2015). Antioxidant and anticorrosive properties of oil palm frond lignins extracted with different techniques. Ann. For. Sci., 72(1): 17–26. https://doi.org/10.1007/s13595-014-0405-1

Chang Y, Park TE, Lee SW, Lee EH (2022). Colorimetric detection of urease-producing microbes using an ammonia-responsive flexible film sensor. Biosensors, 12(10): 886. https://doi.org/10.3390/bios12100886

Chiaravalli M, Rapetti L, Graziosi AR, Galassi G, Crovetto GM, Colombini S (2019). Comparison of faecal versus rumen inocula for the estimation of NDF digestibility. Animals, 9(11): 1–10. https://doi.org/10.3390/ani9110928

Chowdhury ZZ, Hamid SBA (2016). Preparation and characterization of nanocrystalline cellulose using ultrasonication combined with a microwave-assisted pretreatment process. BioResources, 11(2): 3397–3415. https://doi.org/10.15376/biores.11.2.3397-3415

Imsya A, Laconi. EB, Wiryawan KG, Widyastuty Y (2013). Identification of phenolic compounds and its antioxidant activity from lignin and palm oil frond fermented with *Phanerochaete chrysosporium*. Di dalam: Proceedings of The 4th International Conference On Sustainable Animal Agriculture For Developing Countries (SAADC).

Imsya A, Riswandi, Malik B, Yakup (2023). Fermentation of blood meal absorbed by oil palm fronds with Bacillus amyloliquefaciens and Lactobacillus plantarum. Online J. Anim. Feed Res., 13(1): 39–45. https://doi.org/10.51227/ojafr.2023.7

Islam N, Khan MF, Khatun MR, Nur S, Hanif NB, Kulsum U, Arshad L, Lyzu C, Cacciola NA, Capasso R, Haque MA (2021). Neuropharmacological insights of African oil palm leaf through experimental assessment in rodent behavioral model and computer-aided mechanism. Food Biosci., 40: 100881. https://doi.org/10.1016/j.fbio.2021.100881

Jamarun N, Pazla. R, Zain M, Arief A (2020). Milk quality of Etawa crossbred dairy goat fed combination of fermented oil palm fronds, tithonia (*Tithonia diversifolia*) and elephant grass (*Pennisetum Purpureum*). Di dalam: IOP Conf. Ser. J. Phys. Conf. Ser., 1469: 012004. https://doi.org/10.1088/1742-6596/1469/1/012004

Jayanegara A, Yogianto Y, Wina E, Sudarman A, Kondo M, Obitsu T, Kreuzer M (2020). Combination effects of plant extracts rich in tannins and saponins as feed additives for mitigating *in vitro* ruminal methane and ammonia formation. Animals, 10(9): 1–14. https://doi.org/10.3390/ani10091531

Karnatam KS, Mythri B, Nisa WU, Sharma H, Meena TK, Rana P, Vikal Y, Gowda M, Dhillon BS, Sandhu S (2023). Silage maize as a potent candidate for sustainable animal husbandry development perspectives and strategies for genetic enhancement. Front Genet., 14: 1150132. https://doi.org/10.3389/fgene.2023.1150132

Kholif AE (2023). A review of effect of saponins on ruminal fermentation, health and performance of ruminants. Vet. Sci., 10(7): 450. https://doi.org/10.3390/vetsci10070450

Kopeć M, Gondek K, Mierzwa-Hersztek M, Antonkiewicz J (2018). Factors influencing chemical quality of composted poultry waste. Saudi J. Biol. Sci., 25(8): 1678–1686. https://doi.org/10.1016/j.sjbs.2016.09.012

Kuittinen S, Hietaharju J, Kupiainen L, Hassan MK, Yang M, Kaipiainen E, Villa A, Kangas J, Keinänen M, Vepsäläinen J, Pappinen A (2021). Bioethanol production from short rotation S. schwerinii E. Wolf is carbon neutral with utilization of waste-based organic fertilizer and process carbon dioxide capture. J Clean Prod., 293:126088. https://doi.org/10.1016/j.jclepro.2021.126088

Kumneadklang S, O-Thong S, Larpkiattaworn S (2019). Characterization of cellulose fiber isolated from oil palm frond biomass. Mater Today Proc., 17: 1995–2001. https://doi.org/10.1016/j.matpr.2019.06.247

Langner M, Hui S (2000). Effect of free fatty acids on the permeability of 1,2-dimyristoyl-sn-glycero-3-phosphocholine bilayer at the main phase transition. Biochim. Biophys. Acta-Biomembranes, 1463(2): 439–447. https://doi.org/10.1016/S0005-2736(99)00236-9

Li X, Xin Y, Mo Y, Marozik P, He T, Guo H (2022). The bioavailability and biological activities of phytosterols as modulators of cholesterol metabolism. Molecules, 27(2): 523. https://doi.org/10.3390/molecules27020523

Li XX, Wang SP, Sun ZY, Wang ST, Shuai WL, Shen CH, Tang YQ (2021). Performance and microbial community dynamics during rice straw composting using urea or protein hydrolysate as a nitrogen source: A comparative study. Waste Manage., 135: 130–139. https://doi.org/10.1016/j.wasman.2021.08.026

Liu K, Zhang Y, Yu Z, Xu Q, Zheng N, Zhao S, Huang G, Wang J (2021). Ruminal microbiota–host interaction and its effect on nutrient metabolism. Anim. Nutr., 7(1): 49–55. https://doi.org/10.1016/j.aninu.2020.12.001

Lopes ASM, De Oliveira JS, Santos EM, Medeiros AN, Givisiez PEN, Lemos MLP, Santos FNS, Silva NMV, Azevedo PS, Sousa LS, Pereira DM, Oliveira CJB (2020). Goats fed with non-protein nitrogen: Ruminal bacterial community and ruminal fermentation, intake, digestibility and nitrogen balance. J. Agric. Sci., 158: 781–790. https://doi.org/10.1017/S0021859621000162

Lourenco, Jeferson M. Kieran TJ, Seidel, Darren S. Glenn, Travis C. Silveira, Magali F. da Callaway, Todd R. Stewart, R. Lawton Duarte M de S (2020). Comparison of the ruminal and fecal microbiotas in beef calves supplemented or not with concentrate. PLoS One, 15(4): e0231533. https://doi.org/10.1371/journal.pone.0231533

Ma L, Cui Y, Cai R, Liu X, Zhang C, Xiao D (2015). Optimization and evaluation of alkaline potassium permanganate pretreatment of corncob. Bioresour. Technol., 180: 1–6. https://doi.org/10.1016/j.biortech.2014.12.078

Madhubala L., Santhi G (2019). Phytochemical and GC-MS analysis on leaves of selected medicinal plants in boraginaceae family *Cordia dichotoma* L. Pramana. Res. J., 9: 688–707.

Makmur M, Zain M, Marlida Y, Khasrad K, Jayanegara A (2020). *In vitro* ruminal biohydrogenation of C18 fatty acids in mixtures of Indigofera zollingeriana and Brachiaria decumbens. J. Indones. Trop. Anim. Agric., 45(2): 124–135. https://doi.org/10.14710/jitaa.45.2.124-135

Marlina ET, Hamdani H, Ridwan R, Ikhsani L, Hidayati YA, Rahmah KN, Harlia E (2020). Potential of microbial inoculum from buffalo feces in activating lignite coal bed methane. J. Environ. Biol., 41: 650–657. https://doi.org/10.22438/jeb/41/3/MRN-1212

McDonald P, Edwards RA, Greenhalgh J, Morgan C, Sinclair LRW (2002). Animal Nutrition. 8th Edition. Pearson Ltd. (Singapore). https://eliasnutri.wordpress.com/wpcontent/

uploads/2020/07/animal-nutrition-7th-edition.pdf.
Medvedev AA, Kustov AL, Beldova, DA, Polikarpova SB, Ponomarev VE, Murashova EVSPV, Kustov LM (2023). A synergistic effect of potassium and transition metal compounds on the catalytic behaviour of hydrolysis lignin in CO_2-assisted gasification. Energies, 16: 4335. https://doi.org/10.3390/en16114335
Mendoza Lara DF, Hernández-Caballero ME, Terán JL, Ramírez JS, Carrasco-Carballo A (2025). Anticancer activities of natural and synthetic steroids: A review. ACS Omega, 10: 7493–7509. https://doi.org/10.1021/acsomega.4c08577
Mikut R, Ruden S, Reischl M, Breitling F, Volkmer R, Hilpert K (2016). Improving short antimicrobial peptides despite elusive rules for activity. Biochim. Biophys. Acta Biomembr., 1858(5): 1024–1033. https://doi.org/10.1016/j.bbamem.2015.12.013
Mohamad Said KA, Ismail AF, Abdul Karim Z, Abdullah MS, Hafeez A (2021). A review of technologies for the phenolic compounds recovery and phenol removal from wastewater. Process Saf. Environ. Prot., 151: 257–289. https://doi.org/10.1016/j.psep.2021.05.015
Moradi A, Sung CTB, Goh KJ, Hanif AHM, Ishak C (2012). Our soil conservation practices in a nonterraced oil palm plantation. Agron. J., 104: 1727–1740. https://doi.org/10.2134/agronj2012.0120
Mulianda R, Tafsin M, Hanafi ND, Yulinas (2018). Utilizing local microorganisms with different dosages and duration of fermentation towards digestibility and rumen activity of oil palm frond *in vitro*. In: E3S Web of Conferences. 52: 36. https://doi.org/10.1051/e3sconf/20185200036
Munoz P, Bosch SM (2019). Vitamin E in plants: Biosynthesis, transport, and function. Trends Plants Sci., 24: 1040–1051. https://doi.org/10.1016/j.tplants.2019.08.006
New EK, Wu TY, Voon KS, Procentese A, Shak KPY, Teoh WH, Lim JW, Jahim MJ (2021). A Utilization of choline chloride-based deep eutectic solvent integrated with alkaline earth metal hexahydrate in the pretreatment of oil palm Fronds. Ind. Eng. Chem. Res., 60(5): 2011–2026. https://doi.org/10.1021/acs.iecr.0c05052
Nie T, Cooper GJS (2021). Mechanisms underlying the antidiabetic activities of polyphenolic compounds: A review. Front. Pharmacol., 12:798329. https://doi.org/10.3389/fphar.2021.798329
Nordin N, Khan H, Ahsan J (2023). The antibacterial activity of palm oil (*Elaeis guineensis*) leaf extracts against Staphylococcus aureus. Compend. Oral. Sci., 10(1): 45–56. https://doi.org/10.24191/cos.v10i1.21622
Novianti S, Anissa N, Ilman NZ, Pandji P, Hiroaki SYK (2015). Low-potassium fuel production from Empty Fruit Bunches by hydrothermal treatment processing and water leachin. Energy Procedia. 75: 584-589. https://doi.org/10.1016/j.egypro.2015.07.460
Okoye CO, Wang Y, Gao L, Wu Y, Li X, Sun J, Jiang J (2023). The performance of lactic acid bacteria in silage production: A review of modern biotechnology for silage improvement. Microbiol. Res., 266: 127212. https://doi.org/10.1016/j.micres.2022.127212
Omar L, Ahmed OH, Jalloh MB, Majid NMA (2021). Rice husk compost production and use in mitigating ammonia volatilization from urea. Sustain, 13(4): 1–23. https://doi.org/10.3390/su13041832
Palma CF (2012). Characterisation, kinetics and modelling of gasification of poultry manure and litter: An overview. Energy Convers Manage., 53(1): 92–98. https://doi.org/10.1016/j.enconman.2011.08.017
Petrie A, Watson PF (2013). Statistics for veterinary and animal science. Third edition. Wiley-Blackwell. Chichester, West Sussex.
Ping BTY, Yusof M (2009). Characteristic and properties of fatty acid distillates from palm oil. Oil Palm Bul. 59: 5–11.
Putri EM, Zain M, Warly L, Hermon H (2021): Effects of rumen-degradable-to-undegradable protein ratio in ruminant diet on in vitro digestibility, rumen fermentation, and microbial protein synthesis. Veterinary World, 14, 640648. https://doi.org/10.14202/VETWORLD.2021.640-648.
Rahman MM, Lourenço M, Hassim HA, Baars JJP, Sonnenberg ASM, Cone JW, De Boever J, Fievez V (2011). Improving ruminal degradability of oil palm fronds using white rot fungi. Anim. Feed Sci. Technol., 169(3–4): 157–166. https://doi.org/10.1016/j.anifeedsci.2011.06.014
Rizvi S, Raza ST, Ahmed F, Ahmad A, Abbas S, Mahdi F (2014). The role of vitamin E in human health and some diseases. Sultan Qaboos Univ. Med. J., 14(2): 157–165. https://doi.org/10.18295/2075-0528.1566
Rusli ND, Azmi MA, Mat K, Hasnita CH, Wan-Zahari M, Azhar K, Zamri-Saad M, Hassim HA (2019). The effect of physical and biological pre-treatments of oil palm fronds on *in vitro* ruminal degradability. Pertanika J. Trop. Agric. Sci., 42(2): 791–805.
Rusli ND, Ghani AAA, Mat K, Yusof MT, Zamri-Saad M, Hassim HA (2021). The potential of pretreated oil palm frond in enhancing rumen degradability and growth performance: A review. Adv. Anim. Vet. Sci., 9(6): 811–822. https://doi.org/10.17582/journal.aavs/2021/9.6.811.822.
Rustamaji H, Prakoso T, Devianto H, Widiatmoko P, Saputera WH (2022). Urea nitrogenated mesoporous activated carbon derived from oil palm empty fruit bunch for high-performance supercapacitor. J. Energy Storage, 52: 104724. https://doi.org/10.1016/j.est.2022.104724
Safira, Nahrowi N, Diapari D, Ridla M (2024). Physical, chemical, and *in vitro* digestibility of complete feed block silage based on oil palm fronds. Di dalam: IOP Conf. Ser. Earth Environ. Sci., pp. 1359. https://doi.org/10.1088/1755-1315/1359/1/012076
Saminathan M, Mohamed WNW, Noh AM, Ibrahim NA, Fuat MA, Ramiah SK, Chung ELT, Dian NLH (2022). Treated oil palm frond and its utilisation as an improved feedstuff for ruminants: An overview. J. Oil Palm Res., 34: 591–607. https://doi.org/10.21894/jopr.2021.0041
Santoso B, Hariadi BT, Lekitoo MN (2024). Fermentation characteristics, *in vitro* nutrient digestibility, and methane production of oil palm frond-based complete feed silage treated with cellulase. Adv. Anim. Vet. Sci., 12(7): 1394–1403. https://doi.org/10.17582/journal.aavs/2024/12.7.1394.1403
Shamsuddin R, Singh G, Kok HY, Rosli MH, N. A. Dawi Cahyono. MKL, Lim JW, Low A (2021). Palm oil industry processes, by-product treatment and value addition. Di dalam: Sustainable Bioconversion of Waste to Value Added Product. Advances in Science, Technology & Innovation. Springer. https://doi.org/10.1007/978-3-030-61837-7_8
Sharif M, Qamar H, Wahid AA (2019). Effect of rumen degradable protein concentrations on nutrient digestibility, growth performance and blood metabolites in Beetal kids. Concepts Dairy Vet. Sci., 2(5): 249–253.
Shen J, Zheng W, Xu Y, Yu Z (2023). The inhibition of high

ammonia to *in vitro* rumen fermentation is pH dependent. Front. Vet .Sci., 10: 1163021. https://doi.org/10.3389/fvets.2023.1163021

Shitandi A, Abdul F, Anakalo MG (2009). Enhancement of the nutritive value of bagasse using chicken manure. African J. Food, Agric. Nutr. Dev. 9:1713–1724. https://doi.org/10.4314/ajfand.v9i8.48409

Simeh MA (2004). Comparative advantage of the European rapeseed industry vis a vis other oils and fats producers. Oil Palm Ind. Econ. J., 4: 14–22.

Sio, S., Bira GF, Batu MS, Pardosi L, Mau RJ, Klau MO, Hoar J (2022). Organoleptic quality and nutrition of rice straw silage utilizing local microorganisms (MOL) of cattle rumen fluid at different inoculum levels. J. Adv. Vet. Res., 12: 36–40.

Sordillo LM (2016). Nutritional strategies to optimize dairy cattle immunity. J. Dairy Sci., 99(6): 4967–4982. https://doi.org/10.3168/jds.2015-10354

Srivastava R, Singh. P, Tiwari. S, Mishra DB, Kumar G (2021). Sub-acute ruminal acidosis: Understanding the pathophysiology and management with exogenous buffers. J. Entomol. Zool. Stud., 9(2): 593–599. https://doi.org/10.22271/j.ento.2021.v9.i2i.8537

Sujani S, Seresinhe RT (2015). Exogenous enzymes in ruminant nutrition: A review. Asian J. Anim. Sci., 9: 85–99. https://doi.org/10.3923/ajas.2015.85.99

Sukri SSM, Rahman RA, Illias RM, Yakob H (2014). Optimization of alkaline pretreatment conditions of oil palm fronds in improving the lignocelluloses contents for reducing sugar. Rom Biotechnol. Lett., 19: 9006–9018.

Suyitman, Warly L, Rahmat A, Pazla R (2020). Digestibility and performance of beef cattle fed ammoniated palm leaves and fronds supplemented with minerals, cassava leaf meal and their combinations. Adv. Anim. Vet. Sci., 8(9): 991–996. https://doi.org/10.17582/journal.aavs/2020/8.9.991.996

Syapura M, Bata WS, Pratama (2013). Improving of rice straw quality and its effect on ability nutrient digestibility and rumen metabolism products of buffalo *in-vitro* with feces as inoculum source. Agripet. 13(2): 59–67. https://doi.org/10.17969/agripet.v13i2.822

Tadele Y, Amha N (2015). Use of different non protein nitrogen sources in ruminant nutrition: A review. Adv. Life Sci. Technol., 29:100–105.

Tahir PM, Fei AA, Ashaari Z, Lee SH, Al-Edrus SSA (2022). Oil palm empty fruit bunch as a potential feedstock for composting. Lett. Appl. Nano Biosci., 11: 3961–3974. https://doi.org/10.33263/LIANBS114.39613974

Tilley JMA, Terry RA (1963). A two-stage technique for the *in vitro* digestion of forage crops. Grass Forage Sci., 18: 104–111. https://doi.org/10.1111/j.1365-2494.1963.tb00335.x

Van Soest PJ, Robertson JB, Lewis BA (1991). Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci., 74: 3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2

Wang E, Wang J, Lv J, Sun X, Kong F, Wang S, Wang Y, Yang H, Cao Z, Li S, Wang W (2021). Comparison of ruminal degradability, indigestible neutral detergent fiber, and total-tract digestibility of three main crop straws with alfalfa hay and corn silage. Animals, 11(11). https://doi.org/10.3390/ani11113218

Wang X, Guo C, Xu X, Zhang L, Li S, Dai D, Du W (2023). Effect of alkaline mineral complex buffer supplementation on rumen fermentation, rumen microbiota and rumen epithelial transcriptome of newborn calves. Fermentation. 9(11): 1–16. https://doi.org/10.3390/fermentation9110973

Wang Z, Li X, Zhang L, Wu J, Zhao S (2022). Digestibility, fermentation characteristics and rumen microbial community. Animals, 12: 118. https://www.mdpi.com/journal/animals. https://doi.org/10.3390/ani12010118

Wu G (2019). Management of metabolic disorders (including metabolic diseases) in ruminant and nonruminant animals. Elsevier Inc. https://doi.org/10.1016/B978-0-12-817052-6.00027-6

Xia. C, Rahman MAU, Yang H, Shao T, Qiu, Su H, Binghai C (2018). Effect of increased dietary crude protein levels on production performance, nitrogen utilisation, blood metabolites and ruminal fermentation of Holstein bulls. Asian-Australas J. Anim. Sci., 31(10): 1643–1653. https://doi.org/10.5713/ajas.18.0125

Xiao J, Khan MZ, Ma Y, Alugongo GM, Ma J, Chen T, Khan A, Cao Z (2021). The antioxidant properties of selenium and vitamin E; their role in periparturient dairy cattle health regulation. Antioxidants, 10(10): 1555. https://doi.org/10.3390/antiox10101555

Yin Y, Yang C, Tang J, Gu J, Li H, Duan M, Wang X, Chen R (2021). Bamboo charcoal enhances cellulase and urease activities during chicken manure composting: Roles of the bacterial community and metabolic functions. J. Environ. Sci., 108: 84–95. https://doi.org/10.1016/j.jes.2021.02.007

Ying W, Shi Z, Yang H, Xu G, Zheng Z, Yang J (2018). Efect of alkaline lignin modifcation on cellulase–lignin interactions and enzymatic saccharifcation yield. Biotechnol. Biofuels, 11: 214. https://doi.org/10.1186/s13068-018-1217-6

Zain M, Ningrat RWS, Suryani H, Jamarun N (2022). Effect of various feed additives on the methane emissions from beef cattle based on an ammoniated palm frond feeds. IntechOpen., siap terbit. https://doi.org/10.5772/intechopen.100142

Zeng., Yining S, Zhao S, Yang S, Ding Y (2014). Lignin plays a negative in the biochemical process for producing lignocellulosic biofuels. Curr. Opin. Biotechol., 27: 38–45. https://doi.org/10.1016/j.copbio.2013.09.008

Zewdie AK (2019). The different methods of measuring feed digestibility: A review. EC Nutr., 1(2019): 68–74.

Zhang F, Li B, Ban Z, Liang H, Li L, Zhao W, Yan X (2021). Evaluation of origanum oil, hydrolysable tannins and tea saponin in mitigating ruminant methane: *In vitro* and *in vivo* methods. J. Anim. Physiol. Anim. Nutr. (Berl.), 105(4): 630–638. https://doi.org/10.1111/jpn.13501

Zhang M, Bai H, Zhao Y, Wang R, Li G, Zhang G, Zhang Y (2022). Effects of dietary lysophospholipid inclusion on the growth performance, nutrient digestibility, nitrogen utilization, and blood metabolites of finishing beef cattle. Antioxidants, 11:1486. https://doi.org/10.3390/antiox11081486

Zhang Z, Wei W, Yang S, Huang Z, Li C, Yu X, Qi R, Liu W, Loor JJ, Wang M, Zhang X (2022). Regulation of dietary protein solubility improves ruminal nitrogen metabolism *in vitro*: Role of bacteria–protozoa interactions. Nutrients, 14(14). https://doi.org/10.3390/nu14142972

Zhong H, Zhou J, Abdelrahman M, Xu H, Wu Z, Cui L, Ma Z, Yang L, Li X (2021). The effect of lignin composition on ruminal fiber fractions degradation from different roughage sources in water buffalo (*Bubalus bubalis*). Agriculture, 11: 1015. https://doi.org/10.3390/agriculture11101015

Zhong H, Zhou J, Wang F, Wu W, Xiong H, Yin H, Li X

(2023). 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. Front. Microbiol., 14: 1103652. https://doi.org/10.3389/fmicb.2023.1103652

Zurak D, Kristina K, Aladrović J (2023). Metabolism and utilisation of non-protein nitrogen compounds in ruminants : A review Metabolizam i iskorištenje neproteinskih dušićnih spojeva u preživača: Pregled. J. Cent. Eur. Agric., 24(1): 1–14. https://doi.org/10.5513/JCEA01/24.1.3645