Potential of cumin essential oil as inhibitor of deamination during ensiling process: A meta-analysis and in-silico approach

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Abstract. Silage produced from high-protein forage is susceptible to amino acid deamination, resulting in ammonia production. One strategy in the development of novel additives is to employ the in-silico method and meta-analysis. Cumin essential oils (EOs) contain metabolite chemicals that may serve as deamination inhibitors, necessary for further research both in vitro and in vivo. This study intends to conduct virtual screening through molecular docking simulations of compounds derived from cumin essential oil as deamination inhibitors in silico, alongside a meta-analysis to validate their efficacy on fermentative products during ensiling. This work examines the relationship between the ligand of Cumin EOs and the particular glutamate dehydrogenase (GDH) receptor specific from Clostridium sp., a common contaminant in silage. The observed metrics included energy values derived from the Vina program, pharmacokinetic analysis, and free ammonia concentration in silage. The meta-analysis results indicated that cumin essential oil supplementation effectively decreased free ammonia during ensiling ($P < 0.05$). However, computer simulations showed that the α-hederin molecule compound was the most effective ligand tested as a deamination inhibitor. In conclusion, it is proposed that Eos cumin might act as a deamination inhibitor in silage while it is being stored.

1 Introduction

Silage is green fodder that is preserved through a fermentation process and used as feed for ruminant livestock. The purpose of making silage is to preserve and maintain the quality of nutrient substances in feed ingredients for a long time, so that livestock feed needs are still

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met even in the dry season. The basic principle of making silage is to be in anaerobic and acidic conditions for a certain time. Preventing oxygen access and the growth of fungus and decaying bacteria, eliminating air as soon as possible, and lowering pH by generating lactic acid during the storage period are crucial stages in achieving these conditions. [1]. However, in practice, the presence of rotting bacteria often causes the silage process to deteriorate. The presence of these rotting bacteria will cause the physical and chemical quality of the nutrients in the feed ingredients to decrease, thus affecting the palatability and digestibility of feed by livestock. One of the rotting bacteria that is often found in silage is Clostridium sp. The presence of Clostridium sp. can accelerate rotting and cause the deamination process of feed protein, this will certainly affect the adequacy of feed nutrients for livestock [2]. In this case, the activity of the glutamate dehydrogenase (GDH) enzyme found in Clostridium sp. is one of the enzymes that plays a role in the deamination process.

One way that can be done is by adding additives in making silage to prevent the growth of rotting bacteria and support the maintenance of the nutritional content of silage raw materials. Recent research conducted by Susanto et al. [3] shows that the use of cumin essential oil is effective in preventing the growth of fungi and rotting bacteria. Cumin has antibacterial activity that is effective in preventing bacterial growth through the mechanism of damaging bacterial cell membranes [4]. *α-hederin, nigellidine, nigellicine, nigellimine, thymoquinone, and thymohydroquinone* are some of the active compounds in cumin. They stop the growth of pathogenic bacteria [5]. The content of active compounds in cumin, which is abundant in nigella sativa, is also influenced by the location where the plant is planted [6]. Several studies on the use of cumin in silage have also begun to be carried out to assist the fermentation process of silage during storage [7]. However, the results are still quite varied compared to other papers on the use of cumin in silage. The purpose of this study was to find out if cumin essential oil could be used to stop deamination using a meta-analysis method and to find out which compounds in cumin can decrease deamination by using a bioinformatic molecular doking or in silico method.

2 Method

2.1 Meta-analysis

The initial stage of this study involved conducting a meta-analysis, which involved searching for data and creating a database of primary studies conducted by researchers on the use of cumin in silage production. Literature searches were conducted using the keywords "cumin" and "silage" in the Scopus database (https://www.scopus.com/search/, accessed on September 10, 2024) using the institutional IPB University access and several other websites. Nutrient content, such as fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL); chemical composition of feed; and silage fermentation results, such as pH, water-soluble carbohydrates (WSC), NH3-N, and lactic acid (LA). Furthermore, the microbial population parameters include lactic acid bacteria (LAB), yeast, and mold populations. A screening process selected four journals, each containing eight studies (comparisons), which were then tabulated into a data set using Microsoft Excel and presented in Table 1. During the tabulation process, the units were equalized for each parameter.

Table 1. Studies included as a database for meta− analysis of cumin on silage

Exp.	Reference	Substrate	Level Cumin (mg/kg)	Ensiling period (days)
1	Onenc and Turgud [7]	Alfalfa	600	60
2	Turan and Onenc [8]	Alfalfa	300	120

3	Turan and Onenc [8]	Alfalfa	500	120
4	Hodjatpanah-Montazeri et al. [9]	Whole crop corn	120	45
5	Hodjatpanah-Montazeri et al. [9]	Whole crop corn	240	45
6	Akinci and Onenc [10]	Vetch-oat	200	70
7	Akinci and Onenc [10]	Vetch-oat	300	70
8	Akinci and Onenc [10]	Vetch-oat	500	70

The collected data were then analyzed qualitatively and quantitatively. The data obtained were summarized and presented in the form of tables and diagrams, forming qualitative data. The quantitative data obtained were analyzed using meta-analysis techniques and presented in the form of effect sizes, referring to the method carried out by Susanto et al. [11]. The term "effect size" refers to the magnitude of a variable's influence on another variable, as well as the magnitude of the relationship or difference that is independent of sample influence. Effect size as 'Hedges' (d) is applied to measure the effectiveness of cumin essential oil. This method was chosen because of its ability to calculate effect sizes, regardless of the heterogeneity of sample sizes, units of measurement, and statistical test results, as well as its suitability for estimating the effects of paired control and treatment [3,12].

2.2 In-silico

The next research area, bioinformatics, employs the molecular docking or in silico method. The molecular docking method utilizes the three-dimensional structure of glutamate dehydrogenase (GDH) specific to Clostridium sp., which we obtained from the RSCB database (https://www.rcsb.org/) under the PDB code 1BGV (Figure 1). As a comparison ligand, bithionol (2,2'-Thiobis (4,6-dichlorophenol)) is used. The test ligands in this study are cumin compound derivatives like α-hederin, nigellidine, nigellicine, trans-4-methoxy-thujane, nigellimine, thymoquinone, and thymohydroquinone. These were sourced from PubChem (https://pubchem.ncbi.nlm.nih.gov/), SwissADME (http://www.swissadme.ch/), Knapsack (https://www.knapsackfamily.com/KNApSAcK/), and a literature review. The software used was AutoDock Vina version 1.1.2, which was used to analyze binding energy, and PyMol 1.3 for visualization of molecular docking results.

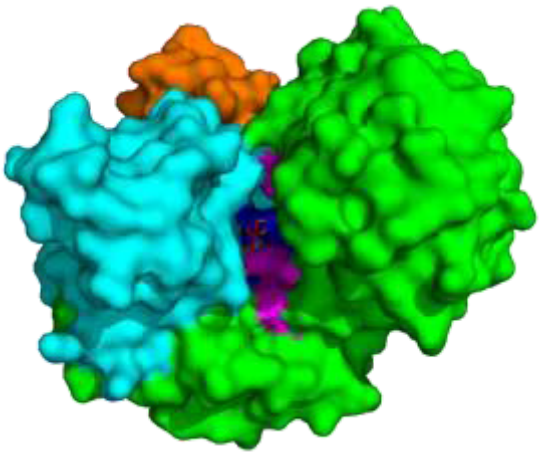

Fig. 1. Domains of glutamate dehydrogenase (GDH)

This study has several stages. The first step involves searching and downloading the three-dimensional structures of GDH, bithionol, and secondary metabolite compounds of cumin that meet the Lipinski Five criteria. These criteria include α-hederin, nigellidine,

nigellicine, trans-4-methoxy-thujane, nigellimine, thymoquinone, and thymohydroquinone. Second, processing molecular docking between GDH receptors and secondary metabolites of cumin with AutoDock software. Third, analyze the binding energy by comparing it with the binding energy of the GDH receptor. Fourth, analyze the binding location by visualizing the results of molecular docking using the PyMol program. Finally, look for secondary metabolites that have smaller bonds than bithionol and have the same binding site between the test ligand and the reference ligand. The secondary metabolites possess the capability to hinder Clostridium sp.'s deamination activity.

3 Results and Discussion

The results of the meta-analysis in Table 2. show a comparison between the control and silage supplemented with cumin. Based on the nutrients in the silage, adding cumin changed the rise in DM, CP, and EE levels. However, only ADL levels decreased significantly ($p < 0.05$) compared to the control group when it came to fiber levels. These results are in accordance with the results of the meta-analysis conducted by Susanto [3], where the use of essential oils affected the increase in nutrient content of silage compared to the control. Cumin supplementation during ensiling did not affect the pH of the silage produced. These results prove that the addition of cumin during the ensiling process does not affect the dynamics of silage pH production. Furthermore, in the microbial analysis, the yeast and mold populations decreased significantly ($p <0.01$) in the silage produced. This result can be attributed to the broad spectrum of antimicrobial activity of cumin [13].

Table 2. Meta-analysis effect cumin on nutrient quality, fermentative, microbial population silage

Variabel	unit	Est.	Lower	Upper	Std.E	p-value	tau^2	Q	Het.p-value	I^2
Nutrien quality										
DM	%	2.11	0.30	3.91	0.92	0.022	4.49	37.20	< 0.001	81.18
pH		-0.75	-2.42	0.92	0.85	0.381	4.15	37.63	< 0.001	81.40
CP	%DM	4.74	2.07	7.41	1.36	< 0.001	10.86	49.62	< 0.001	85.89
EE	%DM	4.08	0.81	7.35	1.67	0.015	13.07	36.12	< 0.001	86.16
CF	%DM	-7.36	-19.65	4.94	6.27	0.241	179.9	69.99	< 0.001	92.86
CA	%DM	-0.08	-3.86	3.71	1.93	0.968	13.80	44.12	< 0.001	88.67
NDF	%DM	-2.66	-7.11	1.78	2.27	0.24	30.23	76.32	< 0.001	90.83
ADF	%DM	-5.83	-13.55	1.89	3.94	0.139	76.53	67.24	< 0.001	92.56
ADL	%DM	-2.27	-4.38	-0.15	1.08	0.035	5.01	26.21	< 0.001	80.93
Fermentation quality										
WSC	g/kg DM	-16.0	-23.98	-8.01	4.07	< 0.001	59.39	26.60	< 0.001	81.21
LA	g/kg DM	21.29	9.97	32.60	5.77	< 0.001	119.3	46.39	< 0.001	89.22
NH3-N	g/kg N	-9.99	-15.95	-4.03	3.04	0.001	34.01	72.80	< 0.001	90.39
Microbial analysis										
LAB	log10 CFU/g	67.33	28.88	105.8	19.62	< 0.001	1738	44.70	< 0.001	88.81
Yeast	log10 CFU/g	-21.4	-30.67	-12.24	4.70	< 0.001	83.19	19.77	0.001	74.71
old	log10 CFU/g	-17.7	-24.63	-10.76	3.54	< 0.001	41.29	14.04	0.02	64.40

Different superscript letters in the same column indicate significant effect ($p<0.05$).

Deamination is the process of breaking down amino acids in feed protein, which is closely related to the free ammonia content of the protein. Making silage from high-protein feed ingredients causes rapid protein degradation and can reduce the value of silage protein [14]. The results of the meta-analysis are presented in Figure 2. The results indicate a significant decrease in the ammonia content when compared to the control group. This shows that cumin has quite good activity in inhibiting deamination without affecting the fermentation process. The effect of cumin supplementation on silage fermentation products shows that cumin has an effect on increasing LA and reducing NH3-N content significantly ($p < 0.01$). These results indicate that cumin has the ability to reduce NH3-N content, which allows for deamination inhibition activity without disrupting the activity of lactic acid bacteria during fermentation. The increased LA content is also followed by the results of the LAB population parameters, which experienced a significant increase ($p < 0.01$) compared to the control.

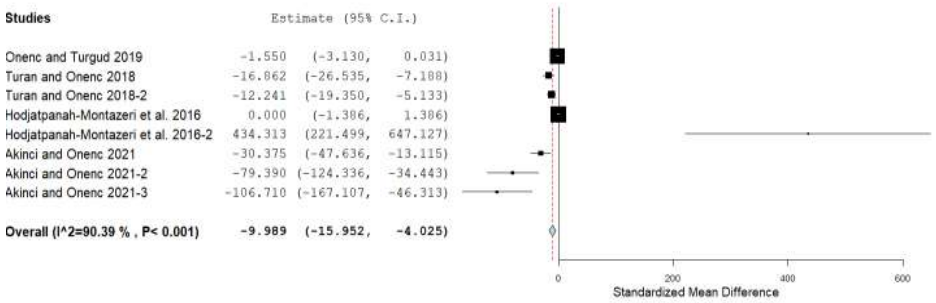

Fig. 2. Forest plot of the effect of cumin on the free ammonia content of silage

Amino acids that undergo deamination are converted into α-keto acids that then undergo decarboxylation, which produces CO2, NH3, and short-chain fatty acids. The end product of protein deamination in the form of NH3 can reduce the quality of silage by reducing the palatability of livestock [15]. The Autodock Vina tool, based on the virtual screening assessment between the glutamate dehydrogenase receptor and the cumin derivative compound test ligand, is highly suitable for scoring the docking process on complex molecules like GDH. The scoring results indicate that cumin group test ligands, such as -hederin, nigellidine, and nigellicine, have the highest binding affinity, with values of -11.3, -8.1, and -7.4 kcal/mol, respectively. These values are more negative than the bithionol control ligand, which has a binding affinity of -7.2 kcal/mol. It is expected that the cumin compound has a better binding affinity with the glutamate dehydrogenase receptor and can inhibit the enzymatic function of hydrogen dehydrogenase. The lowest affinity energy indicates an increased interaction strength in the complex. Furthermore, the bioavailability results via SwissADME (Figure 3) show that bithionol has a smaller molecular size compared to α-hederin. The purpose of using SwissADME is to determine the level of affinity of a compound as a drug or additive when applied to living creatures.

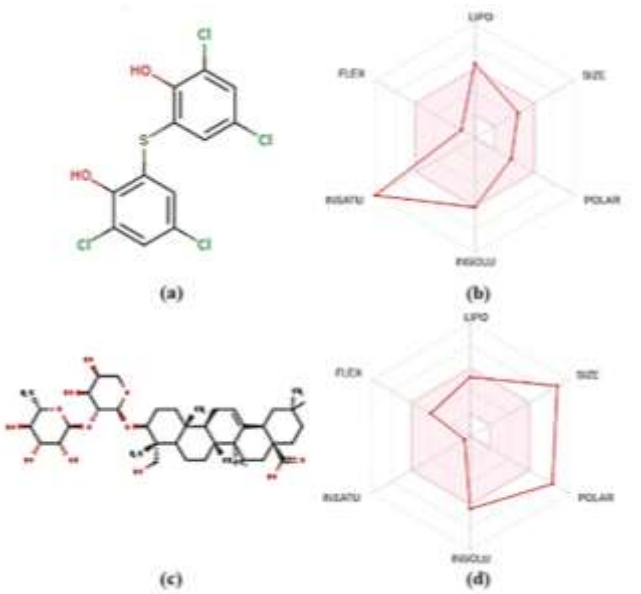

Fig. 3. The compound and bioavailability of (a) control ligand bithionol, (b) bioavailability bithionol, (c) test ligand α-hederin, and (d) bioavailability test ligand α-hederin.

4 Conclusions

The meta-analysis results showed that cumin EOs supplementation effectively increased nutrient content and prevented the growth of spoilage bacteria during ensiling. The fermentation products from silage that had cumin added to it also had higher values, which were linked to lower levels of ammonia. This kept the silage from deaminating. Furthermore, in silico results showed that the specific compound α-hederin was the most effective test ligand as a deamination inhibitor. These results suggest that cumin EOs have the potential to act as a deamination inhibitor in silage during the ensiling process.

The Acknowledgements, This study was supported and provided facilities by the Directorate General of Higher Education, Ministry of Education, Culture, Research, and Technology, on the PMDSU research scheme, No. 001/E5/PG.02.00/PL.PMDSU/2024, date: 3 May 2024.

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