



Valorization of Grape (*Vitis vinifera* L.) by Products as Sustainable Nutrient Source in Livestock Feed Formulation

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ABSTRACT

Background: The viticulture industry generates significant amounts of waste products, primarily grape pomace, consisting of seeds and peels. These byproducts pose a disposal challenge but hold immense potential as a valuable resource for animal feed formulation. This study explored the nutritional value of grape seed and peel extracts derived from *Vitis vinifera* L. (commonly grapevine) in the context of animal feed.

Methods: Freeze drying preserved the integrity of these compounds, making it an attractive method for extraction. Freeze-dried grape seed and peel extract from grape pomace were orally administered to Wistar rats in 5 gm/250 ml dose for two weeks followed by two days washout period and various physiological and biochemical parameters were assessed to elucidate their potential pharmacological effects. Nuclear magnetic resonance (NMR) been used to identify the metabolite compounds that helped to assess the mechanism of action of the metabolic pathways through enrichment analysis.

Result: The potential benefits of incorporating these extracts into animal diets, including improved nutrient utilization, enhanced gut health and antioxidant activity are discussed. High feed efficacy proved the cellular growth and proliferation through body weight enhancement. The biochemical profile also align with NMR analysis that revealed the presence of Medium-chain fatty acids (MCFAs) and Short-chain fatty acids (SCFAs), aligning with enrichment analysis findings. These findings indicate that grape seed and peel extracts possess valuable bioactive compounds, including terpinolene, lipoic acid and tocopherol, which can enhance animal health and feed utilization.

Key words: Animal feed, Grape peel extract, Grape seed extract, NMR, Sustainable livestock production, *Vitis vinifera* L., Waste valorization.

INTRODUCTION

Viticulture industry is a thriving economic sector, producing vast quantities of grapes for wine, juice and raisin production (Zwingelstein *et al.*, 2020). This process generates significant amounts of waste material, primarily grape pomace (Hassan *et al.*, 2019). Pomace consists of skins, seeds, stems and pulp remaining after grape pressing (Beres *et al.*, 2017). Some efforts are underway to utilize pomace for applications such as bio-fuel production or composting, a substantial portion remains unutilized, posing a disposal challenge and environmental concern (Zheng *et al.*, 2012).

At least 14.5 million tons of solid residues are produced annually by the winemaking sector (Costa-Pérez *et al.*, 2023), primarily in the form of stalks (2-4%), wine lees (2-4%) (Ruggieri *et al.*, 2009) and grape (*Vitis vinifera*) pomace (20-30% of processed grapes) (Rodrigues *et al.*, 2022). Generally, the disposal of waste from wineries has been limited to landfills, incinerators and composting, which has negative effects on the environment, human welfare and the economy (Ahmad *et al.*, 2020). However, there's been an increase in interest in turning these biowastes into high-value animal source food (ASF) through the circular economy (Priya *et al.*, 2023). More than 70% of the bioactive phytochemicals in the grape peel (Mini *et al.*, 2021), which include fiber, sugars, proteins, minerals, polyunsaturated fatty acids, tocopherols, β -carotenoids and phenolics, are

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retained during the wine extraction process. To enhance animal health and production performance, as well as the quality of eggs, meat and milk and to lower methane and nitrogen emissions, grape seeds and their extracts have been utilized as feed ingredients and bio-preservatives (Chikwanha *et al.*, 2022). The practical value of grape seed oil, stalks and wine lees in the ASF is not well established, despite an increase in the reporting of these waste uses. Thus, the exploration of these byproducts as a source of nutrients for animal feed formulation (Rangasami *et al.*,

2024) aligns with the principles of circular bio-economy, promoting waste valorization (Readh *et al.*, 2023) and sustainable livestock production (Sadhukhan *et al.*, 2020) (Fig 1).

Grape seeds and peels are valuable sources of nutrients and bioactive compounds with potential benefits for animal health. Protein content ranges from 6-18% in seeds and 3-10% in peels, with a balanced amino acid profile. F-box protein VviKFB07 regulates flavonoid metabolism in grapes by targeting and degrading CHS enzymes through the ubiquitin-proteasome pathway (Zhao *et al.*, 2023). Carbohydrates, especially eleven type of sugar found in NMR study (Scettri *et al.*, 2024), are primarily in the form of dietary fiber (30-60% in seeds and 50-70% in peels), promoting gut health. Nan *et al.*, 2024 explored that after 125 days of flowering shading treatment reduced the sucrose content but higher glucose and fructose content of grape berries and the weight of the berries also increased. Lipids are relatively low (1-5%), but grape seed oil is rich in unsaturated fatty acids, particularly linoleic acid, which can be beneficial for animal health (Javed *et al.*, 2023). Studies reported that decanoic acid (Kong *et al.*, 2021) pentadecanoic acid, myristic acid (Wang *et al.*, 2024), stearic acid, palmitoleic acid, stigmasterol, sitosterol, fenchol (Sadoughi *et al.*, 2015), myrtenoletc macro-metabolites are beneficial for controlling animal fat profile. Bioactive compounds, particularly phenolics (flavonols, stilbenes, anthocyanins), tannins and organic acids, contribute to antioxidant properties to feed that enhance the quality of animal products like meat and eggs by improving their shelf life and reducing oxidative deterioration (Kadhim *et al.*, 2017). Grape pomace is a good source of dietary fiber, with both insoluble (50% to 70%) and soluble fractions (10% to 30 %) that help in

digestion, nutrient absorption and satiety (Zhang *et al.*, 2017). Minerals like potassium (1000-2000 mg/kg), calcium (200-500 mg/kg), magnesium (100-200 mg/kg) and phosphorus (50-100 mg/kg) are abundant, along with trace minerals. The specific composition varies depending on grape variety, growth conditions and processing methods.

This study delves into the potential bioactive effect of grape seed and peel extracts from *Vitis vinifera* L. as a valuable and underutilized resource for enhancing animal feed. We explore the pharmacological effect, the composition and quality of waste material from viticulture through Nuclear magnetic resonance (NMR). By identifying the structure of molecule, we can analyze the metabolic reactions for future studies.

MATERIALS AND METHODS

The study was employed to investigate the pharmacological potential of freeze-dried grape peel and seed extracts (Fig 1). The pilot study is prepared for the test extracts through a standardized freeze-drying process, ensuring the preservation of critical phytochemicals. Wistar rats were selected as the animal model, as they are commonly used in preclinical studies to evaluate the efficacy and safety of test compounds. The experimental design focused on treatment groups, each receiving specific doses of grape peel and seed extracts. Control groups were included for comparison. This structured protocol enabled to evaluate the pharmacological effects of the test samples, including potential therapeutic benefits and any adverse reactions.

Plant material collection and extraction

Waste *Vitis vinifera* L. peel and seeds were obtained from a locally originated wine farm, Madhampatty, Coimbatore

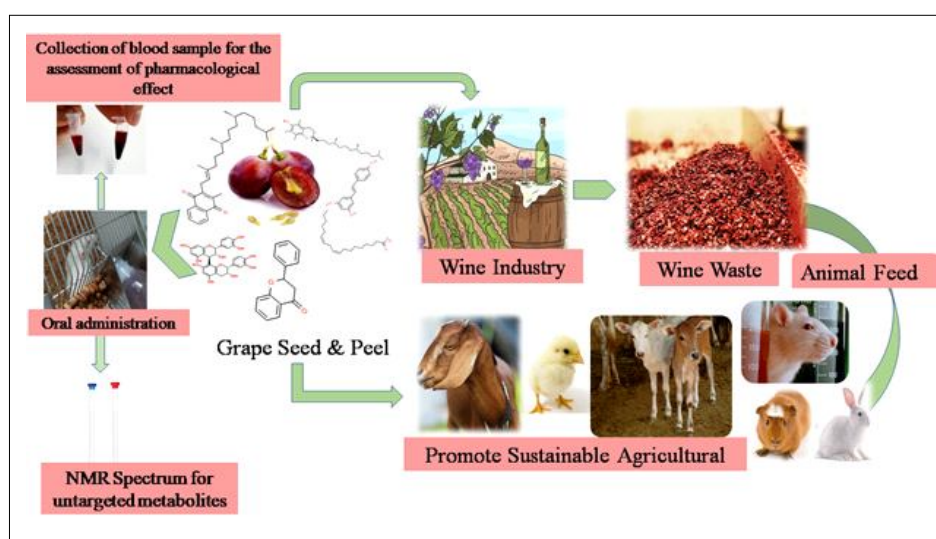


Fig 1: Waste product from the viticulture- grape seed and peel is assessed through blood sample collection and untargeted metabolites are identified that can be useful as feed for small and large animal which promote sustainable agriculture.

as a form of pomace. The peel and seeds were separated, cleaned and subjected to freeze drying to preserve the integrity of bioactive compounds. The freeze-dried materials were ground into a fine powder using a mortar and pestle. Extraction was performed using ethanol to obtain freeze-dried peel and seed extracts.

Animal study design

Male and female Wistar rats (Ethical No.-AIW: IAEK.2023:02) that are 6 weeks old, weighing between 150-200 g were randomly divided into two groups as treatment group. The rats were housed in standard laboratory conditions with ad libitum access to food and water in the animal house of Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, Tamil Nadu, India. The experimental groups received oral administration of freeze-dried *Vitis vinifera* L. peel and seed extract of 5 mg in 250 ml with the water at the replacement of water for 2 weeks with 2 days washout period at the weekend, while the control groups received normal food chow. Twenty rats divided into four groups-control male with normal chow (CMN), control female with normal chow (CFN), treatment male with extract (TME) and treatment female with extract (TFE). The duration of the

study and the frequency of extract administration (Kakaiy *et al.*, 2015) were determined based on previous literature and pilot studies (Kim *et al.*, 2015).

Assessment of pharmacological effects

Physiological (Table 1) and biochemical parameters (Table 2) were assessed to evaluate the pharmacological effects of *Vitis vinifera* L. peel and seed extracts. Physiological parameters including body weight, food intake were monitored throughout the study. Blood samples were collected from the tail vein for biochemical analysis, including glucose level, lipid profile and kidney function test.

Blood samples were kept at 37°C for 30 min and then centrifuged at 500 rpm, 4°C, for 15 min, the pale yellow supernatants (serum samples) were collected in centrifuge tubes and stored at -80°C before use. Animals were rehabilitated by treating washout period for further studies.

Metabolite analysis in NMR

Rat fecal samples were collected after feeding the sample. A total of 300 µL serum of each sample was diluted with 300 µL of phosphate buffer of 20 mM strength with 0.9% saline, containing CDCL₃ for field lock and TSP (0.01%) as a chemical shift reference. The whole mixture was centrifuged (10,000-RPM, at 4°C, for 5 min) to remove the

Table 1: Body weight changes in wistar rats before and after supplementation.

		Weight changes					
		Male			Female		
Sample (5 gm/ 250 ml)	Given	Original	After feeding weight gain (gm)	Feed efficiency (gm)	Original	After feeding weight gain (gm)	Feed efficiency (gm)
Normal feed		±0.154	-	-	±0.213	-	-
Seed and peel sample		±0.188	±0.228 (↑ 74)	±14.8	±0.248	±0.286 (↑ 73)	±14.6
2 days washout							

Table 2: Assessment of pharmacological effect through blood collection from tail vein.

Analyte	Male reference (mg/dl)	Control male with normal chow (CMN) (mg/dl)	Treatment male with extract (TME) (mg/dl)	Female Reference (mg/dl)	Control female with normal chow (CFN) (mg/dl)	Treatment female with extract (TFE) (mg/dl)
Glucose	90-180	±110	±53	82-170	100	±65
Lipid profile						
Serum cholesterol	60-100	±82.5	±110 ↑	62-104	±85.06	±122 ↑
Serum triglyceride	32-78	±58.4	±135 ↑	26-65	±58.6	±165.4 ↑
Serum HDL cholesterol	36-54	±44	±46 ↓	37-68	±44	±48 ↓
LDL cholesterol	15-35	±20	±37 ↑	12-27	±22	±41.72 ↑
Protein	5.1-7.6	±5.1	±9.6 ↑	5.2-8.2	±5.3	±10.1 ↑
Kidney function test						
BUN	12-20	±12.5	±10.27 ↓	13-24	±12.8	±14.47 ↓
Creatinine	0.3-0.6	±0.33	±0.7 ↑	0.3-0.6	±0.36	±1.0 ↑
Calcium	7.5-10.5	±8	±10.8 ↑	7.4-9	±8.5	±11.1 ↑

precipitates. The supernatants of 500 μ L were transferred into 5 mm NMR tubes for ^1H -NMR analysis. NMR spectra (Fig 2 and 3) were recorded at 298 K on Bruker Biospin Avance-III 800 MHz NMR spectrometer operating at a proton frequency of 800.21 MHz. For each sample, two types of one-dimensional (1D) ^1H NMR spectra were recorded using the standard Bruker's pulse program library and chemical shift analyzed with Biological Magnetic Resonance Data Bank (BMRB) in metabolites query search. Matched shifts were listed in Table 3 and 4 in CDCl_3 solvent.

Statistical analysis

Possible mechanism is been analysed in Metabo Analyst 6.0 (<https://www.metaboanalyst.ca/>) with the enrichment pathway analysis.

RESULTS AND DISCUSSION

Changes body weight and feed efficiency

Administration of *Vitis vinifera* L. peel and seed extracts resulted in dose-dependent changes in physiological parameters in Wistar rats. Rats treated with the extracts exhibited improvements in body weight maintenance, food intake compared to control groups.

Feed efficiency (FE) can be viewed as a homeostatic process that represents the net result of “energy expenditure, which is dependent on maintenance needs, certain nutrient restructuring mechanisms and the rate of metabolic processes and intermediary metabolism in tissues and organs and energy intake, which is determined by the voluntary feed intake and the effectiveness of digestive processes (i.e., nutrient digestion and absorption). Feed conversion ratio (FCR), which shows the relationship between feed intake and body weight gain for a particular growth phase, is a common way to express feed efficacy. As a result, animals with high FE usually consume less feed relative to their body weight growth, which may be the result of both improved digestive efficiency and better nutrient absorption and distribution towards anabolic processes (Byrne *et al.*, 2023).

$$\text{Feed efficiency (gm)} = \frac{\text{Body weight gain}}{\text{Food intake}}$$

The observed feed efficacy (Table 1) values of 14.8 gm for male rats and 14.6 gm for female rats, by using seed and peel samples, are indicative of exceptional feed utilization in smaller livestock species. This suggests that

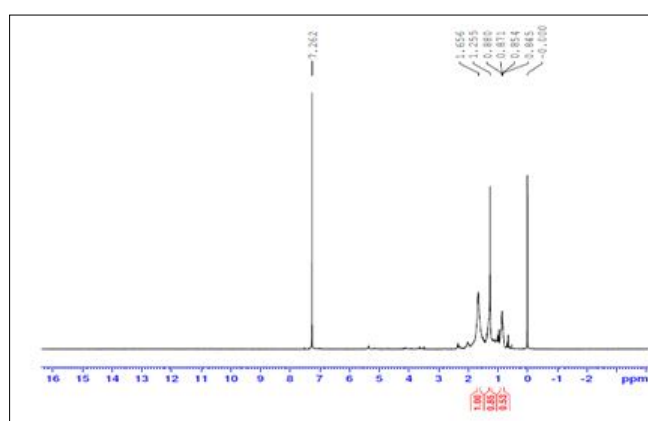


Fig 2: Spectra of female wistar rat fecal sample analyzed in ^1H -NMR CDCl_3 solvent.

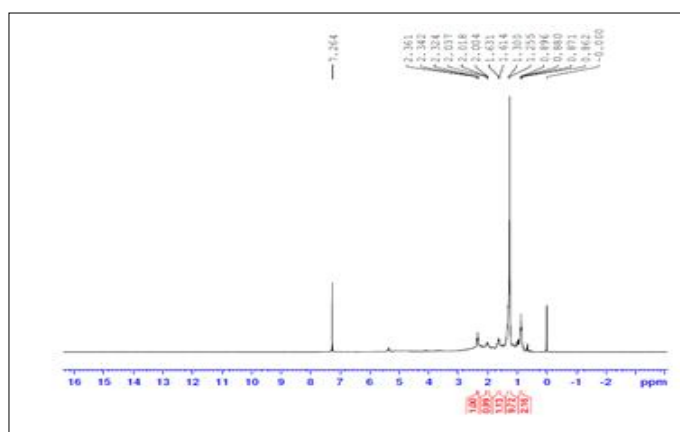


Fig 3: Male wistar rat fecal sample analyzed in ^1H -NMR in CDCl_3 solvent.

these materials are highly digestible and provide a well-balanced nutrient profile for optimal growth in animal. The efficacy might be considered moderate in larger livestock, such as cattle or sheep, it's important to note that these animals typically have different dietary requirements and metabolic rates. A moderate feed conversion in larger livestock could still be economically viable, especially if the seed and peel samples are a cost-effective feed ingredient.

Biochemical analysis revealed that glucose tests lowered the insulin modulation indicating potential hepatoprotective effects.

The extracts also demonstrated higher effects on lipid profile, with enhancement of cholesterol, low-density lipoprotein cholesterol (LDL-C) and triglyceride levels and lower the high-density lipoprotein cholesterol (HDL-C). blood urea nitrogen (BUN) test proved the healthy kidney filtration process of the waste food product.

As water was replaced with 5 mg of sample in 250 ml of water, creatinine and calcium level shows mild higher range than the reference range that occurred due to mild dehydration.

NMR spectra reflected the composition of unsaturated fatty acids that suggested the dietary impact on the animal feed. The chemical shift of the 7 ppm is considered an aromatic group and 1 ppm of the shift is considered an alkyl CH group on the up field side. Hence, it also proved the bioactive composition of seed and peel and the bioavailability of their therapeutic equivalence.

The plot (Fig 4) visualizes the results of an enrichment analysis, likely performed on a set of metabolites. The x-

axis represents the statistical significance of the enrichment, with higher values indicating lower p-values and thus stronger evidence of enrichment. The y-axis lists different groups of metabolites, such as "Fatty acids and conjugates" or "Quinone and hydroquinone lipids." The color of each dot corresponds to the p-value, with red indicating the lowest p-values and yellow indicating higher p-values. Finally, the size of each dot represents the enrichment ratio, with larger dots indicating a greater proportion of metabolites from the analyzed set within that compared group. The analysis reveals significant enrichment of Lipoamides, characterized by the largest dot size and a low p-value, suggesting a strong overrepresentation of this metabolite. This finding strongly implies a crucial role for lipoamide metabolism in the observed biological changes. Additionally, Fatty acids and conjugates exhibit high enrichment and low p-value, indicating significant involvement of fatty acid metabolic pathways. Moderate enrichment with low p-values is observed for Quinone and hydroquinone lipids, suggesting less contribution. In contrast, Alkanes show moderate enrichment with a moderate p-value, suggesting a less prominent role. Finally, Tetraterpenoids and Monoterpenoids exhibit low enrichment and higher p-values, indicating minimal or negligible involvement in the biological pathway.

The findings of this study suggested that freeze-dried extracts derived from *Vitis vinifera* L. peel and seeds from the grape pomace possess pharmacological potential, as evidenced by their beneficial effects on physiological

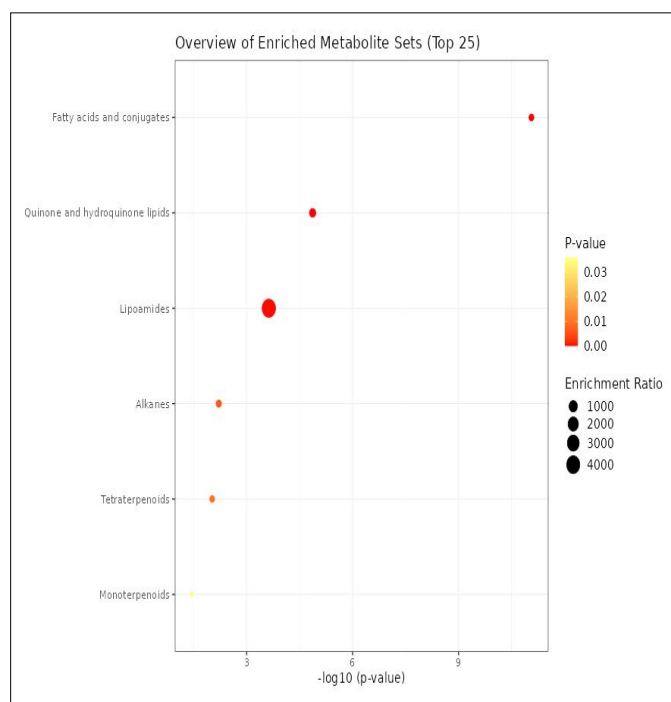


Fig 4: Enrichment analysis using Metaboanalyst 6.0.

Table 3: Identified compounds from NMR spectrum peak of female wistar rat faecal sample.

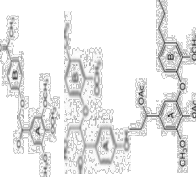
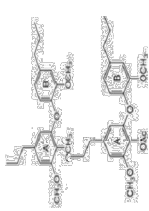
Peak no.	BMRB ID	Compound name	Structure
7.262(7.26)	1. bmse010214	1. 2-(4-Acetylphenoxy)-1-(3,4,5-trimethoxyphenyl)ethanone	
	2. bmse010442	2. H-b-S-OH	
1.656(1.65)	1. bmse010183	1. G-5-O-4-G diacetate	
	2. bmse010193	2. lignin_cw_compound_286	
	3. bmse010195	3. lignin_cw_compound_288	
1.255	1. bmse000737	1. Myristic acid	
	2. bmse000753	2. Polyoxyethylene 23 lauryl ether	
0.880(0.88)	3. bmse000764 bmse000370	3. Nonadecane	     
	bmse000485	2. Stearic acid	
	bmse000509	3. Dodecanoic acid	
	bmse000558	4. Pentadecanoic acid	
	bmse000565	5. Cis-5-dodecenoic acid	
	bmse000737	6. Myristic acid	
	bmse000753	7. Poxoxyethylene 23 lauryl ether	
	bmse000764 bmse010193	8. Nonadecane	
	-	9. lignin_cw_compound_286	
	bmse000600	-	
0.871	-	(+)alpha-tocopherol	
0.854 (0.85)	-	G-5-O-4-G diacetate	
0.845 (0.84)	bmse010183		

Table 4: Identified compounds from NMR spectrum peak of male wistar rat faecal sample.

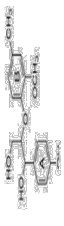
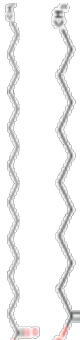
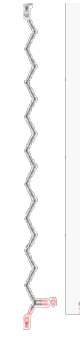
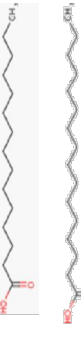
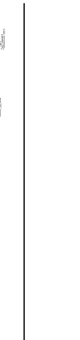
Peak no.	BMRB ID	Compound name	Structure
7.264(7.26)	1. bmse010214	1. 2-(4-Acetylphenoxy)-1-(3,4,5 trimethoxyphenyl)ethanone	
	2. bmse010442	1. H-b-S-OH	
	1. bmse000472	1. 17-alpha-hydroxyprogesterone	
2.361	2. bmse000540	2. 3-methylbenzyl alcohol	
	3. bmse000542	3. alpha-lipoic acid	
2.342 (2.34)	1. bmse010017	1. lignin_cw_compound_26	
	2. bmse010241	2. 1-(4-acetoxy-3,5-dimethoxyphenyl)-2-(4-acetylphenoxy)-3-hydroxypropan-1-one	
	3. bmse010242	3. 1-(4-acetoxy-3,5-dimethoxyphenyl)-(4-acetoxy-2-methoxyphenoxy)-3-hydroxypropan-1-one	
2.324 (2.32)	4. bmse010253	4. 1-(4-acetoxy-3,5-dimethoxyphenyl)-3-hydroxy-2-(2-methoxyphenoxy)propan-1-one	
	1. bmse000504	1. Terpinolene	
	2. bmse010040	2. 1-(4-acetoxy-3,5-dimethoxyphenyl)-1,3-diacetoxy-2-[4-(1-acetoxyethyl)phenoxy]propane	
	3. bmse010237	3. lignin_cw_compound_76	
	4. bmse010245	4. 3-(4-Acetoxy-3-methoxyphenyl)acrylic acid 4-[1,3-diacetoxy-2-(2-methoxyphenoxy)propyl]phenyl ester	
	5. bmse010258	5. 1-(4-acetoxy-3,5-dimethoxyphenyl)-1,3-diacetoxy-2-[4-(1-acetoxyethyl)-2-methoxyphenoxy]	
	6. bmse010259	1-(4-acetoxy-3,5-dimethoxyphenyl)-1,3-diacetoxy-2-[4-(1-acetoxyethyl)-	
		1,3-diacetoxy-2-[4-(1-acetoxyethyl)-	

Table 4: Continue...

Table 4: Continue....

2.037	bmse000535	2,6-dimethoxyphenox[propane 21-hydroxyprogesterone	
2.018	-	-	
2.004	bmse000528	Stigmasterol	
1.631	1. bmse000485	1. Stearic acid	
	2. bmse000509	2. Dodecanoic acid	
	3. bmse000558	3. Pentadecanoic acid	
	4. bmse000590	4. Palmitic acid	
	5. bmse000737	5. Myristic acid	
1.614	1. bmse000832	1. beta-carotene	
1.300	2. bmse010350	2. lignin_cw_compound_3026	
1.255	-	-	
	1. bmse000737	1. Myristic acid	
	2. bmse000753	2. Polyoxyethylene 23 lauryl ether	
	3. bmse000764	3. Nonadecane	
	4. bmse000485	4. Stearic acid	
	5. bmse000558	5. Pentadecanoic acid	
0.896	6. bmse000590	6. Palmitic acid	
0.888	-	-	
0.871	-	-	
0.862	bmse000547	Delta-tocopherol	

and biochemical parameters in Wistar rats. The observed improvements in glucose level, lipid profile and presence of plant sterol, volatile compounds, aromatic groups, essential fatty acids, antioxidants and flavonoids indicated potential therapeutic applications. These effects may be attributed to the presence of bioactive compounds such as terpinolene, lipoic–tocopherol, decanoic and pentadecanoic acid, myrtenol, palmitoleic acid, stigmasterol, sitosterol which have been previously reported for their health-promoting properties. The NMR dataset (Tables 3 and 4) demonstrated the presence of Medium-chain fatty acids (MCFAs) and Short-chain fatty acids (SCFAs), which aligned with the findings from enrichment analysis (Fig 4). The elevated lipid profile observed in response to the seed extract, coupled with increased lipoamide concentrations, suggests enhanced cellular growth and proliferation in healthy male and female Wistar rats. These metabolic changes are likely associated with improved feed efficacy, indicating a potential positive impact of the viticulture waste extract on nutrient utilization and overall animal performance.

Future research directions

Research effort focus on Morein *in vivo* studies are needed to evaluate the effects of grape seed and peel extracts on animal performance, health parameters and gut microbiome composition through *in-vitro*. fermentation and gastrointestinal digestion. Optimization of doses on different animal species can be established in future studies for better feed formulation. Research like anti-nutrient studies can be performed as the viticulture waste is the mixture of all nutrients. Depending on the grape variety, growing conditions, processing methods and storage conditions, it is challenging to formulate consistent and reliable animal feeds with standardized nutrient profiles.

A cost-benefit analysis is necessary to determine the optimal dosage that balances the desired benefits with economic considerations. For example, a lower dosage with a proven positive impact on animal performance or health might be more economically viable than a higher dosage with marginal benefits. Investigating the potential synergistic effects of combining grape seed and peel extracts with other feed additives, such as pre-biotic or pro-biotic, may offer enhanced benefits for animal health and performance among livestock.

CONCLUSION

Grape seed and peel extracts represent a promising source of valuable nutrients and bioactive compounds for animal feed formulation based on the sample feed efficacy. These extracts offer potential benefits for improved nutrient utilization, fibre aids digestion, enhanced gut health, and antioxidant protection in animals. Also, the small amount of sample consumption can help in the growth of body weight in a very limited period of time in a healthy manner

that helps in production cost and affect positively in economic sustainability as well as reduce the carbon emission, thereby enhance human food security. Life cycle assessment can be a valuable tool for evaluating the environmental and economic sustainability of incorporating these extracts into animal feed production systems. By addressing these challenges and conducting further research, grape seed and peel extracts have the potential to become a sustainable and valuable resource for the animal feed industry for livestock, contributing to a more circular economy within the viticulture sector.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal Care and Handling Techniques were approved by the University of Animal Care Committee.

Ethical No.-AIW: IAEC.2023:02

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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