UNIVERSITÀ DEGLI STUDI DI PADOVA

Dipartmento of Biomedicine Comparata e Alimentazione

Department of Comparative Biomedicine and Nutrition

Corso di laurea magistrale/Second Cycle Degree (MSc)

in Italian Food and Wine

Enhancing poultry meat Quality through Farm-to-Fork Traceability: A Novel Use of Post-Distillation Wine Lees

Relatore/Supervisor: Dr. Marta Castrica

Co-supervisor: Dr. Alberto de Iseppi

Dr. Mattia Pravato

Laureanda/o /Submitted by:

Aurora da Silva Melo

Matricola n./Student no.: 2107565

ANNO ACCADEMICO 2024/2025

UNIVERSITÀ DEGLI STUDI DI PADOVA

Department of Comparative Biomedicine and Nutrition

Second Cycle Degree (MSc)

in Italian Food and Wine

Enhancing poultry meat Quality through Farm-to-Fork Traceability: A Novel Use of Post-Distillation Wine Lees

Supervisor: Dr. Marta Castrica

Co-supervisor: Dr. Alberto de Iseppi

Dr. Mattia Pravato

Submitted by

Aurora da Silva Melo

Student no. 2107565

ACADEMIC YEAR: 2024/2025

INDEX

ABSTRACT

The global wine industry, a significant contributor to agricultural economies and cultural heritage, inherently generates substantial quantities of by-products, among which post-distillation wine lees, commonly known as vinasse, represent a major environmental challenge. These residues, characterized by their high organic load, acidity, and diverse chemical composition, demand sustainable and economically viable management strategies to mitigate their environmental impact and unlock their intrinsic value. It is precisely due to this rich presence of organic compounds, including residual sugars, proteins, fibers, and minerals, that vinasse presents a promising potential for valorisation. This intrinsic nutritional value makes it particularly interesting to explore its application as a novel ingredient in animal feed, specifically for broilers, offering a sustainable pathway to enhance feed formulations while addressing an environmental concern.

The study proposes the innovative use of Feed from Food technology to dehydrate and stabilize this material, aiming to transform it into a safe and effective feed ingredient, evaluating the effects of vinasse inclusion in broiler diets on various performance parameters, meat yield, and meat quality. To achieve this, the methodology was split into three phases. First, dehydration and stabilization of the material using feed from food technology. The second part consisted in the characterization of the material, covering the analysis of its nutritional composition (including proteins, fats, carbohydrates, minerals, amino acid and fatty acid profile), as well as the evaluation of protein and polysaccharide extraction under different conditions. Regarding the broiler feeding trial, the inclusion of 3% vinasse in the diet resulted in zootechnical performance, carcass yield, and meat quality traits comparable to the control group. However, the inclusion of 6% vinasse led to a significant reduction in weight gain and feed conversion efficiency, although it did not negatively impact carcass yield or meat quality parameters. These findings suggest that low-level inclusion of vinasse can be considered nutritionally safe, while higher inclusion levels may impair growth performance without compromising final product quality. The inclusion of vinasse in 3% could be a cost feed reduction in broiler production and a sustainable option for the distillers and chicken meat production.

Keywords: Broilers; Feed from food; Sustainability; Wine By-product; Vinasse.

1. Introduction

Wine is one of the most appreciated beverages in the world. According to the International Organization of Vine and Wine, in 2024 the organization estimated a World production of 231 million hectoliters of wine, of which around 60% was produced in Europe (OIV, 2022). This high amount of product also generates high amounts of residue, the estimation is that from the total wine production 14% of the weight is grapes stalk, 13% is grape pomace (seeds, stems, skins and pulp after the pressing process) and 6% is of wine yeast lees, the remain product on the bottom of the tank after fermentation, sedimentation and decanting process (Bonamente et al., 2015; Lucarini et al., 2018).

The growing concern with sustainability and the search for solutions that minimize the environmental impacts of industrial activities have driven the development of more conscious and efficient practices in the use of natural resources in recent decades. In this context, wine residues, such as grape stalks and pomace, have been studied and explored to add value to the by-product, preventing it from being used solely as a soil fertilizer. Additionally, they are utilized to produce biogas and extract bioactive compounds, antioxidants, and food colorants (Ferrara et al., 2025; López-Astorga et al., 2023; Salgado-Ramos et al., 2024a). Regarding wine yeast lees, according to the European Council Regulation (EC) 479/2008, the sub-product should be sent to distilleries to produce ethanol. This approach facilitates the implementation of the circular economy concept, reducing waste production. The question is, for every liter of ethanol produced, an average of 10 to 15 liters of vinasse are generated, a semi-solid material with a significant environmental challenge due to its high production volume and complex composition (Santos et al., 2020). The challenge and high pollutant potential is due to the low pH (around 4), high organic matter concentration, like carbohydrates, total volatile solids, nitrogen, and total phenols, and other recalcitrant compounds, with soluble COD (chemical oxygen demand). If discarded directly into the environment, the vinasse can cause ecological imbalances and affect biodiversity (Lafka et al., 2007; Petta et al., 2017;Vlyssides et al., 2005) .

But, as it is a residue from winemaking, vinasse also can contain polysaccharides from the grapes and the walls of yeast cells, such as cellulose, hemicellulose, and pectin. These compounds, which would otherwise be wasted, could represent a nutritional innovation in animal and human feed, serving as technological properties in the process of food or generating positive effects in animal performance and on reducing the environmental impacts generated by the wine industry.

As a technological food product, the presence of extractable high-value components such as polyphenols, tartrates, and yeast biomass on vinasse has attracted the attention of researchers

for the treatment and recovery of it (De Iseppi et al., 2020). The extraction of these compounds is traditionally carried out using large quantities of organic or acidified aqueous solutions that can generate waste and require treatment steps, posing a risk to the environment and human health. In this context, Natural Deep Eutectic Solvents (NADES) have emerged as a promising and environmentally friendly alternative for extracting phenolic compounds from wine distillation by-products. NADES are mixtures made up of natural compounds such as sugars, organic acids, and amino acids, which, when combined in specific proportions, form liquids with solvent properties similar to traditional organic solvents. The use of NADES has potential for presenting non-toxic quaternary ammonium salts and naturally derived uncharged hydrogen-bond donors, which gives it a green profile (Abbott et al., 2004).

In the animal feed, the antioxidant properties and presence of minerals and organic compounds in the vinasse have been seen as an alternative to conventional feed ingredients, such as grains and bran, or as a substitute for chemical performance enhancers. In ruminants' diets, the use of winemaking by-products has reached good results, partially replacing traditional feedstuffs such as corn silage. In sheep feed, the presence of tannin resulted in positive impacts on the lipid composition of meat due to the modulating effect on rumen fermentation (Vasta et al., 2009). But in monogastric animals' diets (such as pigs and poultry), the use of wine distillation by-products should be investigated with caution due to the physiological particularities, especially regarding the fiber digestibility and sensitivity to anti-nutritional compounds. Although these specific challenges exist, it is also a promising opportunity when properly processed and dosed; inclusion of grape pomace in the diet of pigs up to 10% did not affect the growth performance. In chickens, the inclusion of dehydrated grape seed pomace at levels of up to 6 % did not compromise the zootechnical performance of the animals, and the inclusion of wine lees was found to be a promising feed additive in broilers' diets, improving the meat's oxidative status (Brenes et al., 2008, Mavrommatis et al., 2021).

From the distillers' company point of view, the use of the vinasse as a by-product also has an advantage; tracking and documenting the movement of the transformation ensures traceability and avoids the disposal of material. This information can be used to comply with regulatory authorities and identify potential problems or inefficiencies in the waste management process.

However, the high amount of water, the low content of alcohol, and richness in organic compounds make vinasse highly prone to fermentation and deterioration, and stabilizing it is a crucial challenge to ensure its proper storage and use without damage and losing the nutritional and technological properties of the product. One of the most widely described methods to facilitate storage and transportation and prevent the proliferation of microorganisms is drying.

Hot air ovens are used for more solid materials, while for more liquid materials, spray drying or freeze drying can be used. Although effective, these methods have the limitation of possibly losing bioactive compounds, such as volatile phenolics, due to the high temperatures. Refrigeration and freezing are also effective alternatives, especially when it comes to preserving compounds that are sensitive to oxidation or thermal degradation (Bustamante et al., 2008; Spigno & De Faveri, 2007). But, if this process is done on a small scale or controlled environment, it makes the process expensive due to the need for material transportation to the facilities of the drying machine.

The use of a machine with continuous flow, which can be put directly on the facilities of the winery distillery, could be an option. This way, the continuous method optimizes material processing, resulting in greater efficiency, avoiding the transport of material out of the facilities. In addition, precise control of the temperature and air flow inside the machine ensures that a final product with uniform humidity and high quality is obtained and makes it possible to integrate the dehydration process with other production stages, such as packaging, optimizing workflow, and reducing operating costs, making the product ready for distribution. This continuous flow dehydration technology is already a reality and is being applied by Feed from Food, a startup that has developed and uses this machine to process food waste, transforming it into ingredients with high nutritional value for animal feed.

In this context, the proposal to use vinasse as a by-product in chicken feed not only offers an effective solution for the reuse of organic materials but also contributes to the reduction of waste and the development of more sustainable practices in the agro-industry.

2. Objectives

The demand for more sustainable food systems that reduce waste and enhance food safety is increasing across all stages of the agri-food supply chain. Within this context, the concept of a circular economy seeks to establish connections between sectors that generate residues and those that can repurpose them as valuable by-products. Linking the wine distillation industry, responsible for producing vinasse, with broiler chicken production represents a significant challenge. The sustainability of using vinasse in poultry feed depends not only on maintaining animal performance and zootechnical efficiency but also on ensuring full traceability. Such traceability is essential to guarantee food safety for both animals and end consumers. Therefore, this integration requires not just technological innovation but also monitoring systems to support safe and effective valorisation of agro-industrial residues.

The implementation of Feed from Food technology aims to streamline the vinasse production chain by minimizing logistical operations and delivering a dehydrated, stable, and

ready-to-use product. This facilitates its safe and efficient incorporation into downstream applications, particularly in animal nutrition. Given this context, this thesis aims to evaluate the feasibility and impact of including post-distillation wine lees (vinasse) in broiler chicken diets, using Feed from Food technology to increase the potential of vinasse by dehydrating the product to stabilize it and allow its storage; to explore this possibility by analyzing its composition; to assess the effects of its use at different levels (3% and 6%) on the growth performance, feed efficiency, and overall carcass performance of broiler chickens; and promote the integration of vinasse valorisation within a farm-to-fork traceability system, aligned with the principles of the circular economy.

3. LITERATURE REVIEW

The wine production involves cultural and economic significance around the World, and the consumption has remained at high levels with a continuous demand for wine that drives production and generates by-products and waste. The amount, type, and composition of waste produced in the wine production and in wine distilleries vary according to the different stages of grape processing. At the end of the wine process, wine yeast lees are obtained, which are then used as raw material for distillate production, such as grappa. From this, the final product is vinasse (Figure 1).

By its composition described as rich in glucan, chitin, and mannoproteins from the yeast cell wall, and phenolic acids, flavonoids, and oligomeric fractions (Soto et al., 2012; Blasco et al., 2011), vinasse can no longer be considered waste, and its potential as a byproduct must be explored. This review aims to present the state of the art on studies related to the sustainable use of vinasse in the context of the circular economy and wine sustainability, and present the potential of this by-product on the chicken feed.

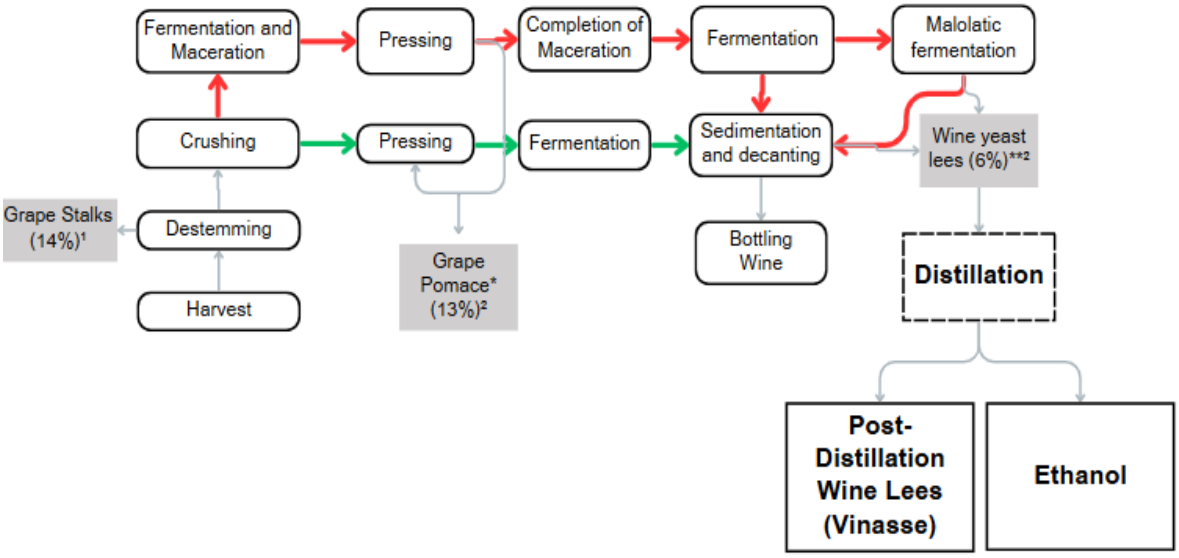

Figure 1. Representing a general process of fabrication of wine, the identification of residue production, and the vinasse production.
Scheme adapted from Evtuguin et al. (2024). Square in grey: solid residues. Red arrow: flow production of red wine. Green arrow: flow production of white wine. *Seeds, stems, skins, and pulp. **5%v/V alcohol (Vlyssides, 2005). [1]Bonamente et al., (2015); [2]Lucarini et al. (2018);

3.1. The Vinasse composition

When surveying the composition of vinasse, the major concern is the toxicity of the material to the environment and the impossibility of releasing it untreated into river affluents or soil. The low pH, high salinity, and high levels of chemical oxygen demand can characterize the vinasse as a waste, targeting the material for fertilizer use, biofuel generation, or bioethanol production (Carrilho & Soares, 2024).

On the other hand, high levels of potassium, nitrogen, and organic compounds, like polyphenols and anthocyanins, attract attention to potential applications not only in the wine sector, but also in the food, biotechnological, cosmetic, and pharmaceutical industries (Sousa et al., 2019;Kojić & Prodanović, 2024).

As the vinasse is a mix of water used in the distillation process, exhausted wine and wine lees, water-soluble compounds of the grape marc, and hydrochloric or sulphuric acid from the wine process, the composition of the final product could vary (Bustamante et al., 2005). The presence of glucan, mannoprotein, and chitin in vinasse, for example, comes from the cell wall of the dead yeasts present in the material. Around 35 to 55% of the wall is made up of glucan, almost the same of mannoprotein and between 1.5-6% of chitin conferring it interesting functional properties that can be acting as modulator of immune system in animal feed and/or be extracted and isolated for use as emulsifiers or stabilizers in the food industry (Russell & Stewart, 2022;Troilo et al., 2021).

Compounds like glucans and lignocellulosic fiber, for example, can aggregate immunomodulatory, antioxidant, anti-inflammatory, and antimicrobial properties to a product containing vinasse. Mannoproteins, when in the digestive system, release mannose, which binds to pathogenic bacteria and prevents them from colonizing the gut (Chioru et al., 2024; Cheng & Kim, 2022). The presence of these bioactive compounds resulted in a strong immune system and healthy intestinal integrity, increasing digestion and absorption of nutrients, and consequently better efficiency of the organism and greater animal performance (Jha & Kim, 2021).

From the food industry point of view, the presence of anthocyanins and flavanols in wine by-products can be isolated and used in food preparation to prevent oxidative stress and increase shelf life (Salgado-Ramos et al., 2024). The same authors also isolated levulinic acid from exhausted grape marc, a versatile chemical compound that can be explored from solvent to use in plastic industries, demonstrating the versatility of the wine by-product**.**

Other compounds were isolated from the vinasse to be used to enrich food for human consumption or production and preserve food, including the production of wine itself. Ahmad et al. (2020) in their extensive literature review quote the isolation of protein-rich fungus biomass, lactic acid, and tartaric acid on vinasse. The presence of proteins and polysaccharides from medium to low molecular weight in samples of wine yeast lees opens the way to the exploitation of the material as an alternative to existing products in the wine industry and can be used for oenological applications, as clarified or as a preservative, since it has antioxidant properties (De Iseppi et al., 2024). To keep the product stable and allow a high extractable amount of polyphenol compounds, high temperature is recommended to treat the material, thus favouring the extraction and quantification of bioactive compounds and facilitating the transport and storage of the material (Delgado et al., 2015)**.**

Due to the complex and variable composition of vinasse, particularly regarding its nutrient content for animal feed and technological properties, further information is essential to understand its nutritional value and optimize its use.

3.2. Use of Vinasse in Animal Feed

The composition of vinasse and the growing pressure for more sustainable food and animal production systems have created a tendency to use the by-product in a way to explore the potential of the vinasse, decrease the cost of production, and put into practice the circular economy concept.

As mentioned above, the presence of phenolic compounds can be beneficial for animal production, but also a problem. Vinasse can contain some anti-nutritional factors, especially for monogastric animals, such as the presence of indigestible fibers and condensed tannins (Erinle

& Adewole, 2022). Process the material, balance the diet to ensure nutrient adequacy, and know the safety level of inclusion is the challenge of the use of vinasse in animal feed.

The use of fermented vinasse in cows demonstrates that the inclusion of not more than 30% in the Guanling Crossbred Cattle increased daily weight gain and beneficial changes in the rumen microbiota, indicating positive effects on intestinal health and production performance (Zhu et al., 2024). The presence of antioxidant activity on wine lees also contributed to reducing the heat stress in milking cows, reducing the oxidative stress, increasing the feed consumption, and improving the milk quality with the increase of the oxidative stability (Yao et al., 2021).

In pigs, studies have indicated that the use of wine by-products can improve production performance and meat quality. The inclusion of fermented yellow wine vinasse improved the gut microbiota and performance of finishing pigs, due to the presence of fibers in yeast that act as prebiotics, promoting intestinal health and improving performance parameters, such as daily weight gain and feed intake, as well as improving meat quality (Zhang et al., 2022).

In the context of broiler production, the inclusion of vinasse or wine by-product in the diet has shown promising effects. An analysis of dried grape pomace showed a high antioxidant capacity (346 to 393 mmol ascorbic acid equivalent/g of dry matter) between different varieties of grape, and the inclusion until 6% didn't affect the production and improved the meat quality parameters, as lipidic oxidation and tenderness of the meat (Turcu et al., 2020). The presence of polyphenols in broilers' diets can ameliorate the negative effects of heat stress due to the antioxidant properties, also resulting in better health and reducing digestion issues due to its effect as a prebiotic, supporting the growth of beneficial gut microbiota and optimizing nutrient absorption (Shakeri et al., 2020; Iqbal et al., 2020).

Studies with grape pomace silage supplementation demonstrated a reduction of the toxic effects induced by oxidative stress and improved the redox status of broilers, preventing radical toxicity as manifested by markers of lipid peroxidation and protein oxidation in blood and tissues of vital organs (Makri et al., 2017).

From human aspects, the antioxidant properties of the wine-making by-products are explored to increase the shelf life and technological properties of the final products. Alarcón et al. (2020) tested the inclusion of 5% of wine yeast lees in the formulation of deer burgers, and the result was an increase of free radical scavenging activity values after 12 days of refrigerated food storage, resulting in a higher protection against lipid and protein oxidation of burgers. The inclusion of 0.2% of dried wine-making yeast cell walls compared with 2.5% of ground grape pomace in broilers feed didn't affect negatively the performance or the oxidative mechanisms in the liver of the animals for both products, in the other side, the content of total phenolic, flavonoid

and tannin content was smaller in the wine yeast lees. Proving that there is variability of composition from different wine-making byproducts, and even so, it represents potential for inclusion in the feed of monogastric animals (Mavrommatis et al., 2021b). Inclusion of Saccharomyces cerevisiae fermentation products in broiler feed increased the performance and improved feed conversion, presenting as alternatives to antimicrobials in poultry feed, due especially to the presence of bioactive compounds in the cell wall of the dead yeasts (Soren et al., 2024).

The inclusion until 5% of grape pomace in broilers' feed resulted in better profitability of the production system by improving feed conversion and reducing feed costs by 3.6%. It not only proves to be a sustainable product for the vinasse producer, but also profitable for the animal producer (Putri et al., 2024).

Further studies are needed to optimize the conditions of the use of vinasse in animal diets to promote more sustainable and efficient feeding practices in animal production; however, it is essential to consider the form of processing and the appropriate dosage to maximize their benefits and avoid possible adverse effects.

3.3 Traceability and food safety

In the context of adding value to agro-industrial by-products, such as vinasse, safety, transparency, and efficiency in the reuse of these residues become crucial aspects for the feasibility of their application in animal feed. The growing concern with sustainability and environmental impact control demands that the entire vinasse transformation process be carefully monitored—not only to comply with legal requirements but also to ensure the quality of the final product and food safety. In this scenario, traceability emerges as an essential tool to ensure that each stage of vinasse processing and destination is properly recorded and controlled.

The high pollutant potential of vinasse requires the industry to implement an effective system for controlling and tracking the material. According to Devesa-Rey et al. (2011), in Spain, the cost charged by municipal authorities for treating vinasse can reach approximately 230 euros per month per cubic meter per hour when the company chooses the legal routes for waste disposal, and variable fee can be apply depending on the type and quantity of alcohol produced. The proper destination of vinasse, maintaining accurate records, proves the reuse of the material and helps to avoid fines and penalties imposed by regulatory agencies.

An important tool to track and monitor the movement of the transformation from the waste to a by-product is traceability, a way to follow the movement in the supply chain that offers transparency and visibility of goods and helps to close the loop in the chain, contributing to the

circular economy, facilitating audits and ensuring compliance with regulatory standards (Gazeau et al., 2024).

Due to the different processing steps from grape to vinasse, the movement of material and transportation between production plants can pose a risk to the control of information essential to the traceability of the process. Discontinuous traceability systems, where data collection occurs only at certain production stages, compromise the visibility and responsiveness of supply chains, especially if they use manual records and fragmented information. That increases the likelihood of human error and weakens auditability, also limiting the ability to respond to operational or sanitary issues, as well as to take timely corrective actions (Bosona & Gebresenbet, 2013).

The implementation of continuous traceability system**s** allows uninterrupted monitoring of data throughout the entire production chain—from the generation of vinasse to its transformation into a co-product for animal feed. This automated and integrated model ensures greater operational control and safety, facilitating the identification of critical parameters such as temperature, pH, and time, which directly influence the quality and stability of the final product (Gazeau et al., 2024).

In addition, continuous traceability aligns with the "farm to fork" concept, which implies the integration of production processes and continuous monitoring of every stage to ensure food safety for the end consumer. In this case, traceability goes beyond regulatory compliance, becoming a strategic component of quality and credibility within the production chain.

In the case of vinasse, which contains approximately 93% water (Carrilho & Soares, 2024), it is necessary to reduce its volume to enable proper storage and stabilization. Continuous processing of vinasse makes it possible to obtain more homogeneous batches, with precise control of moisture and nutritional composition, contributing to improved palatability, shelf life, and zootechnical efficiency in poultry feeding. Moreover, continuous processing significantly reduces energy consumption per unit of final product and minimizes the need for manual interventions, thus optimizing industrial productivity.

In the context, the ability to trace the origin and processing of vinasse ensures that the product is safe and quality-controlled. Feeding animals with materials that are not produced for this purpose can present unacceptable levels of residues that can negatively affect the health of animals and the quality of animal products (Malenica et al., 2023), further increasing the need for traceability and food safety, for the animals and the consumers of their products.

The Feed from Food technology is intrinsically aligned with the principles of traceability and food safety required for transforming agro-industrial residues into safe, high-quality animal

nutrition products. The system is designed with real-time monitoring and full integration of sensor-based data collection at each stage of the process (Feed From Food, 2025). The machine can be adapted to the disposal system, capture the raw vinasse and treat it, and be directed to a continuous thermal concentration unit operating, reducing its moisture content. This step is monitored in real time with sensors measuring temperature, pressure, and residual moisture, ensuring process consistency and compliance with safety parameters. The output is a fine powder with a particle size of 100–300 μm and a moisture content below 10%, processed at a throughput of 200–250 kg/h. This architecture enables a continuous and auditable traceability chain, replacing fragmented, manual systems with a centralized and secure data flow. Technologies such as vacuum thermal concentration, fluidized-bed drying, automated nutrient dosing, and inline near-infrared (NIR) analysis not only ensure product consistency and stability but also reinforce transparency and process responsiveness. As such, the Feed from Food Machine acts not just as a drying and transformation unit, but as a strategic enabler of traceability that supports the "farm to fork" model, aligns with EU food safety frameworks, and promotes circular economy practices by valorising vinasse at the source.

In summary, the literature highlights the substantial potential of vinasse, specifically from wine yeast lees, as a valuable input for sustainable poultry nutrition due to its rich composition of bioactive compounds, functional fibers, and antioxidants. However, its successful integration into animal feed systems hinges on overcoming challenges related to anti-nutritional factors, variability in composition, and stringent food safety and traceability requirements. The need for standardized processing, nutritional consistency, and transparent monitoring is critical to ensure safe and efficient use. Based on these insights, this thesis aims to evaluate the transformation of vinasse into a safe, traceable, and nutritionally viable poultry feed ingredient using the Feed from Food technology. The following section details the materials and methods employed to process vinasse, monitor its transformation, and evaluate its potential as a feed additive through a controlled, traceable system aligned with food safety and circular economy principles.

4. Material and methods

The trial consisted of three phases. The first was to obtain the by-product using Feed from food technology, the second was the laboratory analysis of the vinasse, and the third was a trial with the inclusion of the vinasse in the broilers fed.

4.1. Transformation of the by-product

The raw by-product of distillate production from this study was provided by a company located in Monselice (Pádua-IT). The Vinasse was in a semi-solid state and was transported to

the facilities in a ton capacity container to be subjected to the dehydration process in a continuous fluidized bed machine, the Food from Feed machinery (Figure 2, Image A).

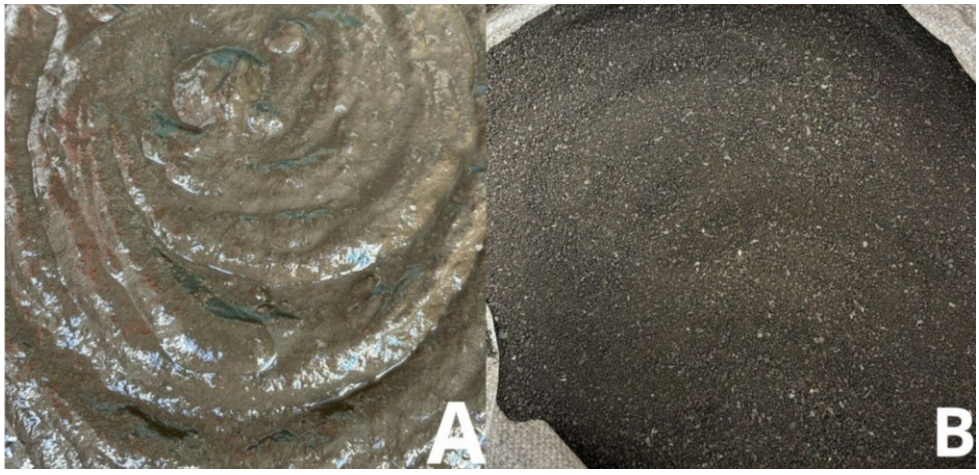

Figure 2. Raw vinasse (A) and powder of vinasse (B), before and after the dehydration process, respectively.

The dehydration process was carried with continuous monitoring of the following operating parameters (Table 1 and figure 3): inlet air temperature (recorded by thermocouples installed at the inlet to the drying chamber), fun speed drying (measured by an anemometer), by-product feed rate into the machine (controlled by a dosing system) and outlet air temperature (recorded by thermocouples at the outlet of the drying chamber). The endpoint of dehydration was defined after obtaining a powder considered adequate for the stability and storage of the dehydrated product (Figure 2, Image B).

Table 1**.** Parameters set on the Feed from Food machinery to obtain the dry vinasse.

Parameters	Values
Temperature Set Point (°C)	135
Maximum Feeding Time (s)	170
Delay Before Feeding Start (s)	3
Duration of Each Feeding Cycle (s)	6
Duration of Each Reverse Feeding Cycle (s)	7
Number of Forward Feedings Before Reversal	3
Minimum Time Between Feedings (s)	50
Maximum Relative Humidity for Feeding (%)	44
Minimum Exhaust Steam Temperature for Feeding (°C)	54
Fan Speed (Hz)	46

s: seconds, Hz: hertz.

The material was stored in polyethylene feed-grade bags under ambient temperature conditions, in a cool, well-ventilated area, and protected from direct sunlight exposure. Samples of the raw

by-product and the final dehydrated product were collected and packed in airtight containers, identified, and stored for subsequent laboratory analysis.

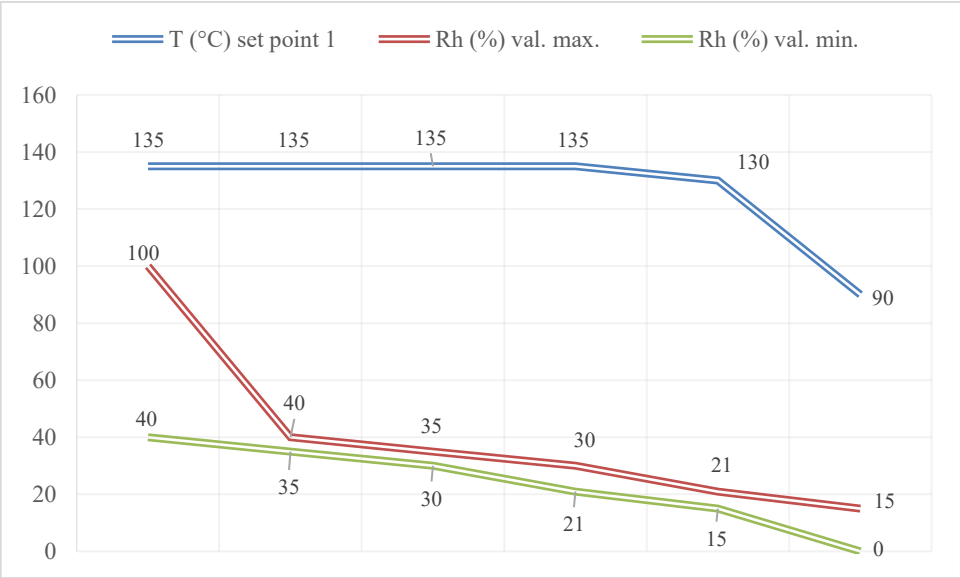

Figure 2. Set parameters and trend during the transformation process of temperature (°C) and relative humidity (%) — maximum and minimum values.

4.2 – Analyses of Vinasses' composition

The centesimal composition of the diets and the Vinasse was followed the methodologies described by (AOAC, 2000) for the analysis of dry matter, ashes, crude protein, ether extract, crude fat, crude fiber, neutral, and acid detergent fiber, total dietary fiber, and acid detergent lignin liquid according to the AIA (Agrarian Italian Association). Two levels of vinasse inclusion in broiler diets were compared with the control diet. The composition of ingredients of the control diet is described on Table 2 and the chemical composition of each diet is described in Table 3.

Table 2. Ingredients (g/kg as fed) of the basal diets.

	Basal diet	
	Starter	Grower
Corn meal	402.3	526.3
Soybean meal (48% CP)	243.0	220.0
Corn gluten feed	70.0	70.0
Jolly Wheat	70.0	70.0
Wheat middlings	75.0	0.0
Full fat soybean	50.0	25.0
Wheat bran	18.0	18.0
Soybean oil	16.7	16.7
Calcium carbonate	16.2	16.6
Monocalcium phosphate	12.0	12.0
Sunflower meal (28% CP)	10.0	10.0
Sodium chloride	2.0	2.0
Sodium bicarbonate	2.0	2.0
L-lysine HCl	2.2	1.4
DL-methionine	2.0	1.4
Liquid coline	0.4	0.4

L-threonine	0.3	0.3
Mineral and vitamin premix[1]	7.9	7.9

CP: crude protein.[1]Premix ingredients (per kg premix): vit. A, 1600000 IU; vit. D3, 500000 IU; vit. E, 5000 mg; iron sulphate (Fe): 7000 mg; calcium iodate anhydrous (I): 100 mg; cupric sulphate (Cu): 1500 mg; manganese oxide (Mn): 21000 mg; zinc oxide (Zn): 8400 mg; sodium selenite (Se): 60 mg; 6-phytase (EC 3.1.3.26): 120000 OTU; Endo-1,4-beta-xilanasi EC 3.2.1.8 (4a7), 112000 TXU; Endo-1,4-beta-glucanasi EC 3.2.1.4 (4a7), 50000 TGU.

Table 3. Chemical composition (% as fed) and mineral content (mg/kg) of the experimental diets and the raw materials (Vinasse).

	Experimental diets						Raw material
	Starter diets (0 to 14 d)			Grower diets (15 to 35 d)			
Composition	F0	F3	F6	F0	F3	F6	Vinasse
Dry matter							
Crude protein	16.8	18.3	18.1	18.3	18.0	18.5	18.2
Ether extract	5.76	5.45	5.57	4.87	4.91	4.89	3.55
Crude fibre	3.67	3.19	3.46	3.01	3.56	3.12	5.44
Ashes	6.76	6.82	7.80	6.89	7.45	8.07	17.6
NDF[1]	19.2	18.1	22.7	18.1	19.1	22.1	33.9
ADF[2]	4.04	4.40	6.71	4.59	5.96	7.34	27.2
ADL[3]	1.11	1.47	3.17	1.31	2.34	3.74	24.2
Al	123	162	240	106	180	340	2624
B	11.0	11.7	12.9	12.6	13.3	14.8	32.9
Ba	2.50	2.75	5.23	4.02	4.67	6.19	40.0
Ca	12607	11906	12064	13652	12860	13341	5281
Cr	3.66	4.2	4.01	3.53	3.54	4.44	9.47
Cu	22.5	26.4	37.4	12.2	27.2	48.2	410
Fe	317	362	437	219	370	783	3888
K	8927	9375	11123	8177	8925	11593	67669
Li	0.124	0.228	0.412	0.165	0.386	0.718	5.95
Mg	2098	2057	1954	1999	1954	1942	923
Mn	163	148	134	147	156	134	47.4
Mo	1.51	1.46	1.4	1.06	1.11	1.08	0.621
Na	1606	1320	1523	1453	1705	1536	327
P	7273	7437	7048	7131	6736	6870	3341
S	2488	2493	2484	2228	2264	2374	4002
Sr	10.8	10.7	10.4	11.9	12.1	14.1	15.0
Ti	3.29	3.93	4.91	2.99	3.68	5.88	38.1
V	4.69	5.28	4.71	2.15	1.84	2.33	2.43
Zn	105	104	101.2	112	88.2	168	35.2

F0: Control diet (100% basal diet); F3: 97% basal diet + 3% vinasse; F6: 94% basal diet + 6% vinasse. [1]Fiber in neutral detergent; [2] 3,64% of fiber in acid detergent, 1,99% of acid detergent lignin.

Tables 4 and 5 are the fatty acid and amino acid profiles of the diets. The fatty acid profile was analyzed after fat extraction by solvent-accelerated extraction (ASE®, Dionex, Sunnyvale, CA, USA, Application Note 334) and using gas chromatography with a 7820A Gas

Chromatograph (Agilent Technologies, Santa Clara, CA, USA) and Supelco OMEGAWAX-TM 250 (Sigma-Aldrich, St. Louis, MO, USA) (Gratta et al., 2019). Fatty acids were identified by comparing the retention time of methylated esters of fatty acids (FAMEs) using a standard (Supelco 37 - component FAME Mix, 47885 - U). Individual fatty acids were expressed as a percentage of total areas.

Table 4. Fatty acid profile (g/100 g of total fatty acids) of the experimental diets and the raw material (vinasse).

	Experimental diets						Raw material
	Starter diets (0 to 14 d)			Grower diets (15 to 35 d)			Vinasse
Fatty acids	C0	C3	C6	C0	C3	C6	
C16:0	13.1	13.0	13.4	12.4	12.9	13.2	28.9
C18:0	3.08	3.04	3.09	2.93	3.00	2.99	6.15
C20:0	0.33	0.32	0.34	0.33	0.35	0.36	1.06
Other SFA	1.14	1.13	1.33	1.06	1.11	1.36	8.70
C18:1 n-9	22.2	22.3	22.1	23.1	23.3	23.0	12.6
C18:1n7	1.14	1.13	1.16	1.06	1.08	1.08	1.15
Other MUFA	0.95	0.96	1.06	2.55	0.98	1.20	5.02
C18:2n6	52.7	52.8	52.2	51.9	52.5	52.0	29.6
C18:3n3	4.99	4.95	4.98	4.35	4.42	4.46	5.49
Other PUFA	0.57	0.48	0.46	0.42	0.45	0.49	1.61
Total SFA	17.5	17.4	18.0	16.6	17.3	17.7	44.5
Total MUFA	24.3	24.4	24.3	26.7	25.3	25.3	18.8
Total PUFA	58.3	58.2	57.7	56.7	57.4	56.9	36.7
n-6	52.9	53.0	52.3	52.0	52.6	52.1	29.8
n-3	5.40	5.29	5.35	4.66	4.78	4.81	6.87
n-6/n-3	9.79	10.02	9.78	11.2	11.0	10.8	4.34

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids. F0: Control diet (100% basal diet); F3: 97% basal diet + 3% vinasse; F6: 94% basal diet + 6% vinasse.

Amino acids were determined after acid hydrolysis and pre-column derivatization with 6-aminoquinolyl-Nhydroxysuccinimidyl carbamate, separated by RPHPLC and analyzed by UV detection following a method adapted from European Pharmacopoeia. Briefly, for alanine, arginine, aspartatic acid, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine, and valine determination, protein of the sample was hydrolyzed with hydrochloride acid (6 M) at 105 °C for 24 h. Cysteine (sum of cystine and cysteine) was determined after reaction with dithiodipropionic acid, producing a mixed disulphide, which then underwent acid hydrolysis. After hydrolysis, the samples were neutralized with sodium hydroxide (8 M), adjusted to volume and filtered at 0.22 mm. Then, the derivatization step was conducted according to the manufacturer's instructions (AccQTag Ultra Derivatization Kit, Waters Corporation, Milford, MA). Tryptophan was determined following a

method adapted from Commission Directive 2000/45/EC (EC, 2000) using a basic hydrolysis with barium hydroxide at 105 °C for 24 h, and after neutralization and filtration analyzed directly by RP-HPLC. Separation and quantification of amino acids were performed using an Agilent 1,260 Infinity HPLC (Agilent Technologies) equipped with a reversed-phase column C18 (CORTECS C18, 2.7 mm, 2.1 £ 150 mm, Waters Corporation) kept at 45 °C, and with a diode array Detector (1260 Series, DAD VL+, Agilent Technologies).

Table 5. Profiles of amino acids (mg/100 g) of the experimental diets and the raw material (vinasse).

	Experimental diets						Raw material
	Starter diets (0 to 14 d)			Grower diets (15 to 35 d)			
mg/100 g	F0	F3	F6	F0	F3	F6	Vinasse
Histidine	500	551	585	495	526	524	449
Arginine	886	928	981	1041	1113	1118	532
Serine	742	769	844	902	921	915	734
Glycine	632	673	734	792	832	853	811
Aspartic acid	1361	1415	1614	1908	2086	2072	1171
Glutamic acid	3053	3126	3446	4124	4371	4214	1676
Threonine	599	619	674	741	771	780	610
Alanine	727	750	847	965	1025	1031	758
Proline	868	892	975	1092	1131	1122	751
Lysine	1026	1008	1146	1419	1464	1471	1011
Methionine	188	198	202	205	219	219	187
Tyrosine	388	355	439	432	454	459	484
Valine	625	668	722	771	841	896	614
Cysteine	266	288	293	294	310	314	197
Isoleucine	519	569	606	636	711	761	507
Leucine	1130	1178	1278	1402	1504	1513	848
Phenylalanine	702	763	791	800	836	850	582
Triptophan	185	180	183	173	181	178	175

F0: Control diet (100% basal diet); F3: 97% basal diet + 3% vinasse; F6: 94% basal diet + 6% vinasse.

In addition, a trial was conducted to determine the optimal method for extracting a higher amount of protein and polysaccharides from the vinasse. Three different buffers were tested as a solvent to extract the polysaccharides, proteins and peptides from the Vinasse, a NADES solvent (Natural Deep Eutectic Solvents) consisted by Betaine, Citric Acid, and H_2O was used for being considered thermally and chemically stable, non-flammable, low-cost, sustainable, and non-volatile in ambient conditions. Then, two different pH McIlvaine buffers were made in different proportions of disodium phosphate (0.2 M Na2HPO4) and citric acid (0.1 M citric acid), reaching 2.4 and 3.4 pH, identified as M2 and M3, respectively. 1,25 grams of the dehydrated vinasse was washed with solvents, and two different treatments were done with this material; one part of the sample was autoclaved at 120ºC for 20 minutes, and the non-autoclaved sample were mixed in

vortex for 10 seconds and centrifugated for 15 minutes at 14000 rpm at 25ºC, this procedure was done more two times. The autoclaved and non-autoclaved samples were left to rest for 24 hours, and then centrifuged, and the solution was extracted. The precipitate material was lyophilized and solubilized in a water solution to analyse the protein content and polysaccharides. This analysis aimed to evaluate the most effective treatment for extracting proteins and polysaccharides from vinasse, using different solvents and the use of high temperatures.

The protein quantification was carried out by spectrophotometry using the Bradford method (Bradford, 1976). The samples were directly diluted in Bradford medium, without an incubation step, and the absorbance was measured immediately at a wavelength of 595 nm in a spectrophotometer (BioTek Synergy HTX Microplate Reader – Multi Mode (Abs + FL + Lum). The protein concentration was determined based on a previously established calibration curve of Bradford reagent, and the results were expressed in g/L and then converted to % (m/v).

The qualitative and quantitative analysis of low, medium, and high molecular weight saccharides and oligosaccharides was carried out using high-resolution size-exclusion chromatography (HRSEC) using an Agilent 1260 series II quaternary pump LC (Agilent Technologies, Santa Clara, CA, U.S.A.). 1 mg of extract samples was solubilized in 1 mL of 50 mM ammonium formate (mobile phase), shaken for 5 min, centrifuged (12 000 rpm for 10 min), and filtered (0.22 μm) before injection (10 μL). The isocratic separation was performed [0.6 mL/ min for 35 min, with a refractive index detector (RID) temperature of 35 °C] with a PL aquagel-OH 50 gel column (Agilent Technologies, Santa Clara, CA, U.S.A.). Pullulans (342−805 000 Da) were used as molecular weight (MW) standards. Polysaccharide quantification was performed using a calibration curve built with pectin and dextran (range of 0−2 g/L). The oligosaccharides were classified into molecular weight ranges according to the following chromatographic intervals: Low molecular weight (LMW): 11.060 to 11.286 min; Medium molecular weight (MMW): 13.187 to 13.208 min; High molecular weight (HMW): 14.804 to 14.955 min; Total oligosaccharides: 16.580 to 16.925 min. The areas of the chromatographic peaks were used for relative quantification. The sum of the areas corresponding to the low, medium, and high molecular weight fractions was used to calculate the polysaccharide concentration (PS). The sum of the areas of all the fractions (low, medium, and high molecular weight and oligosaccharides) was used to determine the content of total complex carbohydrates (or total saccharides; TS). The data was processed using the system's acquisition and analysis software (OpenLab Chromatography Data System).

Samples of the fresh and dehydrated vinasse, as well as after the protein and polysaccharide extraction treatment, were taken under a 20x and 40x light microscope. The

micrographs obtained were used to evaluate the morphological and structural characteristics of the material before and after the process, to identify possible changes in the cell structure and components of the by-product resulting from the process.

4.3 – Effect of vinasse on chicken feed

To evaluate the effect of including the dehydrated by-product in the diet of broilers, a feeding experiment was carried out in the warehouse of the Department of Agronomy, Animal Food, Natural Resources and Environment (DAFNAE) at the Azienda Agraria Sperimentale "L. Toniolo" of the University of Padua. The breeding took place during November and December 2024. A health vacuum had been in place for approximately eight months, and the warehouse was thoroughly cleaned and disinfected before the arrival of the birds.

The warehouse was equipped with a heating and cooling system, forced ventilation and an indoor air exhaust system, windows with a total blackout system, and a light intensity control system with sunrise and sunset effect. For animal husbandry, the place was divided into 18 wire mesh subdivisions, paddocks, of 3 m² (1.20 x 2.50 m, with walls 1.20 m high). The floor was solid concrete, and for the animals' comfort, a bed of absorbent material 5 cm thick, dry and dust-free, was laid out. In each paddock, there were drinking stations with drip-proof cups, suitably raised or lowered according to the size of the animals, and circular feeders with a diameter of 37 cm with manual filling connected to electronic scales to record daily food consumption. To allow the chicks to acclimatize and feed quickly on the first days, some sheets of paper were placed on the floor with a low container with additional feed inside.

From day 1 to day 10, the warehouse was heated using infrared lamps to maintain 30°C at the animals' body level. Ventilation was further increased to 80% of fan capacity from day 11 to day 19 of the trial and to 100% from day 27 to the end of the trial. The light program included 24 consecutive hours of light on the first two days and a progressive increase in hours of darkness from the third day until reaching 6 hours of darkness on the 12th day. On the two days before commercial slaughter, the light program provided was 24 hours of light.

Animal experimental design

A total of 450 male broilers, one day old, of the 600 Ross 308 strain were purchased from a commercial hatchery and delivered by a truck licensed by Regulation (EC) No. 1/2005. In the hatchery, the animals were vaccinated against Marek's disease, infectious bronchitis, and Newcastle disease. Once they arrived at the chicken paddock, the chicks were weighed individually and each one was affixed a label with an identification number and distributed in a completely randomized design, with six replications per treatment. Since day one the birds were fed with the experimental diets formulated to meet the nutritional requirements of each growth

phase, and the formulation of the control diet is present on the Table 2 for each phase, the initial or period one (P1) from day 1 to 21 days, and the final period or period 2 (P2) from day 22 to 36.

In vivo trial

During the experimental period, the health of the animals was monitored, and all mortalities were recorded, as well as the environmental parameters and the correct functioning of the automatic drinkers, and the correct height of the feeders and drinkers was adjusted according to the growth of the birds. Animals were individually weighed once a week throughout the rearing period and immediately recorded in an Excel file to allow immediate monitoring of animal growth (Figure 4).

Figure 4. Animal housing and the details of individual identification.

Commercial slaughter and dissection of carcasses

At the end of the experimental period, at 36 days of age, 6 broilers representative of the average live weight and variability for each paddock were selected for slaughter and evaluation of carcass yield and meat quality analysis. The broilers were fasted for 7 h for food and 4 h for water, individually weighed, and manually placed in transport cages. Slaughter was performed in a commercial slaughterhouse according to the normal procedures of stunning, exsanguination through severing of the carotid arteries and jugular veins, feathering, evisceration, and cooling of carcasses by the responsible personnel, and the carcasses were collected with the legs for individual identification. The carcasses were placed in a cooling chamber and weighed individually after two hours to calculate the carcass yield (Uijttenboogaart & Gerrits, 1982).

Carcass characteristics and meat quality

The carcasses were transported and kept in cold storage at 4°C for 24 h at the LaChi laboratory, then the carcasses were dissected and weighed to obtain the yield of major cuts such as breast, thigh, over-thigh, and wings. In addition, all carcasses were subjected to macroscopic

examination to determine the presence of white stripe breast myopathy according to the criteria proposed by Kuttappan et al. (2012) and Sihvo et al. (2014)

Meat quality was evaluated by pH determination and colorimetric analysis of the left breast fillet (Pectoralis major) of all samples. The pH was measured using a pH meter (Basic 20, Crison Instruments Sa, Carpi, Italy) equipped with a specific electrode for penetration into the meat and a thermal probe (cat. 5232, Crison Instruments Sa, Carpi, Italy). The pH was measured at three different points on the surface of the muscle, and the average value was then calculated. Colorimetry was determined using a Minolta CM-508 spectrophotometer (Minolta Corp, Ramsey, NJ, USA) with a 65D illuminant, and the measurement of brightness (L*), red index (a*), and yellow index (b*) was performed according to the CIELab method (CIE, 1976). The right side of P. major was cut into smaller portions (8 cm x 4 cm x 3 cm), vacuum-packed, and stored at -18°C for subsequent evaluation of thawing losses, cooking losses, texture, and sensory analysis.

To determine thawing and cooking losses, meat samples were thawed at 4°C for 24 h at room temperature, and the final weight of the sample after drying was measured (Petracci & Baéza, 2011). To calculate cooking losses, meat samples were again vacuum-packed and cooked in a water bath at 80°C for 50 min. After cooking, the samples were allowed to cool at room temperature for about 40 min, removed from the packaging, dried from the liquid lost during cooking, and weighed. A smaller portion (4 cm x 2 cm x 1 cm) was subjected to shear force determination with the Ls5 dynamometer (Lloyd Instruments Ltd, Bognor Regis, UK), Allo-Kramer probe (10 blades) (load cell: 500 kg; distance between blades: 5 mm; thickness: 2 mm; cutting speed: 250 mm/min) (Mudalal et al., 2015).

The degree of lipid oxidation in the meat was determined by analysis of thiobarbituric acid reactive substances (TBARS). The left side of P. major was minced using a Grindomix GM 200 (Retsch), ground, and used for extraction with trichloroacetic acid (TCA). The extract was then reacted with thiobarbituric acid (TBA), and the absorbance of the resulting solution was measured in a spectrophotometer at 532 nm. The results were expressed as milligrams of malonaldehyde (MDA) per kilogram of meat.

4.4 – Statistics

The data obtained from the productive evaluation, performance, yield, physicochemical parameters, and carcass characteristics were subjected to tests for homoscedasticity (Levene's Test), heteroscedasticity, and normality (Cramer-von Mises test). Subsequently, an analysis of variance (ANOVA) and Tukey's multiple comparison test ($P<0.05$) were performed. Additionally, the effects of vinasse inclusion levels were assessed using linear and quadratic

regression models, applying the same probability thresholds to detect dose-response trends. The data were analysed using the R statistical software – Development Core Team (2011).

5. RESULTS AND DISCUSSION

The Feed from Food technology proved effective in stabilizing vinasse. The resulting material was transformed into a fine, dark-colored, lightweight, and compact powder. It exhibited good mixing compatibility with other feed ingredients, showing no handling issues during formulation. No clumping or visible moisture reabsorption was observed after storage, indicating adequate physical stability and shelf-life potential under ambient conditions.

5.1. Vinasse composition

Results about the composition of vinasse are scarce, Lafka et al. (2007) analysed the composition of winery waste (grape skin and seeds), found the value of 4,6% of ashes and 6,3 % of fat. The quantification of extractable compounds after using solvents and autoclaving or not is described in Table 3. Statistical analysis revealed significant differences among treatments for the Oligosaccharides (OLIGO), MMW, LMW, PS, and total fractions ($p < 0.05$), except for proteins($p=0.504$) and HMW ($p = 0.781$), indicating that the extraction of proteins and high molecular weight compounds was not influenced by the different methods applied.

Solvent treatment M3A (McIlvaine buffer pH 3.4 plus autoclaving) achieved the highest total saccharides extraction yield (2.341 ± 0.22%), with the highest concentration of oligosaccharides (1.928%). This result suggests that the combination of medium acidic pH and thermal treatment effectively disrupts the yeast cell wall structures present in vinasse, enhancing the release of bioactive compounds. Vinasse samples with M2 solvent and autoclaved also yielded high values (2.057 ± 0.60 %), reinforcing the importance of acidification and thermal processing for the extraction of biomolecules, particularly oligosaccharides and LMW fractions.

In contrast, the treatments NADES NA (NADES solvent without autoclaving) and M2NA (McIlvaine buffer pH 2.4 without autoclaving) reached the lowest extraction yields (<0.8 % of total saccharides), indicating that the absence of thermal processing limits the breakdown of cellular structures and the release of soluble components, even when using a stable solvent such as NADES. The comparison between treatment pairs with and without autoclaving at the same pH underscores the critical role of thermal treatment: for instance, comparing M2NA and M2A reveals nearly a threefold increase in total yield when autoclaving is employed.

Table 6. Quantification of proteins and saccharides extracted (expressed as percentage ± standard deviation) from vinasse after treatment with solvent.

			Polissacharide				
	Total Protein (%)	Oligo (%)	HMW (%)	MMW (%)	LMW(%)	Total (%)	Total Saccharide (%)
NADESNA[1]	0.006± 0.014	0.583± 0.142[d]	0.017± 0.003	0.051± 0.011[c]	0.074± 0.013[e]	0.116± 0.027[d]	0.727± 0.166[d]
NADESA[2]	0.029± 0.0439	0.906± 0.014[c]	0.024± 0.002	0.178± 0.018[a]	0.229± 0.005[bc]	0.432± 0.025[ab]	1.338 0.024[c]
M2NA[3]	0.009± 0.0346	0.453± 0.039[d]	0.018± 0.018	0.089z 0.035[bc]	0.151± 0.042[d]	0.258± 0.095[c]	0.712 0.130[d]
M2A[4]	0.005± 0.0115	1.537± 0.035[b]	0.025± 0.005	0.136± 0.008[ab]	0.358± 0.013[a]	0.519± 0.027[a]	2.056 0.060[b]
M3NA[5]	0.013± 0.0252	0.804± 0.045[c]	0.019± 0.002	0.101± 0.013[b]	0.171± 0.021[cd]	0.294± 0.051[bc]	1.097± 0.079[c]
M3A[6]	0.057± 0.1625	1.927 ± 0.025[a]	0.022± 0.002	0.128± 0.005[ab]	0.262± 0.008[b]	0.413± 0.014[ab]	2.340± 0.022[a]
P-value	0.504	<0.001	0.781	<0.001	<0.001	<0.001	<0.001

[1]NADES not autoclave; [2]NADES_autoclave; [3]McIlvaine buffer pH 2.4_not autoclave; [4]McIlvaine buffer pH 2.4_autoclavate; [5]McIlvaine buffer pH 3.4__not autoclave; [6]McIlvaine buffer pH 3.4_autoclaved. [a, b, c] Different superscripts in a column indicate a significant difference using the Tukey test ($P < 0.05$).

For MMW compounds, thermal treatment proved more efficient than non-thermal methods, suggesting that both NADES and acidic buffers, when combined with heat, facilitate partial cleavage of polymeric chains. There was no significant difference between the solvents used under thermal conditions, indicating that MMW fractions are particularly sensitive to the interaction between solvent type and temperature. The M2 buffer appeared to favour the extraction of MMW compounds, especially when combined with autoclaving. The non-autoclaved NADES treatment was the least effective for MMW extraction.

For LMW compounds, the autoclaved M2 buffer was the most efficient, followed by M3 and NADES. Temperature was again a determining factor in LMW extraction, which includes short peptides and soluble oligomers. Autoclaved treatments outperformed non-autoclaved ones. The solvent type also played a significant role: even at similar pH levels, the nature of the solvent influenced LMW extraction. M2 was more effective than NADES, and although NADES showed performance comparable to non-autoclaved M3, the autoclaved M3 treatment had a superior extraction capacity. The chemical characteristics of the molecule to be extracted affect the efficiency of the NADES, as it is known to the highly efficient in extracting anthocyanins instead of other phenols (Frontini et al., 2024). So, more studies need to be done to understand the real efficacy of NADES to extract proteins and saccharides.

In the case of polysaccharides (PS), thermal processing consistently resulted in greater extraction compared to non-autoclaved treatments, with no significant differences between solvents. The NADESNA treatment yielded the lowest PS concentration (0.116%), highlighting that despite the stability and environmental advantages of NADES, its efficacy is significantly reduced in the absence of thermal processing.

Overall, the data support the premise that vinasse can serve as a rich and viable source of functional biomolecules for feed applications, particularly when subjected to optimized extraction conditions. Oligosaccharides, in particular, function as prebiotics, promoting the growth of beneficial gut bacteria, especially in poultry and swine, leading to improved feed conversion and enhanced immune function. Meanwhile, LMW compounds are easily metabolized and can provide an alternative energy source to glucose and other simple sugars in feed formulations. Additionally, the total polysaccharide (PS) content offers an estimate of the material's overall nutritional potential, making it relevant not only for feed formulation but also for biotechnological applications, such as additive encapsulation carriers. Canalejo et al. (2022) freeze-dried wine yeast lees from red and white wine and dissolved the material in a methanol: water solution (50:50, v/v), finding an amount of crude protein higher than the findings of this research (31.1 and 27.1%, from white and red wine).

The values of extractable compounds obtained in this study were lower than those reported in the literature for raw wine-making residues before vinasse production. For example, Rivas et al., (2021) reported 156.04 mg/g of total neutral sugars in AIR from wine yeast lees—approximately 15% content. Similarly, De Iseppi et al. (2024) found high levels of proteins, oligosaccharides, and polysaccharides in fresh wine lees. Canalejo et al. (2022) reported polysaccharide extraction yields of up to 89% from winery by-products such as grape pomace and yeast lees. These studies highlight a reduction in extractable compounds as the wine production process advances, particularly proteins and medium-to-high molecular weight polysaccharides. Higher values were obtained from early-stage residues such as grape pomace, while lower yields were observed in later-stage by-products like wine yeast lees. This reduction is attributed to factors such as processing methods, grape cultivar, extraction matrix, and the degree of compound degradation during vinification.

The findings of the present study are in line with this trend. The additional thermal and mechanical processing steps applied to vinasse during alcohol distillation and subsequent dehydration contributed to further nutrient depletion and compound denaturation, thereby limiting extraction efficiency. High temperatures, such as those used in distillation (>100 °C), have been shown to promote protein denaturation and degradation of heat-sensitive phenolic

compounds, such as flavonoids and anthocyanins (Pérez-Serradilla & Luque de Castro, 2011). Moreover, thermal processing may negatively impact soluble fiber fractions through Maillard reactions or thermal polymerization, altering the functionality and solubility of polysaccharides like β-glucans and mannans (Troilo et al., 2021).

These alterations are also reflected in the microscopic analysis of the vinasse (Figure 5). In images A and B, taken before dehydration, the yeast cell structures appear largely intact. In contrast, images C and D, taken after the vinasse dehydration process, show significant structural damage, with visible aggregates of fragmented material and bentonite particles adhered to the cell walls. This physical disruption of cellular integrity likely hinders the release and extraction of compounds that were originally embedded within the yeast cell walls, further explaining the lower yields observed in this study.

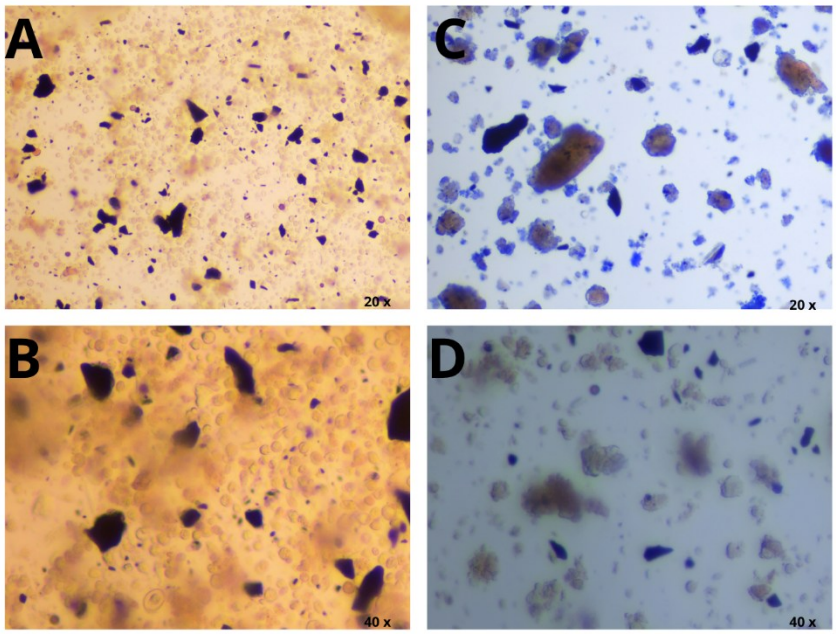

Figure 5. Microscope image of raw vinasse zoomed in 20 and 40 times (A and B) and vinasse after dehydration (C and D).

Microscopic observations of the vinasse samples treated with different solvents, with or without autoclaving, are presented in Figure 6. The solvent treatments did not result in any major morphological changes under optical microscopy, as the clusters of fragmented material remained visibly aggregated, regardless of the method applied. These findings suggest that the dehydration step is the primary factor influencing the structural integrity of the material and, consequently, the availability of polysaccharides and oligosaccharides for extraction. As this dehydration technique represents an innovative approach, further research is warranted to fully evaluate its effects on cell wall disruption and compound recovery efficiency.

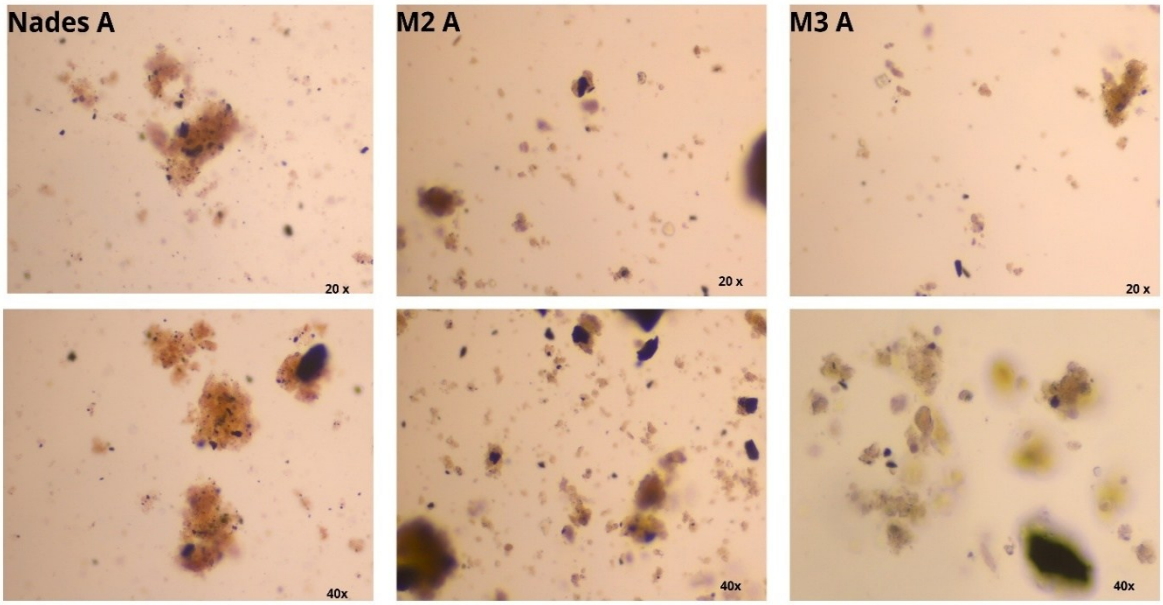

Figure 6 Microscope image of vinasse material treated with different solvents after autoclaving, zoomed in 20 and 40 times. Nades A – Nades autoclave; M2 A - McIlvaine buffer pH 2.4 autoclave; M3 A - McIlvaine buffer pH 3.4 autoclave.

Despite the variability in extraction yields reported in the literature and the influence of different solvents, the presence of oligosaccharides and polysaccharides in the vinasse analysed in this study reinforces its nutritional and functional potential as a feed ingredient for broilers. These compounds, particularly mannooligosaccharides (MOS) and β-glucans, are well recognized for their prebiotic properties, supporting the growth of beneficial gut microbiota such as *Lactobacillus* and *Bifidobacterium* spp. In poultry nutrition, these bioactives contribute to enhanced intestinal health, immune modulation, and nutrient absorption, ultimately improving growth performance, feed conversion efficiency, and resilience against enteric diseases (Jha & Kim, 2021). Additionally, some polysaccharides may act as natural immunomodulators, offering a sustainable alternative to antibiotic growth promoters. Therefore, even in the face of compositional variability, the detection of these functional carbohydrates underscores the value of vinasse as a promising ingredient in sustainable and health-oriented poultry feeding strategies.

5.2 Effect of vinasse on chicken feed

The inclusion of vinasse in experimental diets significantly affected productive performance throughout the broiler chickens' production cycle (Table 7). Statistical analysis revealed a linear negative effect as the inclusion levels increased from 0% to 6% of vinasse, particularly in terms of body weight gain and feed conversion ratio. No significant differences in feed intake were observed in the whole trial.

Table 7. Productive performance (LS means) of broiler chickens fed diets with different inclusion levels of vinasse from hatching until commercial slaughter.

	Experimental diet (D)			P-value	RMSE
	F0	F3	F6		
Chickens (no.)	133	132	130		
Body weight[1] (g)					
1d	46.9	47.1	47.3	0.732	3.89
7d	151[a]	147[ab]	141[b]	<0.001	17.9
14d	447[ab]	436a	414[b]	<0.001	58.3
21d	894[a]	871[a]	827[b]	<0.001	108
28 d	1495[a]	1454[a]	1356[b]	<0.001	171
35 d	2270[a]	2204[a]	2086[b]	<0.001	244
Starter period (1-14 d)					
Weight gain (g/d)	42.4 [a]	41.2 [a]	39.0[b]	<0.001	5.37
Feed intake (g/d)	54.8	54.8	53.6	0.259	1.22
Feed conversion	1.29[b]	1.31[a]	1.39[a]	0.004	0.04
Grower period (15-35 d)					
Weight gain (g/d)	95.0 [a]	92.2[a]	87.3[b]	<0.001	11.4
Feed intake (g/d)	166	174	169	0.167	7.41
Feed conversion	1.75[b]	1.87[ab]	1.97[a]	0.009	0.11
Whole trial (1-35 d)					
Weight gain (g/d)	63.6[a]	61.6[a]	58.3[b]	<0.001	6.95
Feed intake (g/d)	104	108	105	0.226	3.80
Feed conversion	1.63[b]	1.72[a]	1.81[a]	0.002	0.07

RSME, root mean square error. F0: Control diet (100% basal diet); F3: 97% basal diet + 3% vinasse; F6: 94% basal diet + 6% vinasse. [1]Individual data: live weight and daily growth rate. Pen data: feed intake and feed conversion. [a, b, c] Different superscripts within a row indicate a significant difference ($P < 0.05$).

The initial homogeneity of the experiment based on the body weight was confirmed throughout the absence of difference among treatments on the first day (1d). However, by day 7, birds in the F6 group showed a significantly lower average body weight compared to F0 and F3. This trend persisted throughout the trial, culminating at 35 days with birds from the F6 group reaching the lowest result of body weight compared with the other treatments.

Analysing the two periods, start and grower phase, the pattern of average daily weight gain had the same results: F6 showed lower values compared to F3 and F0 ($P < 0.001$). For the P1, the feed conversion of F3 and F6 resulted in high values compared with the F0; meanwhile, in the second period, the F0 and F3 diets had similar results, and the F6 had the worst feed conversion.

In the cumulative analysis, the whole trial, broilers in the F6 group showed the poorest overall performance (average daily gain: 58.3 g/day), while F3 and F0 groups reached 61.6 g/day and 63.6 g/day, respectively ($P < 0.001$). Feed conversion ratio over the entire trial also confirmed the negative impact of vinasse inclusion ($P = 0.002$), indicating progressively reduced feed efficiency with higher inclusion levels. Studies on the inclusion of vinasse in chicken feed are

scarce. When evaluating the inclusion of 2% wine yeast lees in chickens, a product one step ahead of vinasse, Giamouri et al. (2022) also found a reduction in efficiency of broilers fed with wine by-product when compared to control diets in the first phase of the growth, but no impact on the total period for both feed conversion and weight gain, showing that the composition of vinasse can be affect the productive results.

As no difference was observed in feed intake, this suggests that the palatability of the diet was maintained, and that the adverse effects of the inclusion of vinasse resulted in metabolic or digestive limitations. The high amount of fiber like NDF (33.9%) and lignin (ADL: 24.2%) in vinasse, resulting in a high amount in the diets, especially in F6 diets (22.7 and 22.1%) compared with F0 (19.2 and 18.1%) for both periods could be an explanation of that. Fiber can improve the intestinal health and immunity of broilers if included in appropriate doses, but in excessive amounts, the digestibility of nutrients can be compromised (Zhang et al., 2023). The lignified fibers bind with other compounds, such as proteins and carbohydrates, making them less accessible to digestive enzymes, reducing the overall digestibility and utilization of nutrients (Hetland et al., 2004).

As already discussed before, the vinasse is rich in minerals, and some of them, compared to conventional broiler feed ingredients such as corn, soybean meal and wheat, revealed high concentration. These deviations suggest the presence of potential metabolic limiting factors which may explain, at least in part, the performance results observed. The highest presence of minerals like Potassium, Aluminium and Iron resulted in highest value of these minerals in the experimental diets in both periods, especially for the diet with the highest inclusion percentage, which may be related to the lower performance observed in the diets contain vinasse, especially in F6 groups.

The potassium content of vinasse (67,000 mg/kg, or 6.7%) is 20 times higher than that of corn (~3,300 mg/kg) and soybeans (~2,050 mg/kg) (NRC, 1994). Although potassium is essential for normal functions in the organism, its excess can cause electrolyte disturbances, negatively affect the absorption of other minerals such as magnesium, calcium and sodium, and result in imbalance of electrolytes (Underwood & Suttle, 1999) and then reducing performance. The concentration of aluminium in vinasse was 2,642 mg/kg, increasing the amount of this mineral on the diets F3 (162 and 180 mg/k) and F6 (240 and 340 mg/kg) compared with the F0 (123 and 106 mg/kg). The accumulation of Aluminium can interfere the absorption of other minerals leading to lower growth efficiency (Klein, 2019). The same happened with the Iron concentration in the diets formulated with vinasse. High levels of iron in broilers' feed can cause oxidative

stress, leading to structural damage in the intestines of broilers, damaging the integrity of cell membranes, thereby affecting the absorption and performance of the animals (Gou et al., 2018).

This information reinforces that, although vinasse has been reported to possess probiotic potential and nutritional value, particularly due to its crude protein and bioactive compound content, its practical use as a feed ingredient for broilers must be approached with caution.

For the carcass performance parameters, the results are presented in Tables 8 and 9. The multiple comparison (Tukey's test, Table 8) revealed a significant difference between the treatments only for the variables of live weight before slaughter, chilled carcass weight and chilled carcass weight 24 hours post-mortem. The F6 treatment showed lower values compared to the control diet, while the F3 treatment was similar to both for live weight, following the trend results of body weight gain and feed conversion. For chilled carcass weight, the main difference was between F6 and F0, which resulted in a worse performance in chilled carcass weight 24 hours after slaughter for the F6 treatment compared to the control treatment. However, no significant differences were observed among the treatments for other variables such as carcass yield and the yield of cuts, including breast, wing, thigh, drumstick, and *P. major* muscle yield. The reduction in carcass mass is a direct consequence of the impaired growth performance observed during the rearing period, as previously discussed. Since carcass yield (%) and cut yields (breast, wings, thighs, and drumsticks) were not significantly different among treatments, it can be inferred that the carcass composition remained proportionally consistent, and that the decrease in absolute weights is primarily due to reduced muscle deposition and overall live weight.

Table 8. Live weight, carcass and noble cuts yield of broiler chickens fed diets with different inclusion levels of vinasse

Parameters	F0	F3	F6	P-Value
Live weight (g)	2321.75 ± 237.19^{a}	2248.58 ±225.48^{ab}	2141.97 ±235.81^{b}	0.005
Chilled carcass weight (g)	1694.33 ± 172.22^{ab}	1634.80 ±169.23^{a}	1553.00 ± 180.43^{ab}	0.003
Chilled carcass weight 24 h* (g)	1601.25 ± 160.11^{a}	1531.41 ± 167.20^{ab}	1462.58 ± 174.16^{b}	0.003
Carcass yield (%)	72.99±1.59	72.69±1.88	72.46±1.57	0.409
Breast yield (%)	37.69±2.17	38.94±2.40	38.30±2.67	0.096
P. major yield (%)	23.46 ± 2.20^{a}	24.03 ± 1.80^{a}	23.57 ± 2.02^{a}	0.441
Wing yield (%)	10.34±0.70	10.30±0.69	10.12±0.67	0.367
Thigh and drumstick yield (%)	31.50±1.48	31.59±1.91	31.43±2.24	0.946

Chilled carcass weight 24 hours after slaughter; F0: Control diet (100% basal diet); F3: 97% basal diet + 3% vinasse; F6: 94% basal diet + 6% vinasse. $^{a, b}$Different superscripts within a row indicate a significant difference ($P < 0.05$). ± standard deviation

The statistical analysis of the linear and polynomial regression models is described in Table 9. For live weight before slaughter, the linear regression presented a significant difference with a negative linear pattern, but with a very low R^2. Implying that despite affecting the live weight, the variation is only poorly explained by the vinasse inclusion in broiler diets ($p < 0.001$, $Y=2417.21 - 89.89X$; R^2: 0.0920). For cold carcass weight 24 hours post-mortem, significant differences were observed for both the linear model ($Y = 1755.83 - 71.76X$; R^2: 0.105) and the quadratic model ($Y = 1612.31 - 608.94X - 19.80X^2$; R^2: 0.105) regarding vinasse inclusion in broiler diets ($p < 0.05$). For the linear model, the pattern was negative, but despite the significant difference, the R^2 values were low, suggesting a weak relationship between vinasse inclusion in broiler diets and cold carcass weight. No linear or quadratic effects were observed for the variables of carcass yield, breast, thigh, drumstick, wing yield, and P. major. These findings reinforce the hypothesis that the inclusion of vinasse at 6% may have negatively impacted nutrient utilization and metabolic efficiency, potentially due to factors such as high fiber content and mineral imbalances, all of which can impair growth without altering the relative distribution of carcass components.

Table 9. P-values and R^2 for the Linear and Quadratic models of the performance variables of chicken broiler diets with different inclusion levels of vinasse.

	Linear model		Quadratic model	
	P-value	R^2	P-value	R^2
Live weight	<0.005[1]	0.092	0.0057	0.093
Chilled carcass	<0.001	0.101	0.003	0.102
Chilled carcass 24h	<0.001[2]	0.105	0.002[3]	0.105
Carcass yield	0.182	0.016	0.410	0.016
Breast yield	0.038	0.039	0.073	0.048
P. major yield	0.837	<0.001	0.442	0.015
Wing yield	0.146	0.019	0.311	0.022
Thigh and drumstick yield	0.864	<0.001	0.939	0.001

The Y value represents the fitted model equation for each response variable, considering the treatment (vinasse) as a factor. The p-value indicates the statistical significance of each model, and R^2 reflects the fit of the model. [1]Linear model adjusted for live weight: $Y=2417.21-89.89X$ where X is the level of vinasse; [2]Linear model adjusted for 24h cold carcass: $Y=1755.83-71.76X$; [3]Quadratic model adjusted for 24h cold carcass: $Y=1612.31-608.94X-19.80X^2$

No differences were observed between the treatments for all meat quality parameters, neither for multiple comparison analysis (p-value>0.05; Table 10) and polynomial analysis (p-value>0.05; Table 11).

Table 10. Results of the quality parameter of meat chicken broilers fed diets with different inclusion levels of vinasse.

	F0	F3	F6	P-value
Physical traits				
Loss due to thawing (%)	19.43±3.54	18.53±3.64	18.01±3.23	0.220
Cooking loss (%)	29.72±2.57	30.11±2.05	29.51±3.01	0.640
Shear Force (kg/g)	2.11±0.64	2.08±0.52	2.01±0.47	0.618

pH_{24}	5.73±0.11	5.73±0.11	5.70±0.12	0.580
Colour traits				
L*	56.30±1.99	56.34±1.86	56.78±1.62	0.599
a*	0.81±0.69	0.60±0.45	0.66±0.50	0.281
B*	11.25±1.56	10.79±1.47	10.69±1.74	0.283
TBARS[1]	0.07±0.03	0.06±0.02	0.06±0.02	0.713

F0: Control diet (100% basal diet); F3: 97% basal diet + 3% vinasse; F6: 94% basal diet + 6% vinasse. The p-values indicate statistical significance between the treatments. ± standard deviation. L*: lightness; a*: redness; b*: yellowness. [1]TBARS Thiobarbituric Acid Reactive Substances (Milligrams of malonaldehyde (MDA) per kilogram of meat.

The technological quality of broiler meat, reflected in parameters such as cooking loss, thawing loss, tenderness, and pH, is influenced by a range of factors, including genetics, post-mortem metabolism, oxidative stress, and the nutritional balance of the diet (Vlad et al., 2025). Cooking loss and thawing loss can be primarily affected by thermal stress and post-mortem glycolysis, as well as nutritional factors such as protein and amino acid concentrations in the diet, and the presence of probiotics and prebiotics, which may act as antioxidants and reduce cooking and thawing losses in broiler meat (Choi et al., 2023). Meat tenderness is related to rigor mortis, final muscle pH, genetics, and rearing environment, with minimal influence from animal nutrition, except in cases of nutritional imbalances (Kuttappan et al., 2012). As for meat pH, pre- and post-slaughter factors can influence the parameter, with diet only having an indirect effect. However, this parameter is important, as meat pH is directly related to colour and can be an indicator of PSE (pale, soft, and exudative) or DFD (dark, firm, and dry) conditions (Mir et al., 2017).

The finding of the study agrees with those found in Giamouri et al. (2022) e Jonathan et al. (2021), who reported no differences on the inclusion of wine sub products in chicken diets, did not affect the carcass yield, physical traits, and redness, a, and yellowness, b, of the meat fed with these by-products in different levels of inclusion.

Table 11. P-values and R^2 for the Linear and Quadratic Models of the quality parameter cooking and losses of meat chicken broilers diets with different inclusion levels of vinasse

	Linear model		Quadratic model	
	P-value	R^2	P-value	R^2
Physical traits				
Loss due to thawing (%)	0.084	0.029	0.219	0.028
Cooking loss (%)	0.730	0.001	0.641	0.008
Firmness (kg/g)	0.435	0.005	0.625	0.008
pH	0.513	0.004	0.576	0.010
Colour traits				
L*	0.587	0.002	0.856	0.002
a*	0.272	0.011	0.267	0.024
b*	0.144	0.019	0.298	0.022
TBARS[1]	0.415	0.009	0.712	0.009

L*: lightness; a*: redness; b*: yellowness. [1]Thiobarbituric Acid Reactive Substances.

Despite the dark colour and the possible presence of phenolic compounds with potential antioxidant activity, such as flavonoids and polyphenols, there was no significant difference between the treatments for colour (L, a, b) and oxidative stability of the meat, TBARS (Table 10 and 11). The absence of significant differences in these meat quality parameters between the treatments suggests that, despite potential variations on the carcass characteristics due to the mineral content and fiber level on the diets containing vinasse, especially the F6 diet, the inclusion was not sufficient to induce physiological or metabolic stress capable of altering post-mortem muscle biochemistry. These findings align with studies that observed no improvement in oxidative markers when wine by-products were included at low levels in poultry diets (Mavrommatis et al., 2021a) indicating that higher doses may be required to exert functional antioxidant effects in meat, but as already presented on this study, 6% of vinasse in is already enough to decrease the zootechnical performance of broilers. This way, more research is needed to evaluate the economic viability of a potential improvement in meat quality at the detriment of a reduction in performance.

The composition of vinasse has advantages and disadvantages for animals. The protein concentration of the product is considerably relevant (18.2%) with the presence of essential amino acids relevant to animal production such as threonine and lysine, but low levels of methionine and tryptophan. The fatty acid composition of the vinasse is also relevant as it contains 36.7% polyunsaturated fatty acids, including 29.6% linoleic acid (C18:2n6) and 5.5% alpha-linolenic acid (C18:3n3), resulting in a ratio of 4.34 between omega 6 and 3, considered recommendable for broiler diets, and can be a source of meat enrichment for the final consumer (Alagawany et al., 2019). The presence of insoluble fibers and minerals may have been determining factors to affect the performance of animals fed 6% vinasse in the diet, but the inclusion of 3% vinasse in broiler diets proved to be positive in terms of carcass performance, since there was no significant difference for all the variables compared to the control treatment. meaning that, despite reducing feed conversion, considering the total period of the experiment, the live weight of the animals at slaughter, and the carcass yield were not harmed.

The inclusion of 3% of vinasse in broilers' diets does not affect the carcass and quality traits and could reduce feed costs and offer a sustainable alternative for broiler production, as well as a destination for the by-product of distillate production. Feed from Food technology can facilitate the connection between the two production chains, guaranteeing sustainability, traceability, and consequent food safety for animals and end consumers.

Conclusions

Given the inclusion of 3% of vinasse in broilers diets had similar results to control diet in terms of weight gain and not affect negatively the carcass and quality traits, the inclusion of this level could reduce feed costs and offer a sustainable alternative for broiler production, as well as a destination for the by-product of distillate production. Feed from Food technology can facilitate the connection between the two production chains, guaranteeing sustainability, traceability, and consequent food safety for animals and end consumers.

Bibliography

Abbott, A. P., Boothby, D., Capper, G., Davies, D. L., & Rasheed, R. K. (2004). Deep Eutectic Solvents formed between choline chloride and carboxylic acids: Versatile alternatives to ionic liquids. *Journal of the American Chemical Society*, *126*(29), 9142–9147. https://doi.org/10.1021/ja048266j

Ahmad, B., Yadav, V., Yadav, A., Rahman, M. U., Yuan, W. Z., Li, Z., & Wang, X. (2020). Integrated biorefinery approach to valorize winery waste: A review from waste to energy perspectives. *Science of the Total Environment*, *719*. https://doi.org/10.1016/j.scitotenv.2020.137315

Alagawany, M., Elnesr, S. S., Farag, M. R., Abd El-Hack, M. E., Khafaga, A. F., Taha, A. E., Tiwari, R., Iqbal Yatoo, M., Bhatt, P., Khurana, S. K., & Dhama, K. (2019). Omega-3 and omega-6 fatty acids in poultry nutrition: Effect on production performance and health. In *Animals* (Vol. 9, Issue 8). MDPI AG. https://doi.org/10.3390/ani9080573

Alarcón, M., López-Viñas, M., Pérez-Coello, M. S., Díaz-Maroto, M. C., Alañón, M. E., & Soriano, A. (2020). Effect of wine lees as alternative antioxidants on physicochemical and sensorial composition of deer burgers stored during chilled storage. *Antioxidants*, *9*(8), 1–17. https://doi.org/10.3390/antiox9080687

AOAC. (2000). *International Official methods of analysis of AOAC* (17th ed.). Association of Official Analytical Chemists.

Blasco, L., Viñas, M., & Villa, T. G. (2011). Proteins influencing foam formation in wine and beer: The role of yeast. In *International Microbiology* (Vol. 14, Issue 2, pp. 61–71). Sociedad Espanola de Microbiologia. https://doi.org/10.2436/20.1501.01.136

Bonamente, E., Scrucca, F., Asdrubali, F., Cotana, F., & Presciutti, A. (2015). The water footprint of the wine industry: Implementation of an assessment methodology and application to a case study. *Sustainability (Switzerland)*, *7*(9), 12190–12208. https://doi.org/10.3390/su70912190

Bosona, T., & Gebresenbet, G. (2013). Food traceability as an integral part of logistics management in food and agricultural supply chain. In *Food Control* (Vol. 33, Issue 1, pp. 32–48). https://doi.org/10.1016/j.foodcont.2013.02.004

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. . *Analytical Biochemistry*, *72*(1–2), 248–254.

Bustamante, M. A., Moral, R., Paredes, C., Pérez-Espinosa, A., Moreno-Caselles, J., & Pérez-Murcia, M. D. (2008). Agrochemical characterisation of the solid by-products and residues from the winery and distillery industry. *Waste Management*, *28*(2), 372–380. https://doi.org/10.1016/j.wasman.2007.01.013

Bustamante, M. A., Paredes, C., Moral, R., Moreno-Caselles, J., Pé Rez-Espinosa, A., & Pé Rez-Murcia, M. D. (2005). Uses of winery and distillery effluents in agriculture: characterisation of nutrient and hazardous components. *Water Science and Technology*, *51*, 145–151.

Canalejo, D., Guadalupe, Z., Martínez-Lapuente, L., Ayestarán, B., Pérez-Magariño, S., & Doco, T. (2022). Characterization of polysaccharide extracts recovered from different

grape and winemaking products. *Food Research International*, *157*. https://doi.org/10.1016/j.foodres.2022.111480

Carrilho, E. N. V. M., & Soares, M. R. (2024). Vinasse, a byproduct of the bioethanol industry—A valuable resource for sustainable agriculture and renewable energy production. In *Environmental Materials and Waste: Circular Economy and Pollution Abatement* (pp. 193–239). Elsevier. https://doi.org/10.1016/B978-0-443-22069-2.00008-5

Cheng, Y. C., & Kim, S. W. (2022). Use of Microorganisms as Nutritional and Functional Feedstuffs for Nursery Pigs and Broilers. In *Animals* (Vol. 12, Issue 22). MDPI. https://doi.org/10.3390/ani12223141

Chioru, A., Chirsanova, A., Dabija, A., Avrămia, I., Boiştean, A., & Chetrariu, A. (2024). Extraction Methods and Characterization of β-Glucans from Yeast Lees of Wines Produced Using Different Technologies. *Foods*, *13*(24). https://doi.org/10.3390/foods13243982

Choi, J., Kong, B., Bowker, B. C., Zhuang, H., & Kim, W. K. (2023). Nutritional Strategies to Improve Meat Quality and Composition in the Challenging Conditions of Broiler Production: A Review. In *Animals* (Vol. 13, Issue 8). MDPI. https://doi.org/10.3390/ani13081386

CIE. (1976). *Commission Internationale de l'Éclairage (CIE). (1976). Colorimetry (CIE Publication No. 15, 3rd ed.).* (3rd ed., Vol. 15). Commission Internationale de l'Éclairage Central Bureau Bureau.

De Iseppi, A., Curioni, A., Marangon, M., Invincibile, D., Slaghenaufi, D., & Ugliano, M. (2024). Chemical and Electrochemical Assessment of Wine Lees Extracts Tested as Novel Antioxidant Additives in Model Wine. *Journal of Agricultural and Food Chemistry*, *72*(4), 1969–1977. https://doi.org/10.1021/acs.jafc.3c00567

De Iseppi, A., Lomolino, G., Marangon, M., & Curioni, A. (2020). Current and future strategies for wine yeast lees valorization. In *Food Research International* (Vol. 137). Elsevier Ltd. https://doi.org/10.1016/j.foodres.2020.109352

Delgado De La Torre, M. P., Priego-Capote, F., & Luque De Castro, M. D. (2015). Characterization and comparison of wine lees by liquid chromatography-mass spectrometry in high-resolution mode. *Journal of Agricultural and Food Chemistry*, *63*(4), 1116–1125. https://doi.org/10.1021/jf505331f

Devesa-Rey, R., Vecino, X., Varela-Alende, J. L., Barral, M. T., Cruz, J. M., & Moldes, A. B. (2011). Valorization of winery waste vs. the costs of not recycling. *Waste Management*, *31*(11), 2327–2335. https://doi.org/10.1016/j.wasman.2011.06.001

Erinle, T. J., & Adewole, D. I. (2022). Fruit pomaces—their nutrient and bioactive components, effects on growth and health of poultry species, and possible optimization techniques. In *Animal Nutrition* (Vol. 9, pp. 357–377). KeAi Communications Co. https://doi.org/10.1016/j.aninu.2021.11.011

Evtuguin, D., Aniceto, J. P. S., Marques, R., Portugal, I., Silva, C. M., Serafim, L. S., & Xavier, A. M. R. B. (2024). Obtaining Value from Wine Wastes: Paving the Way for Sustainable Development. In *Fermentation* (Vol. 10, Issue 1). Multidisciplinary Digital Publishing Institute (MDPI). https://doi.org/10.3390/fermentation10010024

Feed From Food. (2025, July). *Feed From Food Srl*. Https://Www.Feedfromfood.Com/.

Ferrara, A., D'Auria, G., Barile, D., Baller, M. I., Nitride, C., Mamone, G., & Ferranti, P. (2025). The valorization of grape pomace from Montepulciano winemaking: A new source of functional ingredients for sustainable food industry. *Food Research International*, *200*. https://doi.org/10.1016/j.foodres.2024.115443

Frontini, A., Luvisi, A., Negro, C., Apollonio, M., Accogli, R., De Pascali, M., & De Bellis, L. (2024). Polyphenols Extraction from Different Grape Pomaces Using Natural Deep Eutectic Solvents. *Separations*, *11*(8). https://doi.org/10.3390/separations11080241

Gazeau, B., Zaman, A., Minunno, R., & Shaikh, F. (2024). Developing Traceability Systems for Effective Circular Economy of Plastic: A Systematic Review and Meta-Analysis. In *Sustainability (Switzerland)* (Vol. 16, Issue 22). Multidisciplinary Digital Publishing Institute (MDPI). https://doi.org/10.3390/su16229973

Giamouri, E., Mavrommatis, A., Simitzis, P. E., Mitsiopoulou, C., Haroutounian, S. A., Koutinas, A., Pappas, A. C., & Tsiplakou, E. (2022). Redefining the Use of Vinification Waste By-Products in Broiler Diets. *Sustainability (Switzerland)*, *14*(23). https://doi.org/10.3390/su142315714

Gou, Z. Y., Li, L., Fan, Q. L., Lin, X. J., Jiang, Z. Y., Zheng, C. T., Ding, F. Y., & Jiang, S. Q. (2018). Effects of oxidative stress induced by high dosage of dietary iron ingested on intestinal damage and caecal microbiota in Chinese Yellow broilers. *Journal of Animal Physiology and Animal Nutrition*, *102*(4), 924–932. https://doi.org/10.1111/jpn.12885

Hetland, H., Choct, M., & Svihus, B. (2004). Role of insoluble non-starch polysaccharides in poultry nutrition. *World's Poultry Science Journal*, *60*(4), 415–422.

Iqbal, Y., Cottrell, J. J., Suleria, H. A. R., & Dunshea, F. R. (2020). Gut microbiota-polyphenol interactions in chicken: A review. In *Animals* (Vol. 10, Issue 8, pp. 1–18). MDPI AG. https://doi.org/10.3390/ani10081391

Jha, R., & Kim, S. W. (2021). Editorial: Nutritional Intervention for the Intestinal Health of Young Monogastric Animals. In *Frontiers in Veterinary Science* (Vol. 8). Frontiers Media S.A. https://doi.org/10.3389/fvets.2021.668563

Jonathan, O., Mnisi, C. M., Kumanda, C., & Mlambo, V. (2021). Effect of dietary red grape pomace on growth performance, hematology, serum biochemistry, and meat quality parameters in Hy-line Silver Brown cockerels. *PLoS ONE*, *16*(11 November). https://doi.org/10.1371/journal.pone.0259630

Klein, G. L. (2019). Aluminum toxicity to bone: A multisystem effect? *Osteoporosis and Sarcopenia*, *5*(1), 2–5.

Kojić, N., & Prodanović, R. (2024). By-Products of Wine Production in the Service of the Circular Economy. *Journal of Agronomy, Technology and Engineering Management (JATEM)*, *7*(6), 1245–1251. https://doi.org/10.55817/TPWZ4981

Kuttappan, V. A., Brewer, V. B., Apple, J. K., Waldroup, P. W., & Owens, C. M. (2012b). Influence of growth rate on the occurrence of white striping in broiler breast fillets. *Poultry Science*, *91*(10), 2677–2685. https://doi.org/10.3382/ps.2012-02259

Lafka, T. I., Sinanoglou, V., & Lazos, E. S. (2007). On the extraction and antioxidant activity of phenolic compounds from winery wastes. *Food Chemistry*, *104*(3), 1206–1214. https://doi.org/10.1016/j.foodchem.2007.01.068

López-Astorga, M., Molina-Domínguez, C. C., Ovando-Martínez, M., & Leon-Bejarano, M. (2023). Orujo de Uva: Más que un Residuo, una Fuente de Compuestos Bioactivos. *EPISTEMUS*, *16*(33). https://doi.org/10.36790/epistemus.v16i33.283

Lucarini, M., Durazzo, A., Romani, A., Campo, M., Lombardi-Boccia, G., & Cecchini, F. (2018). Bio-based compounds from grape seeds: A biorefinery approach. In *Molecules* (Vol. 23, Issue 8). MDPI AG. https://doi.org/10.3390/molecules23081888

Makri, S., Kafantaris, I., Stagos, D., Chamokeridou, T., Petrotos, K., Gerasopoulos, K., Mpesios, A., Goutzourelas, N., Kokkas, S., Goulas, P., Komiotis, D., & Kouretas, D. (2017). Novel feed including bioactive compounds from winery wastes improved broilers' redox status in blood and tissues of vital organs. *Food and Chemical Toxicology*, *102*, 24–31. https://doi.org/10.1016/j.fct.2017.01.019

Malenica, D., Kass, M., & Bhat, R. (2023). Sustainable Management and Valorization of Agri-Food Industrial Wastes and By-Products as Animal Feed: For Ruminants, Non-Ruminants and as Poultry Feed. In *Sustainability (Switzerland)* (Vol. 15, Issue 1). MDPI. https://doi.org/10.3390/su15010117

Mavrommatis, A., Giamouri, E., Myrtsi, E. D., Evergetis, E., Filippi, K., Papapostolou, H., Koulocheri, S. D., Zoidis, E., Pappas, A. C., Koutinas, A., Haroutounian, S. A., & Tsiplakou, E. (2021a). *Antioxidant Status of Broiler Chickens Fed Diets Supplemented with Vinification By-Products: A Valorization Approach*. https://doi.org/10.3390/antiox1008

Mavrommatis, A., Giamouri, E., Myrtsi, E. D., Evergetis, E., Filippi, K., Papapostolou, H., Koulocheri, S. D., Zoidis, E., Pappas, A. C., Koutinas, A., Haroutounian, S. A., & Tsiplakou, E. (2021b). *Antioxidant Status of Broiler Chickens Fed Diets Supplemented with Vinification By-Products: A Valorization Approach*. https://doi.org/10.3390/antiox1008

Mir, N. A., Rafiq, A., Kumar, F., Singh, V., & Shukla, V. (2017). Determinants of broiler chicken meat quality and factors affecting them: a review. In *Journal of Food Science and Technology* (Vol. 54, Issue 10, pp. 2997–3009). Springer India. https://doi.org/10.1007/s13197-017-2789-z

Mudalal, S., Lorenzi, M., Soglia, F., Cavani, C., & Petracci, M. (2015). . Implications of white striping and wooden breast abnormalities on quality traits of raw and marinated chicken meat. *Animal*, *9*(4), 728–734.

OIV. (2022). *World Wine Production Outlook—OIV First Estimates: Vol. November*. International Organisation of Vine and Wine.

Pérez-Serradilla, J. A., & Luque de Castro, M. D. (2011). Microwave-assisted extraction of phenolic compounds from wine lees and spray-drying of the extract. *Food Chemistry*, *124*(4), 1652–1659. https://doi.org/10.1016/j.foodchem.2010.07.046

Petracci, M., & Baéza, E. (2011). Harmonization of methodologies for the assessment of poultry meat quality features. . *World's Poultry Science Journal* , *67*(1), 137–153.

Petta, L., De Gisi, S., Casella, P., Farina, R., & Notarnicola, M. (2017). Evaluation of the treatability of a winery distillery (vinasse) wastewater by UASB, anoxic-aerobic UF-MBR and chemical precipitation/adsorption. *Journal of Environmental Management*, *201*, 177–189. https://doi.org/10.1016/j.jenvman.2017.06.042

Putri, B. R. T., Siti, N. W., Villano, R., Ambarawati, I. G. A. A., & Dewi, N. M. A. K. (2024). Wine Pomace as Feed in Native Chicken Farming: A Revenue-Cost Analysis. *Revista de Gestão Social e Ambiental*, *18*(12), e10179. https://doi.org/10.24857/rgsa.v18n12-017

Rivas, M. Á., Casquete, R., Córdoba, M. de G., Ruíz-Moyano, S., Benito, M. J., Pérez-Nevado, F., & Martín, A. (2021). Chemical composition and functional properties of dietary fibre concentrates from winemaking by-products: Skins, stems and lees. *Foods*, *10*(7). https://doi.org/10.3390/foods10071510

Russell, I., & Stewart, G. G. (2022). *Distilling yeast and fermentation. In Whisky and Other Spirits*. Academic Press.

Salgado-Ramos, M., Huertas-Alonso, A. J., Martí-Quijal, F. J., Barba, F. J., Sánchez-Verdú, M. P., Moreno, A., & Cabañas, B. (2024b). Sustainable management of wine-derived leftovers: Enhanced extraction of antioxidants and production of levulinic acid by microwave-assisted processing. *Journal of Environmental Chemical Engineering*, *12*(2). https://doi.org/10.1016/j.jece.2024.112411

Santos, F. O., Eichler, P., Machado, G. M., De Mattia, J. A., & De Souza, G. H. (2020). *By-products of the sugarcane industry. In Sugarcane biorefinery: Technology and perspectives*. Academic Press.

Shakeri, M., Cottrell, J. J., Wilkinson, S., Le, H. H., Suleria, H. A. R., Warner, R. D., & Dunshea, F. R. (2020). A dietary sugarcane-derived polyphenol mix reduces the negative effects of cyclic heat exposure on growth performance, blood gas status, and meat quality in broiler chickens. *Animals*, *10*(7), 1–14. https://doi.org/10.3390/ani10071158

Sihvo, H. K., Immonen, K., & Puolanne, E. (2014). Myodegeneration with fibrosis and regeneration in the pectoralis major muscle of broilers . *Veterinary Pathology*, *51*(3), 619–623.

Soren, S., Mandal, G. P., Mondal, S., Pradhan, S., Mukherjee, J., Banerjee, D., Pakhira, M. C., Amla, Mondal, A., Nsereko, V., & Samanta, I. (2024). Efficacy of Saccharomyces cerevisiae Fermentation Product and Probiotic Supplementation on Growth Performance, Gut Microflora and Immunity of Broiler Chickens. *Animals*, *14*(6). https://doi.org/10.3390/ani14060866

Soto, M. L., Conde, E., González-López, N., Conde, M. J., Moure, A., Sineiro, J., Falqué, E., Domínguez, H., Núñez, M. J., & Parajó, J. C. (2012). Recovery and concentration of antioxidants from winery wastes. *Molecules*, *17*(3), 3008–3024. https://doi.org/10.3390/molecules17033008

Sousa, R. M. O. F., Amaral, C., Fernandes, J. M. C., Fraga, I., Semitela, S., Braga, F., Coimbra, A. M., Dias, A. A., Bezerra, R. M., & Sampaio, A. (2019). Hazardous impact of vinasse from distilled winemaking by-products in terrestrial plants and aquatic organisms. *Ecotoxicology and Environmental Safety*, *183*. https://doi.org/10.1016/j.ecoenv.2019.109493

Spigno, G., & De Faveri, D. M. (2007). Antioxidants from grape stalks and marc: Influence of extraction procedure on yield, purity and antioxidant power of the extracts. *Journal of Food Engineering*, *78*(3), 793–801. https://doi.org/10.1016/j.jfoodeng.2005.11.020

Troilo, M., Difonzo, G., Paradiso, V. M., Summo, C., & Caponio, F. (2021b). Bioactive compounds from vine shoots, grape stalks, and wine lees: Their potential use in agro-food chains. In *Foods* (Vol. 10, Issue 2). MDPI AG. https://doi.org/10.3390/foods10020342

Turcu, R. P., Panaite, T. D., Untea, A. E., Șoica, C., Iuga, M., & Mironeasa, S. (2020). Effects of supplementing grape pomace to broilers fed polyunsaturated fatty acids enriched diets on meat quality. *Animals*, *10*(6). https://doi.org/10.3390/ani10060947

Uijttenboogaart, T. G., & Gerrits, A. R. (1982). Method of dissection of broiler carcasses and description of parts (European Federation Working Group No. 5). *World's Poultry Science Association, Danish Branch.*

Underwood, E. J., & Suttle, N. F. (1999). *The mineral nutrition of livestock* (3rd ed.). CABI Publishing.

Vasta, V., Mele, M., Serra, A., Scerra, M., Luciano, G., Lanza, M., & Priolo, A. (2009). Metabolic fate of fatty acids involved in ruminal biohydrogenation in sheep fed concentrate or herbage with or without tannins. *Journal of Animal Science*, *87*(8), 2674–2684.

Vlad, S. T., Bordei, S. I., Magdalena, G., Pirlea, G., Ianitchi, D., Maftei, M. L., Grigore, D.-M., Moise, E. A., Custura, I., & Tudorache, M. (2025). Factors Influencing the Quality of Chicken Meat: A Review. *Scientific Papers: Animal Science and Biotechnologies*, *58*(1), 1–11.

Vlyssides, A. G., Barampouti, E. M., & Mai, S. (2005). Wastewater characteristics from Greek wineries and distilleries. *Water Science and Technology*, *51*(1), 53–60.

Yao, K., Jiang, L., Liu, J., Wang, D., Liu, H., & Ren, D. (2021). Effect of yellow wine lees supplementation on milk antioxidant capacity and hematological parameters in lactating cows under heat stress. *Animals*, *11*(9). https://doi.org/10.3390/ani11092643

Zhang, C., Hao, E., Chen, X., Huang, C., Liu, G., Chen, H., Wang, D., Shi, L., Xuan, F., Chang, D., & Chen, Y. (2023). Dietary Fiber Level Improve Growth Performance, Nutrient Digestibility, Immune and Intestinal Morphology of Broilers from Day 22 to 42. *Animals*, *13*(7). https://doi.org/10.3390/ani13071227

Zhang, Y., Wang, C., Su, W., Jiang, Z., He, H., Gong, T., Kai, L., Xu, H., Wang, Y., & Lu, Z. (2022). Co-fermented yellow wine lees by Bacillus subtilis and Enterococcus faecium regulates growth performance and gut microbiota in finishing pigs. *Frontiers in Microbiology*, *13*. https://doi.org/10.3389/fmicb.2022.1003498

Zhu, M., Zhang, T., Xu, D., Zhou, B., Wang, K., Liao, C., Cheng, Z., Li, P., & Chen, C. (2024). Impact of fermented wine lees on gut microbiota and metabolic responses in Guanling crossbred cattle. *BMC Microbiology*, *24*(1). https://doi.org/10.1186/s12866-024-03583-z