Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Evaluation of dietary *Jasminum subtriplinerve* leaf powder supplementation on lactation performance, milk composition, and somatic cell count in Saanen dairy goats

Avaliação da suplementação dietética com pó de folhas de *Jasminum subtriplinerve* sobre o desempenho na lactação, a composição do leite e a contagem de células somáticas em cabras leiteiras da raça Saanen

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Abstract

This study evaluated *Jasminum subtriplinerve* (Vằng tea), a traditional medicinal plant, as a potential feed additive for Saanen dairy goats. Phytochemical profiling revealed that J. subtriplinerve leaf powder (CV1) contained high levels of verbascoside (4.69 mg/g) and a balanced flavonoid profile (rutin, astragalin, isoquercitrin), whereas stem inclusion (CV2) diluted these bioactive metabolites. In DPPH radical scavenging assays, CV1 exhibited strong antioxidant activity (SC_{50}=0.03679 mg/mL), comparable to ascorbic acid (SC_{50}=0.02919 mg/mL). Antibacterial testing showed that CV1 ethanol extracts displayed mild to moderate inhibition zones (7.0-10.0 mm) against five mastitis-related bacterial strains. Based on these in vitro results, CV1 was selected for a 90-day feeding trial with 18 lactating goats divided into Control (0%), JS1 (0.5%), and JS2 (1.0% leaf powder) groups. Over 90 days, 1.0% supplementation increased daily milk yield (peaking at 1.618 ± 0.01 kg/day vs. 1.244 ± 0.01 kg/day in control; P = 0) and improved feed conversion ratio (0.932 ± 0.014 vs. 1.196 ± 0.014 kg DM/kg milk; P < 0.05). Meanwhile, main milk parameters including fat (3.869%–4.164%), protein (2.827%–2.963%), and solid-not-fat (8.559%–8.772%) remained unaffected (P > 0.05). The JS2 group achieved the highest average milk yield, peaking at 1.618 ± 0.01 kg/head/day, and demonstrated superior feed conversion efficiency with an average feed conversion ratio (FCR) of 0.932 ± 0.014 kg DM/kg milk, compared to 1.196 ± 0.014 in the Control group (P < 0.05). Furthermore, the JS2 group had no mastitis (0%), whereas the control group had a 50% incidence, with somatic cell counts peaking at 1.678 x 10^6 cells/mL. In conclusion, supplementation with 1.0% *J. subtriplinerve* leaf powder is an potential nutritional strategy to improve milk yield and udder health, offering a promising natural feed supplement to support productivity and udder health in dairy goat production.

Keywords: *Jasminum subtriplinerve,* Saanen dairy goats, milk yield and quality, mastitis, dietary supplementation.

Resumo

Jasminum subtriplinerve (chá Vằng) é uma planta medicinal tradicional avaliada neste estudo como potencial aditivo alimentar para cabras leiteiras da raça Saanen. O perfil fitoquímico revelou que o pó das folhas de *J. subtriplinerve* (CV1) continha elevados níveis de verbascosídeo (4,69 mg/g) e um perfil equilibrado de flavonoides (rutina, astragalina, isoquercitrina), ao passo que a inclusão do caule (CV2) diluiu esses metabólitos bioativos. Nos ensaios de varredura do radical DPPH, o CV1 exibiu forte atividade antioxidante (SC_{50} = 0,03679 mg/mL), comparável à do ácido ascórbico (SC_{50} = 0,02919 mg/mL). Os testes antibacterianos mostraram que os extratos etanólicos de CV1 apresentaram zonas de inibição leves a moderadas (7,0-10,0 mm) contra cinco cepas bacterianas associadas à mastite. Com base nesses resultados *in vitro*, o CV1 foi selecionado para um ensaio de alimentação de 90 dias com 18 cabras em lactação, divididas nos grupos Controle (0%), JS1 (0,5%) e JS2 (1,0% de pó das folhas). Ao longo dos 90 dias, a suplementação com 1,0% aumentou a produção diária de leite (atingindo um pico de 1,618 ± 0,01 kg/dia vs. 1,244 ± 0,01 kg/dia no controle; P = 0) e melhorou a conversão alimentar (0,932 ± 0,014 vs. 1,196 ± 0,014 kg MS/kg de leite; P < 0,05). Enquanto isso, os principais parâmetros do leite, incluindo gordura (3,869%–4,164%), proteína (2,827%–2,963%) e extrato seco desengordurado (8,559%–8,772%), permaneceram inalterados (P > 0,05). O grupo JS2 alcançou a maior produção média de leite, atingindo o pico de 1,618 ± 0,01 kg/cabeça/dia, e demonstrou maior eficiência de conversão alimentar, com uma taxa de conversão alimentar (FCR) média de 0,932 ± 0,014 kg MS/kg de leite, em comparação com 1,196 ± 0,014 no grupo Controle (P < 0,05). Além disso, a incidência de mastite foi eliminada

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Received: April 4, 2026 – Accepted: September 29, 2026
Editor: José Galizia Tundisi

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no grupo JS2 (0%), ao passo que o grupo Controle registrou uma taxa de incidência de 50%, com contagens de células somáticas atingindo o pico de 1,678×10^6 células/mL. Em conclusão, a suplementação com 1,0% de pó das folhas de *J. subtriplinerve* mostrou-se uma estratégia nutricional eficaz para melhorar a produção de leite e a saúde do úbere, oferecendo um suplemento alimentar natural promissor para apoiar a produtividade e a saúde do úbere na produção de cabras leiteiras

Palavras-chave: *Jasminum subtriplinerve*, cabras leiteiras Saanen, produção e qualidade do leite, mastite, suplementação alimentar.

1. Introduction

The goat farming industry in Vietnam has experienced rapid growth in recent years, with the total population exceeding 2.8 million head in 2023. However, production is still primarily characterized by small-scale farming, with 73.4% of households raising fewer than 10 goats. Specialized dairy goats account for only a small proportion of the total herd (approximately 2–5%). Meanwhile, domestic demand for goat milk has increased due to its perceived nutritional value, driving prices to 1.5 to 3 times higher than those of cow's milk. This imbalance has resulted in a domestic supply that fails to meet consumer demand, largely due to the low milk yield of current herds (Nguyen et al., 2023). Additionally, the goat production systems are frequently affected by health disorders such as diarrhea, parasites, bloat, and particularly mastitis, which severely impacts milk production. The management of mastitis or diarrhea often involves the use of antibiotics, which may lead to concerns regarding antibiotic residues in milk and the development of antimicrobial resistance.

Recent strategies in ruminant nutrition focus on utilizing plant secondary metabolites and phytogenic feed additives as natural alternatives to antibiotics to optimize ruminal nitrogen metabolism and enhance animal productivity (Jackson et al., 2026; Mohai Ud Din et al., 2026). *J. subtriplinerve* (Vàng tea), a traditional Vietnamese medicinal plant commonly used to support lactation and postpartum recovery, represents a potential candidate for such applications. This species is an herbaceous climbing plant belonging to the Oleaceae family and is widely distributed in Vietnam and other Southeast Asian countries (Tan et al., 2024). Among its commonly recognized forms, the small-leaf Vàng tea is considered to possess higher medicinal value and is frequently used in traditional medicine and functional foods. Previous scientific investigations on *J. subtriplinerve* have primarily focused on its crude extract characterization, chemical constituents, and *in vitro* antioxidant or antibacterial activities in laboratory settings. However, its application as a phytogenic feed additive in ruminant nutrition—specifically in dairy goats or dairy cattle—remains unexplored. Phytochemical studies have indicated that *J. subtriplinerve* contains various bioactive compounds, including flavonoids and water-soluble polysaccharides, which are associated with anti-inflammatory and antioxidant activities (Minh Ngoc et al., 2022; Ngan et al., 2008). At the physiological level, the high metabolic demand during peak lactation in dairy goats accelerates the accumulation of reactive oxygen species (ROS), inducing oxidative stress and damaging mammary epithelial tissues (Lu et al., 2025; Mohai Ud Din et al., 2026). Natural bioactive metabolites, particularly phenylethanoid glycosides (such as verbascoside) and flavonoids, possess recognized antioxidant and anti-inflammatory properties that can scavenge free radicals and support cellular integrity (Pesce et al., 2015; Minh Ngoc et al., 2022).

We hypothesized that dietary inclusion of standardized *J. subtriplinerve* leaf powder would mitigate lactation-induced oxidative stress, thereby supporting udder health indicators and milk performance in dairy goats without altering core milk composition. To test this hypothesis, this study evaluated the phytochemical profile, in vitro antioxidant and antibacterial properties of *J. subtriplinerve* preparations, and subsequently investigated the effects of dietary supplementation with selected leaf powder on milk yield, milk quality, feed conversion efficiency, and clinical udder health parameters in lactating Saanen goats (Huu, 2016; Ngan et al., 2008; Pham and Nguyen, 2018; Ruegg, 2017).

2. Materials and Methods

2.1. Materials

The study examined three preparations of *J. subtriplinerve*, including commercial leaf powder (CV1), self-harvested leaf and stem powder (CV2), and an aqueous concentrated extract (CV3). Based on the bioactive profile, the superior sample was selected for the in vivo feeding trial at two inclusion levels: 0.5% (JS1) and 1.0% (JS2) in the goat's diet. The research subjects consisted of 18 purebred Saanen dairy goats, strictly selected from the Goat and Rabbit Research Center in Son Tay, Hanoi, Vietnam, to ensure uniformity.

The diet was designed as a vehicle to deliver the herbs into the goats' bodies, combining green forage (Guinea grass, *Trichanthera gigantea* leaf) and concentrate feed formulated from corn, soybean, and minerals. Specifically, the concentrate feed was supplemented with *J. subtriplinerve* powder at different concentrations according to the nutritional experimental design.

The study utilized four common pathogenic bacterial strains alongside standard antibiotics such as ciprofloxacin and streptomycin. Simultaneously, 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used to determine antioxidant activity. Mastitis status was diagnosed using the California Mastitis Test (CMT) rapid kit. The Ekomilk system was employed to analyze the physicochemical components of the milk and somatic cell counts (SCC).

2.2. Extract preparation

The extraction procedure for *J. subtriplinerve* samples was performed uniformly using two solvents—40% ethanol and distilled water—to prepare for biological activity

analyses. Initially, 10 g of each ground and homogenized sample was placed in an Erlenmeyer flask with 100 mL of solvent and underwent maceration by shaking at 100 rpm for 24 hours at room temperature. Following this stage, the resulting extract was filtered through filter paper to completely remove residue. The subsequent step involved concentration using a Rotavapor R300 vacuum rotary evaporator at temperatures below 50°C to evaporate the solvent and enrich the bioactive compound concentration. After concentration, the sample was re-dissolved in 10 mL of DMSO to produce a concentrated extract for direct use in antibacterial and antioxidant assays. Finally, these extracts were stored in dark vials and preserved in a refrigerator at 0 to 4°C for a maximum of 7 days to ensure bioactive stability (Ngan et al., 2008; Anh et al., 2019).

2.3. Bioactive compound analysis

Target bioactive metabolites in *J. subtriplinerve* preparations (CV1, CV2, and CV3) were quantified using validated chromatographic and spectrophotometric methods. For individual phytochemicals, samples were extracted with methanol via ultrasonication and filtered through a 0.45 µm membrane. Chromatographic analysis was performed on an UHPLC Ultimate 3000 system equipped with DAD and MSQ Plus mass spectrometry detectors (Thermo Scientific, USA). Specifically, verbascoside and epigallocatechin gallate (EGCG) were analyzed via UPLC-DAD on a Hypersil GOLD C18 column (150×2.1 mm, 3 µm) using gradient elution with 0.1% formic acid and acetonitrile or methanol at flow rates of 0.35 to 0.45 mL/min. Flavonoids (rutin, astragalin, isoquercitrin) and gallic acid were quantified via LC-MS in Selected Ion Monitoring (SIM) mode on the same Hypersil GOLD C18 column, whereas oleanolic acid and betulinic acid were evaluated using LC-MS on a Luna C8 column (150×2.0 mm, 3 µm). For group parameters, total tannins were measured colorimetrically using the Folin–Ciocalteu assay at 725 nm (BioTek PowerWave HT), and total alkaloids were extracted via acid-base liquid-liquid extraction with chloroform and quantified gravimetrically (Kraus, 2003; Ngan et al., 2008).

2.4. Biological activity assays

The biological activity of *J. subtriplinerve* was evaluated through two main tests: antibacterial ability and antioxidant activity. Antibacterial testing was performed using an agar well diffusion assay (50 µL into 6 mm wells) in Mueller–Hinton Agar against five standard strains: *Escherichia coli* VTCC 12272, *Staphylococcus aureus* VTCC 12275, *Salmonella enterica* VTCC 12277, *Pseudomonas aeruginosa* VTCC 12273, and *Streptococcus agalactiae* ATCC 12386. Bacterial density was standardized to 0.5 McFarland before adding 50 µL of extract into the wells and incubating at 37 °C for 24 hours. The net diameter of the zone of inhibition was calculated using the following formula1 IZD (mm) = D_{total} - D_{well}, where D_{total} is the total diameter of the clear inhibition zone (mm) measured with a digital caliper, and D_{well} is the diameter of the agar well (6 mm). All assays were performed in triplicate (n=3). Antibacterial results were determined by measuring the inhibition zone diameter (mm) and directly compared with standard control antibiotics such as ciprofloxacin and streptomycin. Simultaneously, antioxidant activity was quantified by the DPPH method based on the ability to reduce stable purple free radicals to colorless compounds at a wavelength of 517 nm. Extracts at concentrations from 0.01 to 1 mg/mL were reacted with 0.2 mM DPPH solution in the dark for 30 minutes before measuring absorbance with a UV-Vis spectrophotometer. The final SC_{50} index was calculated through a four-parameter logistic (4PL) regression model to accurately determine the concentration required to scavenge 50% of the free radicals of the research sample (Brand-Williams et al., 1995).

2.5. Animal feeding trial

To ensure uniformity and minimize error due to individual factors, 18 Saanen dairy goats were selected from the Goat and Rabbit Research Center in Son Tay. To make baseline comparability across treatment groups, 18 Saanen goats were balanced for parity (3rd and 4th parity), days in milk (averaging 60 ± 5 days in milk, corresponding to their 3rd month of lactation), body weight (39 ± 3.5 kg), and baseline milk yield (1.03 ± 0.30 kg/head/day). All experimental goats were vaccinated and dewormed according to current veterinary regulations.

The experiment was conducted over 90 days following a single-factor model. The goats were randomly assigned to three control and experimental groups (6 goats per group) and housed separately in individual pens to accurately monitor feed intake. The three groups included: 1) Control: Basal diet without *J. subtriplinerve* powder supplementation (0%); 2) JS1: Basal diet supplemented with 0.5% *J. subtriplinerve* powder in the concentrate; 3) JS2: Basal diet supplemented with 1.0% *J. subtriplinerve* powder in the concentrate. The supplementation levels of 0.5% and 1.0% were selected based on preliminary palatability observations in Saanen goats and established inclusion ranges for phytogenic feed additives.

The diets were designed based on nutritional standards, meeting the requirements for dry matter, energy, and protein for dairy goats, including concentrate and basal green forage. The concentrate consisted of primary ingredients such as corn meal, soybean meal, DCP, Premix-Vitamin, Urea, and *J. subtriplinerve* powder, which were thoroughly mixed, pelleted, and dried using a specialized cold dryer to a moisture content of < 14% (Table 1) (NRC, 1989). Subsequently, the feed was stored in vacuum-sealed plastic bags to ensure the stability of the bioactive compounds throughout the experiment. The feeding regimen was performed four times daily as described in Table 2, with concentrate provided at 7:00 and 14:00, and green forage (Guinea grass and *Trichanthera gigantea* leaves) provided at 8:30 and 15:30. Clean drinking water was provided ad libitum throughout the study.

2.6. Monitoring performance and health indicators

2.6.1. Feed efficiency

This group of indicators helps evaluate the ability to convert the diet (supplemented with *J. subtriplinerve*)

Table 1. Ingredient composition and calculated nutrients of the concentrate feed.

Ingredients	Control (0%)	JS1 (0.5%)	JS2 (1%)
	Proportion of ingredients (%)		
Corn meal	79.0	79.0	79.0
Soybean meal	16.0	15.5	15.0
Che Vang powder	0	0.5	1.0
DCP	3.5	3.5	3.5
Premix-Vitamin	0.5	0.5	0.5
Urea	1	1	1
Total	100	100	100
	Nutritional composition on a DM basis		
DM (%)	87.5	87.5	87.5
ME (MJ/kg VCK)	10.5	10.6	10.5
Protein (% VCK)	14.6	14.5	14.4
CF (% VCK)	15.6	15.2	14.9
Ca (% VCK)	0.84	0.84	0.85
P (% VCK)	0.62	0.62	0.63

Note: DM: Dry matter; ME: Metabolizable energy; CP: Crude protein; CF: Crude fiber; DCP: Dicalcium phosphate. Control: Basal diet; JS1: 0.5% *J. subtriplinerve* supplementation; JS2: 1.0% *J. subtriplinerve* supplementation.

Table 2. Daily basal diet and nutritional composition for experimental goats.

Ingredients	Control group (0%)	JS1 (0.5%)	JS2 (1%)
	Ingredients (kg/goat/day)		
Grass (Guinea/King grass)	2	2	2
Gigantea leaf	0.2	0.2	0.2
Concentrate feed Control	1.3		
Concentrate feed JS1		1.3	
Concentrate feed JS2			1.3
	Nutritional composition on a DM basis		
DM (kg)	1.64	1.64	1.64
ME (MJ/kg VCK)	9.94	9.93	9.91
Protein (% VCK)	14.1	14.04	13.97
CF (% VCK)	20.07	19.82	19.58
Ca (% VCK)	0.68	0.69	0.69
P (% VCK)	0.53	0.53	0.53

Note: DM: Dry matter; ME: Metabolizable energy; CP: Crude protein; CF: Crude fiber; DCP: Dicalcium phosphate. Control: Basal diet; JS1: 0.5% *J. subtriplinerve* supplementation; JS2: 1.0% *J. subtriplinerve* supplementation.

into milk of the goat herd throughout the experimental period. Feed intake was determined daily by weighing the difference between the amount offered and the refused feed, then calculating the total and converting it to monthly dry matter (DM) consumption. Milk production was recorded daily for each goat. The FCR was defined as the amount of dry matter consumed per unit of milk produced and calculated using the following formula: FCR=daily DM intake/daily milk yield (kg DM/kg milk).

2.6.2. Milk yield and composition analysis

Goats were hand-milked twice daily (at 8:00 and 16:00). Monitoring milk samples (approximately 150 mL) were collected immediately after milking for 3 consecutive days; morning and afternoon samples were thoroughly mixed to form a single representative composite sample. Milk samples were refrigerated at 0–4°C and analyzed on the same day using the Ekomilk Milkana KAM 98-2A automatic milk analyzer. Core parameters measured included the dry

matter percentage, fat content, protein content, and milk density. An Ekomilk Scan automatic counter was used to determine the SCC in the milk. The SCC index reflects both the hygienic quality of the milk and serves as an indicator for assessing mammary gland inflammation.

2.6.3. Clinical assessment of udder health

Daily in the morning, goat udders and milk were observed to detect clinical abnormalities such as swelling, hard lumps upon palpation, or pain; clotted, purulent, or bloody milk; and a sudden decrease in milk yield. Additionally, systemic signs such as fever, lethargy, loss of appetite, or limping due to udder pain were also recorded. Goats with suspicious clinical signs were tested using the California Mastitis Test (CMT kit - DeLaval, Poland) (Ruegg, 2017). The procedure involved discarding the first few milk squirts, collecting milk samples into the tray, adding CMT solution at a 1:1 ratio, and rotating gently in a circular motion for 10–15 seconds. The scoring scale for mastitis severity was assessed according to the instructions from the test kit manufacturer. In addition, somatic cell counts (SCC) were measured to detect subclinical inflammation, with a threshold set at > 1.0 x 10^6 cells/mL.

2.7. Statistical analysis

Experimental data were pre-processed using Excel 2011 and statistically analyzed with Minitab 16.0 (Minitab Inc., State College, PA, USA). For variables measured repeatedly over time (e.g., milk yield and feed intake), a repeated measures analysis was performed using a mixed-effects model, with dietary treatment as a fixed effect and individual animal as a random effect. Time (month) and the interaction between treatment and time were also included in the model.

For variables measured once or as averaged values (e.g., feed conversion ratio, milk composition, and SCC), a one-way analysis of variance (ANOVA) was applied using the General Linear Model (GLM) for a single-factor design: $y_{ij} = \mu + \alpha_i + e_{ij}$, where y_{ij} is the dependent variable, μ is the overall mean, αi is the dietary effect, and eij is the random error.

When significant effects were detected, differences among treatment means were compared using Tukey's post hoc test. Statistical significance was declared at $P < 0.05$, and trends were discussed at $0.05 \leq P < 0.10$. Results are presented as means ± standard error of the mean (SE).

For antioxidant activity (DPPH assay), non-linear regression analysis was performed using a four-parameter logistic (4PL) model in GraphPad Prism (GraphPad Software, San Diego, CA, USA) to estimate the half-maximal scavenging concentration (SC_{50}). Model goodness-of-fit was evaluated using the coefficient of determination (R^2).

3. Results and Discussion

3.1. Phytochemical profile and bioactive composition

The quantification of major bioactive compounds in *J. subtriplinerve* establishes a critical scientific foundation for determining its optimal inclusion level in dairy goat diets (Table 3).

Among the detected constituents, verbascoside was identified in all three samples, peaking in the concentrated extract (CV3: 7.76 mg/g), followed by the leaf powder (CV1: 4.69 mg/g), and reaching its lowest point in the mixed leaf–stem powder (CV2: 0.44 mg/g). The markedly lower concentration in CV2 confirms that the inclusion of stems dilutes the potency of the raw material, highlighting *J. subtriplinerve* leaves as the primary reservoir of biologically active metabolites. Verbascoside, a prominent phenylethanoid glycoside, is renowned for its diverse pharmacological profile—including antioxidant, anti-inflammatory, and antimicrobial activities—which are essential for enhancing animal health and productivity (Rossi et al., 2024).

A key finding was the presence of several flavonoids—rutin, astragalin, and isoquercitrin—exclusively or most abundantly in the leaf powder (CV1). Notably, these compounds were not detected (KPH) in the concentrated traditional extract (CV3). This absence suggests that intensive extraction or thermal processing methods may

Table 3. Bioactive compound concentrations in different preparations of *J. subtriplinerve*.

Nr.	Bioactive compounds	Unit	Concentration		
			CV1	CV2	CV3
1	Verbascoside	mg/g	4.69	0.44	7.76
2	Rutin	mg/g	0.06	ND	ND
3	Astragalin	mg/g	0.02	0.021	ND
4	Isoquercitrin	mg/g	0.04	0.02	ND
5	Gallic acid	µg/mL	ND	ND	ND
6	EGCG	µg/mL	ND	ND	ND
7	Oleanolic acid	µg/mL	ND	ND	ND
8	Betulinic acid	µg/mL	ND	ND	ND
9	Total Alkaloid	%	1.52	1.70	9.64
10	Total Tannin	%	1.37	1.6	2.11

Note: CV1: *J. subtriplinerve* leaf powder; CV2: Mixed leaf and stem powder; CV3: Aqueous concentrated extract; ND: Not detected.

compromise the stability of these flavonoids, which are vital for protecting cells against oxidative damage. The detection of astragalin is particularly important due to its reported anti-inflammatory properties that may help alleviate mammary gland inflammation.

Our results are consistent with previous phytochemical reports on *J. subtriplinerve*. (Kraus, 2003) identified terpene glycosides (Chevangin A–D) with antimicrobial properties, while (Minh Ngoc et al., 2022), recently identified 39 compounds, including verbascoside and stigmasterol, highlighting the plant's potential in metabolic regulation. Furthermore, the presence of total alkaloids and tannins in all samples—with CV3 showing the highest levels—indicates that extraction processes effectively enrich these specific groups.

Organic acids such as gallic acid, EGCG, oleanolic acid, and betulinic acid were not detected in any of the analyzed samples. In contrast, total alkaloids and total tannins were present in all three preparations, with the highest levels observed in the concentrated extract (CV3). This indicates that the extraction and concentration processes effectively increase the relative proportion of these compounds, whereas the organic acids mentioned above are either absent in this species or lost during preparation.

Based on this comprehensive profile, CV1 (leaf powder) was selected as the optimal dietary supplement. Although CV3 exhibited higher verbascoside levels, CV1 provided a more balanced synergistic profile of verbascoside and protective flavonoids. This combination is hypothesized to be sufficient to influence biological responses, enhance milk production, and improve udder health in dairy goats.

3.2. In vitro biological activity of J. subtriplinerve extracts

3.2.1. Antibacterial activity against mastitis-related pathogens

The antibacterial efficacy of *J. subtriplinerve* extracts against five common pathogenic strains, including those primarily responsible for bovine and caprine mastitis, is summarized in Table 4. The results demonstrated that the ethanol extract (96%) exhibited significantly higher antibacterial activity compared to the aqueous extract, as evidenced by the formation of larger inhibition zones. Among the tested preparations, CV1 (leaf powder) showed a positive inhibitory effect across all strains, with inhibition zones ranging from 7.0 to 10.0 mm. CV1 was the only sample capable of inhibiting *Staphylococcus aureus* (8.0 mm) and *Streptococcus agalactiae* (8.0 mm)—the two most critical pathogens associated with mammary gland infections in dairy goats. In contrast, CV2 (mixed leaf–stem powder) and CV3 (concentrated extract) showed very limited or no activity against several strains. The superior performance of CV1 suggesting that the synergistic combination of verbascoside and flavonoids found predominantly in the leaves is essential for antimicrobial defense.

While the inhibition zones of *J. subtriplinerve* were smaller than those of the positive controls (streptomycin and ciprofloxacin, which ranged from 21.25 to 36.25 mm), they are classified as weak to moderate. However, it is important to note that crude plant extracts often exhibit lower diffusion rates in agar media compared to purified, highly soluble commercial antibiotics. However, because minimum inhibitory concentration / minimum bactericidal concentration values were not determined in this preliminary screening, these *in vitro* results should be interpreted as preliminary indicators of mild antimicrobial activity.

Our findings align with previous studies by Nguyen et al., 2012, and Ngan et al., 2008, which identified verbascoside, protocatechuic acid, and gallic acid as the primary agents responsible for the antimicrobial properties of *J. subtriplinerve*. The observed inhibition of both Gram-positive (*S. aureus, S. agalactiae*) and Gram-negative (*E. coli, S. enterica, P. aeruginosa*) bacteria supports the broad-spectrum potential of the genus *Jasminum* (Balkrishna et al., 2021)

3.2.2. Antioxidant activity of J. subtriplinerve

The antioxidant potential of *J. subtriplinerve* preparations was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay. To ensure statistical robustness, a four-parameter logistic (4PL) nonlinear regression model was employed, which provided an excellent fit for the antioxidant response curves (R^2 values ranging from 0.9824 to 0.9895) (Figure 1).

All three samples demonstrated radical-scavenging activity at a concentration of 0.1 mg/mL, though their efficacy varied significantly across treatments ($P < 0.05$). Sample CV1 exhibited the most potent antioxidant activity, characterized by the lowest SC_{50} value (0.03679 mg/mL). Notably, the scavenging percentage of CV1 (89.10 ± 0.92%) showed no significant difference compared to the positive control, ascorbic acid (90.44 ± 0.58%; SC_{50} = 0.02919 mg/mL),

Table 4. Antibacterial ability of *J. subtriplinerve* ethanol extracts (n=3).

Samples		Diameter of inhibition zone (mm)				
		***Escherichia coli* VTCC 12272**	***Salmonella enterica* VTCC 12277**	***Staphylococcus aureus* VTCC 12275**	***Pseudomonas aeruginosa* VTCC 12273**	***Streptococcus agalactiae* ATCC 12386**
Ethanol extract	CV1	7.0±0.3	8.65±0.1	8.0±0.08	10.0±0.32	8.0±0.15
	CV2	-	-	-	3.5±0.12	-
	CV3	2.75±0.1	5,75±0.1	-	-	-
Positive control	Streptomycin	21.25±0.2	28.25±0.2	27.5±0.3	28.5±0.25	28.5±0.15
	Ciprofloxacin	36.25±0.3	33.5±0.4	35.65±0.26	36.15±0.34	35.25±0.2

Values are expressed as mean (n=3). Disk diameter: 6 mm. (-): No inhibition zone. Two-way ANOVA analysis with p<0.0001.

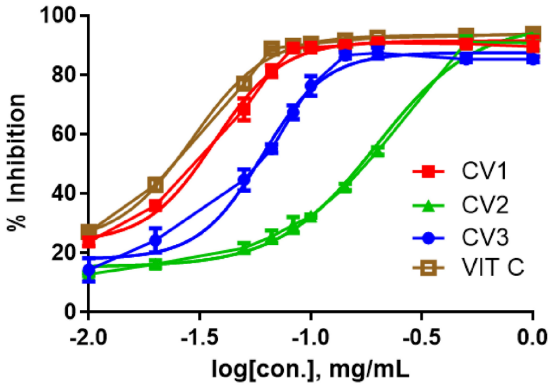


Figure 1. DPPH free radical scavenging activity of *J. subtriplinerve*. The data points represent the mean values (n=3), and the solid lines indicate the four-parameter logistic (4PL) regression fit.

indicating that CV1 possesses a near-equivalent capacity to neutralize free radicals as the standard antioxidant. Conversely, the concentrated extract (CV3) showed moderate antioxidant capacity (SC_{50} = 0.06036 mg/mL), while the mixed leaf–stem powder (CV2) presented the weakest activity (SC_{50} = 0.1972 mg/mL). The performance of CV1 is highly correlated with its rich and balanced content of verbascoside and flavonoids (rutin, isoquercitrin, and astragalin).

These findings align with previous reports by Nguyen et al. (2012), and Anh et al. (2019), which highlighted the role of polyphenols in *J. subtriplinerve* for inhibiting lipid peroxidation and protecting cells from oxidative stress. The bioactive compounds in *J. subtriplinerve*, particularly verbascoside, function as efficient electron donors, neutralizing reactive oxygen species (ROS) that are often elevated during peak lactation in dairy animals. Furthermore, although anti-inflammatory activity was not directly quantified in the present *in vitro* study, the robust antioxidant capacity of CV1 serves as a significant pharmacological indicator. Previous studies by Nguyen et al. (2012), and Do et al. (2022), demonstrated that ethanol extracts of *J. subtriplinerve* could significantly reduce experimental paw edema in animal models. Therefore, the high radical scavenging activity observed in CV1 suggests a strong potential for mitigating oxidative damage and supporting inflammatory control in the mammary tissues during the subsequent *in vivo* trials.

3.2.3. Nutritional and phytochemical profile of experimental diets

To ensure scientific rigor, the experimental diets were formulated for nutritional uniformity across all treatment groups while providing a precise, quantifiable dose of bioactive compounds from *J. subtriplinerve* powder. As detailed in Table 5, the basal diets were well-balanced, with crude protein and lipid levels optimized to support both casein synthesis and milk fat secretion in accordance with established standards for lactating goats (NRC, 1989). The lack of significant variation in dietary energy and fiber fractions confirms that any subsequent differences in animal performance are attributable to the herbal supplementation rather than nutritional discrepancies.

Regarding the experimental variables, the inclusion of *J. subtriplinerve* leaf powder at 0.5% (JS1) and 1.0% (JS2) established a distinct gradient of secondary metabolite intake (Table 6). Specifically, the JS2 group received a 2-fold higher daily loading of verbascoside, total alkaloids, and total tannins compared to JS1, alongside a consistent supply of protective flavonoids such as rutin and astragalin. The 0.5% supplementation level provided 30.49 mg of verbascoside, 97.5 mg of alkaloids, and 89.05 mg of tannins per head per day. The 1.0% supplementation level provided 60.97 mg of verbascoside, 195 mg of alkaloids, and 178 mg of tannins per head per day. Despite the characteristic bitterness typically associated with high concentrations of alkaloids and tannins, total feed intake remained stable across all treatments. This absence of feed refusal or reduced dry matter intake demonstrates that *J. subtriplinerve* supplementation at these levels is highly palatable for Saanen goats, providing a reliable physiological baseline for evaluating its impact on milk yield and mammary gland health.

3.2.4. Impact of J. subtriplinerve supplementation on lactation performance and feed efficiency

The dietary supplementation of *J. subtriplinerve* leaf powder significantly enhanced both the lactation performance and nutrient utilization efficiency of Saanen goats over the 90 day experimental period. As illustrated in Table 7, the milk yield of goats in both treatment groups (JS1 and JS2) was significantly higher than that of the control group throughout the trial ($P < 0.01$). In April, the daily milk yield already showed a positive response to supplementation, with JS1 (1.183 ±0.046 kg) and JS2 (1.234 ± 0.05 kg) outperforming the Control group (1.036 ±0.024 kg; P = 0.003). The most pronounced effect was observed in May, where the JS2 group reached its peak production at 1.618 ± 0.01 kg/head/day, which was statistically superior to both JS1 (1.472 ± 0.008 kg) and the Control (1.244 ± 0.01 kg; P = 0.000). The data suggest a dose-dependent relationship; while the 0.5% inclusion level (JS1) was sufficient to trigger a significant increase in production, the 1.0% level (JS2) provided a further boost in yield and sustained higher lactation persistence into June (1.507 ±0.02 kg for JS2 vs. 1.429 ± 0.015 kg for JS1).

The efficiency of converting dietary dry matter into milk is summarized in Table 8. The results indicate that the inclusion of *J. subtriplinerve* leaf powder markedly optimized feed utilization without requiring an increase in total dry matter intake. The average DM consumption per kilogram of milk produced was significantly lower in the treated groups ($P < 0.05$). The JS2 group achieved the most optimized feed efficiency, requiring only 0.932 ± 0.014 kg DM/kg milk, compared to 1.043 kg DM/kg milk in JS1 and 1.196 kg DM/kg milk in the Control group. A significant difference between the two treatment levels emerged in May and June ($P < 0.05$), where the higher dose of bioactive compounds in JS2 enabled the goats to utilize nutrients more effectively for milk synthesis during peak lactation. The observed enhancements in feed conversion efficiency and milk yield without compromising core milk composition align with recent findings in lactating dairy goats, where plant polyphenol supplementation linearly

Table 5. Nutritional composition on a DM basis of experimental diets.

Diets		Analysis results				
		DM	CP	Ash	Lipid	CF
		(%)	(%)	(%)	(%)	(%)
Green feed		20.33	11.94	16.11	1.82	19.87
Concentrate feed	Control	87.58	14.71	10.16	3.15	8.20
	JS1	87.32	14.86	10.20	3.18	8.27
	JS2	87.52	14.78	10.24	3.17	7.57

DM: Dry matter; CP: Crude protein; CF: Crude fiber; Control: Basal diet without supplementation; JS1: Basal diet + 0.5% J. subtriplinerve leaf powder; JS2: Basal diet + 1.0% J. subtriplinerve leaf powder.

Table 6. Estimated dose of *J. subtriplinerve* powder supplement in the goat diet.

Bioactive compounds	Concentration (mg/g)	(%)	Supplemental dose	
			0,5%	1%
			(6.5 g/head/day)	(13 g/head/day)
Verbascoside	4.69	0.469	30.49 mg/day	60.97 mg/day
Rutin	0.06	0.006	0.39 mg/day	0.78 mg/day
Astragalin	0.02	0.002	0.13 mg/day	0.26 mg/day
Isoquercitrin	0.04	0.004	0.26 mg/day	0.52 mg/day
Alkaloid	15.0	1.50	97.5 mg/day	195.0 mg/day
Tannin	13.7	1.37	89.05 mg/day	178.0 mg/day

JS1: 0.5% *J. subtriplinerve* supplementation (6.5 g/head/day); JS2: 1.0% *J. subtriplinerve* supplementation (13 g/head/day); mg/day: Grams per head per day.

Table 7. Effects of *J. subtriplinerve* powder on daily milk yield (n=18).

Experimental Time	Mean ±SE (kg/head/day)			P value
	Control	JS1 (0.5%)	JS2 (1%)	
April	1.036^{b}±0.024	1.183^{a}±0.046	1.234^{a}±0.05	0.003
May	1.244^{c}±0.01	1.472^{b}±0.008	1.618^{a}±0.01	0.000
June	1.251^{c}±0.012	1.429^{b}±0.015	1.507^{a}±0.02	0.000

SE: Standard error of the mean; Control: Basal diet; JS1: 0.5% *J. subtriplinerve* supplementation; JS2: 1.0% *J. subtriplinerve* supplementation. Means within the same row with different superscript letters (a, b, c) differ significantly ($P < 0.05$) according to Tukey's test.

Table 8. Feed conversion ratio (kg DM/kg milk) of goats supplemented with *J. subtriplinerve* powder.

Experimental Time	DM intake (kg)/kg milk		
	Mean±SE		
	Control	JS1	JS2
April	1.248^{a} ± 0.03	1.098^{b}±0.04	1.003^{b}±0.03
May	1.172^{a} ± 0.015	0.986^{b}±0.016	0.885^{c}±0.011
June	1.177^{a} ± 0.015	1.044^{b}±0.016	0.908^{c}±0.011
Average	**1.196^{a} ± 0.014**	**1.043^{b} ± 0.016**	**0.932^{c} ± 0.014**

SE: Standard error. Means within a row with different superscripts (a, b, c) differ significantly ($P < 0.05$) according to Tukey's test.

increased fiber digestibility, boosted total antioxidant capacity, and reduced lipid peroxidation markers such as malondialdehyde (Lu et al., 2025).

The feed efficiency recorded in the JS2 group (0.932 kg DM/kg milk) is highly competitive and aligns with or exceeds international benchmarks. Oliveira et al. (2014) reported an average efficiency of 1.06 kg milk per kg dry matter intake (approximately 0.94 kg DM/kg milk) for Saanen goats under similar confinement. Furthermore, our results demonstrate a substantially better efficiency than the values reported by (Abdullah et al., 2012) where goats required approximately 2.9 kg of feed to produce 1 kg of milk under different forage regimes.

3.2.5. Influence of J. subtriplinerve on milk composition and udder health protection

Dietary inclusion of *J. subtriplinerve* leaf powder did not significantly alter the core nutritional parameters, including protein (2.827%–2.963%), non-fat dry matter (8.559%–8.772%), and milk density ($P > 0.05$). This indicates that the substantial increase in milk volume (observed in the lactation performance section) did not compromise the synthetic capacity of the mammary gland or lead to a dilution effect. The JS2 group (1.0%) exhibited a numerical trend toward higher milk fat content (4.164 ± 0.226%) compared to the Control (3.875 ± 0.299%), suggesting that bioactive polyphenols may support lipid metabolism and energy partitioning without negatively influencing milk's nutritional profile (Table 9).

The observation of udder health during the trial indicated a lower occurrence of clinical signs in the supplemented groups. As detailed in Table 10, no mastitis cases were observed in the JS2 group during the 90-day trial period. In contrast, the Control group showed a 50% incidence rate (3 out of 6 goats), featuring cases ranging from mild (+) to moderate (++). The JS1 group (0.5%) recorded only one mild case that resolved spontaneously. The SCC served as a helpful indicator of udder condition. In the Control group, goats exhibiting clinical signs showed higher SCC levels, peaking at 1.678 x 10^6 cells/

Table 9. Nutritional values and somatic cell counts of experimental goat milk (n=9).

Values	Mean ±SE			P value
	Control	JS1	JS2	
NFDM (%)	8.566 ± 0.127	8.559 ± 0.135	8.772±0.125	0.421
Protein (%)	2.963±0.271	2.827 ± 0.195	2.958±0.208	0.892
Fat (%)	3.875±0.299	3.869 ± 0.095	4.164±0.226	0.431
Density (g cm^{-3})	27.533±0.61	27.48 ± 0.644	27.933±0.59	0.851
Somatic cells (10^3/mL)	168.0±48.9	106.3±16.3	104.0±14.0	0.32

NFDM: non-fat dry matter.

Table 10. Clinical mastitis incidence and severity scores.

Groups	April			May			June		
	No. of infected	Incidence Rate %	Score	No. of infected	Incidence Rate %	Score	No. of infected	Incidence Rate %	Score
Control	1	16.67	+	2	33.33	++	1	16.67	++
JS1	0	0	0	1	16.67	+	0	0	0
JS2	0	0	0	0	0	0	0	0	0

Note: Score (+): Mild mastitis; (++): Moderate mastitis according to California Mastitis Test (CMT). (-): Negative/No infection.

Table 11. Individual somatic cell counts (cells/mL) in goats diagnosed with mastitis.

Groups	Goat No.	Somatic cells/ml		
		April	May	June
Control	5162	760.000	-	1.678.000
	5050	-	844.000	-
	5168	-	885.000	-
JS1	1806	-	437.000	-

mL (Table 11). Conversely, the treated goats maintained lower and relatively stable SCC levels, suggesting that the supplementation may be associated with reduced subclinical and clinical udder inflammation. These results align with previous research where phytogenic additives supported animal health and performance without altering the fundamental chemical composition of milk (Tedesco et al., 2003), further quantitative and diagnostic studies are required to confirm the causal pathways of secondary metabolites in *J. subtriplinerve*.

No clinical mastitis cases were observed in the 1.0% group during the study period. The lower somatic cell counts observed in the supplemented groups may be associated with the antioxidant properties of the bioactive compounds present in *J. subtriplinerve*, although further studies measuring specific inflammatory biomarkers and immune responses are needed to confirm these mechanisms. Polyphenols such as verbascoside, flavonoids (rutin, astragalin), and alkaloids have been reported in literature to possess antioxidant and anti-inflammatory potential, which may help reduce oxidative stress and support tissue health in lactating animals (Pesce et al., 2015).

Furthermore, the flavonoids (rutin, astragalin) and alkaloids present in *J. subtriplinerve* may act synergistically to provide direct antimicrobial effects while neutralizing reactive oxygen species (ROS) generated during periods of intense milk secretion, thereby supporting overall metabolic stability (Rossi et al., 2024). More importantly, the maintenance of udder health, reflected by low SCC, indirectly supported sustained milk yield. Given that mastitis typically induces epithelial damage and impairs milk secretion, implementing a protective nutritional strategy through the diet not only ensures superior hygienic quality but also optimizes the total production potential of Saanen goats throughout their lactation cycle.

Our results demonstrating enhanced milk yield and improved feed conversion efficiency (FCR) alongside core milk constituent stability in Saanen goats are strongly supported by recent findings on phytogenic feed additives in dairy ruminants. Specifically, Khayya et al. (2022) reported that dietary inclusion of dried rosemary or laurel leaves at 1% of concentrate significantly increased daily milk yield (1.420 kg/day and 1.156 kg/day in controls) and improved feed conversion without adversely affecting basic milk chemical composition in Zaraibi goats. Similarly, Wójtowski et al. (2025) observed that multi-herbal dietary supplementation in Polish White Improved goats maintained baseline milk production and chemical composition while significantly improving milk fat percentage and fat-to-protein ratio.

Furthermore, comprehensive reviews on phytogenic feed additives in ruminants confirm that plant secondary metabolites, particularly polyphenols, flavonoids, and essential oils, enhance overall lactation performance and feed utilization efficiency primarily by optimizing rumen fermentation and improving nutrient digestibility. In agreement with these systemic responses, Saleh et al. (2023) demonstrated that herbal formulation supplementation in dairy cows significantly lowered milk and plasma cholesterol levels while elevating lactose content and beneficial unsaturated fatty acid proportions. Additionally, systematic evaluations of phytogenic modulation in caprine physiology show that bioactive herbal inclusion enhances fiber digestibility by up to 25%, mitigates lipid peroxidation (reducing malondialdehyde by over 65%), and boosts endogenous antioxidant enzyme defenses (such as superoxide dismutase). These physiological mechanisms provide a solid rationale for the superior milk yield, enhanced FCR, and complete elimination of clinical mastitis observed in our *J. subtriplinerve*-supplemented Saanen goats.

Due to the small group size ($n = 6$ goats per treatment, 18 in total), the statistical power of this study may be somewhat limited. We chose animals with very strict criteria—such as balancing parity, lactation stage, and baseline milk yield—to minimize individual variation and enhance statistical sensitivity. Consequently, this investigation should be considered a pilot study, and future large-scale trials involving larger herd sizes across diverse farming environments are recommended to confirm these preliminary findings and support broader field applications.

4. Conclusion

The findings of this pilot study indicate that *J. subtriplinerve* is a promising natural feed supplement for Saanen dairy goats. Based on the experimental data, the 1.0% inclusion level produced the most favorable responses among the tested treatments in improving production parameters. This is demonstrated by the improved feed conversion ratio (0.932 ± 0.014) and a sustained daily milk yield of 1.618 ± 0.01 kg, representing a significant enhancement compared to the control group. Beyond lactation performance, this supplementation level was associated with improved udder health indicators and a lower incidence of mastitis-related symptoms during the study period, without adversely affecting the measured milk composition parameters. In conclusion, J. subtriplinerve leaf powder represents a promising natural feed supplement that may contribute to dairy goat productivity and udder health.

Acknowledgements

This work was supported by the project "Dietary supplement of *Jasminum subtriplinerve* powder to improve milk yield, milk quality and reduce mastitis in dairy goats" funded by the KU-KOICA-VNUA.

Data Availability Statement

Data is available on demand from referees.

Ethics and consent

All animal procedures were approved by the Ethics Committee of the Faculty of Animal Science, Vietnam National University of Agriculture (Approval No. FAS-VNUA-2025/01; Date: April 05, 2025). The trial was conducted at the Goat and Rabbit Research Centre (National Institute of Animal Sciences, Vietnam) in accordance with institutional guidelines for animal care and use.

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