EDITORIAL CSIC

Spanish Journal of Agricultural Research
24 (1) January-March 2026, 21426
ISSN-L: 1695-971X, eISSN: 2171-9292
https://doi.org/10.5424/sjar/2026241-21426

ARTICLE

RESEARCH ARTICLE

Dietary incorporation of exogenous fibrolytic enzymes cocktail and wormwood (*Artemisia absinthium* L.) herb synergistically enhances immunity and carcass traits in sheep

La incorporación a la dieta de una mezcla de enzimas fibrolíticas exógenas y ajenjo (*Artemisia absinthium* L.) mejora sinérgicamente la inmunidad y las características de la canal en las ovejas

Yasir A. Beigh
Division of Animal Nutrition, Faculty of Veterinary Sciences & Animal Husbandry, Shuhama, Alusteng, Srinagar 190006, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir, Jammu & Kashmir, India
Corresponding author: vetyasir1@gmail.com, https://orcid.org/0000-0002-2814-9179

Abdul M. Ganai
Division of Animal Nutrition, Faculty of Veterinary Sciences & Animal Husbandry, Shuhama, Alusteng, Srinagar 190006, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir, Jammu & Kashmir, India
https://orcid.org/0000-0002-0656-0258

Haidar A. Ahmad
Division of Animal Nutrition, Faculty of Veterinary Sciences & Animal Husbandry, Shuhama, Alusteng, Srinagar 190006, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir, Jammu & Kashmir, India
https://orcid.org/0009-0001-1491-6971

Majid Shafi
Division of Veterinary Pathology, Faculty of Veterinary Sciences & Animal Husbandry, Shuhama, Alusteng, Srinagar 190006, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir, Jammu & Kashmir, India
https://orcid.org/0009-0002-5130-7451

Abstract: *Aim of study:* To assess the effects of exogenous fibrolytic enzymes (EFE) and wormwood (*Artemisia absinthium* L.) herb (WH) added individually and in-combination in diets of sheep on immune responses and in vivo carcass traits. *Area of study:* Mountain Research Centre for Sheep and Goat, Srinagar, Jammu & Kashmir, India. *Material and methods*: Crossbred sheep (n = 20) were distributed equally to 4 dietary groups fed total mixed rations for three months as basal feed (control), or added with EFE cocktail and WH either individually at 6.0 g/kg (EFE) and 45.0 g/kg dry matter (WH), respectively or in combination (EFE + WH) after which the animals were tested. *Main results:* Monoterpenes hydrocarbons (Chrysanthenyl acetate and L- β- Pinene) were the most abundant bioactive components found in WH. In humoral immune assay, WH added diets (WH and EFE + WH) increased ($P < 0.01$) mean antibody titers at different days post-immunization. Similarly, the cell mediated immune response of sheep fed WH added diets was higher ($P < 0.01$) on average as well as at each time point post-challenge. Ultrasonographic scanning revealed larger ($P < 0.05$) longissimus dorsi muscle area and wider rib flank streaking, while body biometric measurements depicted higher height at withers, thoracic circumference ($P < 0.05$), and heart girth ($P < 0.01$) in sheep of EFE+WH group compared to those of the control group. Paunch girth was higher ($P < 0.01$) in EFE incorporated groups (EFE and EFE + WH) compared to those of the control group. *Research highlights:* Dietary combination of EFE and WH synergistically could enhance carcass characteristics of sheep compared to the individual additions, while WH beneficially modulates immune response either incorporated alone or in-combination.

Keywords: body biometrics; exogenous enzymes; immunity; perennial herb; ultrasonography.

Resumen: *Objetivo del estudio:* Evaluar los efectos de las enzimas fibrolíticas exógenas (EFE) y el ajenjo (*Artemisia absinthium* L.) (WH), añadidos individualmente y en combinación a la dieta de las ovejas, en las respuestas inmunitarias y las características de la canal in vivo. *Área del estudio:* Centro de Investigación de Montaña para Ovejas y Cabras, Srinagar, Jammu y Cachemira, India. *Material y métodos:* Se distribuyeron ovejas mestizas (n = 20) equitativamente en 4 grupos de alimentación a los que se les suministró raciones mixtas completas durante tres meses como alimento base (control), o con la adición de un cóctel de EFE y WH, ya sea individualmente a 6,0 g/kg (EFE) y 45,0 g/kg de materia seca (WH), respectivamente, o en combinación (EFE + WH), tras lo cual se realizaron las pruebas en los animales. *Resultados principales:* Los hidrocarburos monoterpénicos (acetato de crisantenilo y L-β-pineno) fueron los componentes bioactivos más abundantes encontrados en WH. En el ensayo de inmunidad humoral, las dietas suplementadas con WH (WH y EFE + WH) aumentaron ($P < 0,01$) los títulos medios de anticuerpos en diferentes días posteriores a la inmunización. De manera similar, la respuesta inmune mediada por células de las ovejas alimentadas con dietas suplementadas con WH fue mayor ($P < 0,01$) en promedio, así como en cada momento posterior al desafío. La ecografía reveló un área del músculo longissimus dorsi mayor ($P < 0,05$) y una mayor infiltración de grasa en el flanco, mientras que las mediciones biométricas corporales mostraron mayor altura a la cruz, circunferencia torácica ($P < 0,05$) y perímetro torácico ($P < 0,01$) en las ovejas del grupo EFE+WH en comparación con las del grupo control. El perímetro abdominal fue mayor ($P < 0,01$) en los grupos que recibieron EFE (EFE y EFE + WH) en comparación con el grupo control. *Aspectos destacados de la investigación:* La combinación dietética de EFE y WH podría potenciar sinérgicamente las características de la canal de las ovejas en comparación con la adición individual de cada componente, mientras que el WH modula de forma beneficiosa la respuesta inmunitaria, tanto si se incorpora solo como en combinación.

Palabras clave: biometría corporal; enzimas exógenas; hierba perenne; inmunidad; ecografía.

Received: 05-10-2024 / **Accepted**: 05-11-2025 / **Published**: 23-06-2026

Citation: Beigh, YA; Ganai, AM; Ahmad, HA; Shafi, M (2026). Dietary incorporation of exogenous fibrolytic enzymes cocktail and wormwood (*Artemisia absinthium* L.) herb synergistically enhances immunity and carcass traits in sheep. Spanish Journal of Agricultural Research, Volume 24, Issue 1, 21426. https://doi.org/10.5424/sjar/2026241-21426

Supplementary information ↓

1. INTRODUCTION

Dietary incorporation of natural feed additives has been documented as an effective approach to enhance the ruminant health and production efficiency (Arsenos & Giannenas, 2023). This serves as an alternative feeding strategy considering the public and scientific concerns of drug resistance, residual effects and environmental issues over the use of conventional feed additives (antibiotics, hormones, etc.). Besides, it will help the small livestock holders in developing countries for sustainable organic animal production. Among the natural feed additives, herbs (medicinal plants) and exogenous enzymes have attracted much emphasis due to their satisfactory effects on nutrient metabolism, health promotion and enhanced production.

Exogenous fibrolytic enzymes (EFE) application improves ruminal fermentation and increases nutrient digestion (Kholif & Patra, 2024), enhance the nutritional status to support better body weight gain (Anil et al., 2023) and lactation performance (Martinez et al., 2023), beneficially influences the body immune functioning (Roque et al., 2019) and increase dietary energy availability for higher carcass traits (Vargas et al., 2013). Moreover, several medicinal herbs have been used to improve animal performances

(Ramdani et al., 2023) due to their beneficial effects on feeding pattern and stimulation of digestive enzymes secretion by aromatic properties (Pliego et al., 2020), digestion enhancement by favoring the growth of beneficial ruminal microbiota (Lee et al., 2020), reduction in the incidence of digestive disturbance due to their medicinal properties (Batiha et al., 2020), inhibition of methane production (Lambo et al., 2024), and modulation of immune response (Lakhani et al., 2019), thereby enhancing productive performances of animals.

Wormwood (*Artemisia absinthium* L.) herb (WH), a hard perennial subshrub, belongs to the family Asteraceae (Compositae), and is native to temperate zones of Asia, Europe, and North America. In India, the herb is prevalent in the Himalayan region across Jammu and Kashmir, and is natively called 'Tethwen' in Kashmir valley. It has discrete incense and medicinal properties due to its several valuable biological effects including antioxidant, anti-parasitic, antimicrobial, antiviral, hepato-protective, metabolic modifier and immune-modulator (Wani et al., 2014; Mohammed, 2022). However, scanty information is available with regards to the effect of dietary addition of EFE or WH on immune assays and carcass traits in sheep. In this context, the present study has been conducted with the objective to assess the effect of feeding complete diet without or with inclusion of EFE cocktail and WH either alone and in-combination on immune responses and carcass characteristics in sheep.

2. MATERIAL AND METHODS

2.1. Experimental feed additives

A commercially available product of EFE (ALLENZIMIXEP) in powder form was assayed for enzymatic activities as per Valdes et al. (2015) at pH 6.5 and 39 °C to emulate optimal rumen conditions. The experimental EFE product contained (in $\times 10^3$ IU/g of product) 800 cellulase, 450 βglucanase, 400 xylanase, 400 phytase, and 350 pectinase.

The aerial parts (twigs, leaves and footstalks) of WH were collected from wild growing shrubs during early summer season in the Aharbal area (33° 38' 45.5" N, 74° 46' 50.5" E) of district Kulgam in South Kashmir (a zone classified to have a typical temperate climate). The herb was authenticated by the Centre for Biodiversity and Taxonomy, University of Kashmir. The samples of WH were dried at 50 °C for 72 h in forced-air oven (Narang Private Ltd, India: SE929), ground in a Wiley mill (Philadelphia, USA: Arthur H. Thomas Co.) to pass through a 0.50 mm filter, packed and stored until chemical analysis and experimental use.

To determine the bioactive components in WH, a representative sample of the experimental WH was extracted and analyzed according to Wani et al. (2014) by gas chromatography-mass spectrometry [Varian Gas Chromatograph series 3800 fitted with a VF-5 ms fused silica capillary column (60 m × 0.26 mm, film thickness 0.25 µm) coupled with a 4000 series mass detector]. The bioactive components of WH were detected by the combination of retention index data and mass spectra data using the Mainlib library.

2.2. Experimental design, animals and diets

Twenty crossbred (Hampshire × Fec-B gene carrying strain) male sheep (4-6 months age) were used in the study to avoid the confounding effects of gender difference. The experimental animals were fed to meet their nutrient requirements (ICAR, 2013) a basal total mixed ration (TMR) consisting 400 g/kg dry matter (DM) of oats straw (Avena sativa L.), 200 g/kg DM of mixed legume-grass hay, and 400 g/kg DM of concentrate feed mixture. Mixed hay comprised of clover (Trifolium repens L.) and rye (Lolium perenne L.) in equal proportions. The ingredients and chemical composition of the basal TMR is presented in Table 1.

Table 1. Ingredients and chemical composition (g/kg dry matter) of the experimental basal total mixed ration (TMR) for sheep

Attribute	Basal TMR
Physical composition	
Oats straw	400
Mixed grass hay	200
Crushed yellow maize	110
Wheat bran	50
De-oiled rice bran	40
Mustard oil cake	60
Whole soybean	114
Molasses	10
Mineral mixture[‡]	6
Common salt	4
Urea	6
Chemical composition	
Organic matter	905
Crude protein	166
Neutral detergent fibre	448
Acid detergent fibre	284
Acid detergent lignin	43.3

[‡] Contained per kg mineral mixture: Calcium 180 g, Phosphorous 120 g, Magnesium 2 g, Sulphur 2.3 g, Zinc 210 mg, Iron 55 mg, Iodine 10 mg, Copper 60 mg, Manganese 210 mg, Cobalt 8 mg, Fluorine 30 mg.

All sheep were fed the same basal TMR without any additives for 10 days as an adaptation period, and were then distributed equally into four dietary treatments (n=5 sheep /treatment) in a completely randomized design and fed for 90 days: control (fed the basal TMR with no additive), EFE, WH, and EFE+WH fed the basal TMR added with EFE at 6.0 g/kg DM, dried WH at 45.0 g/kg DM, and a combination of EFE and WH at 6.0 plus 45.0 g/kg DM, respectively. The adjustment of the amount of offered ration was revised at 15-day intervals depending on the total amount of weighed back refusals and allowing refusal amount not exceeding 10% of daily feed intake. The inclusion levels of the feed additives selected were based on the results of *in vitro* nutrient degradability assays carried out prior to the *in vivo* feeding trial (Beigh et al., 2023 & 2024).

The feed additives were offered at the time of feeding by first hand-mixing with concentrate feed portion, and then with chaffed roughage portion to obtain the total mixed diets. The animals were fed the respective experimental diets twice daily at 09:00 and 17:00 h in equal portions individually in stalls. Clean and fresh drinking water was offered ad libitum. Daily feed intake was recorded by weighing the diets offered and refusals of the previous day.

2.3. Immunological assays

At the end of the feeding trial, four animals from each treatment were chosen randomly and fed the experimental diets of respective treatment groups for 28 additional days to test for humoral as well as cell-mediated immune responses.

2.3.1. Humoral immune (HI) assay

The HI response was assessed through hemagglutination assay (HA) (Hudson & Hay, 1991) using chicken erythrocyte (C-RBC) as antigen. For immunization, 1 mL of 1% C-RBC suspension in phosphate-buffered saline (PBS, pH 7.4) adjuvanted with Montanide ISA206 (Seppic, Paris, France) was injected intra-dermally in the experimental sheep. A booster (challenge) dose was injected on the 7th-day after the first injection. Blood samples were collected from the jugular vein in ethylene diamine-tetra acetic acid (EDTA) vials (Hi-media, Mumbai) from animals before immunization on day 0, and 7, 14, 21, and 28 days post- first immunization. Blood samples were kept at room temperature for 2 h and then overnight at 4 °C followed by centrifugation at 2500 × g for 10 minutes. The sera were collected, incubated at 56 °C for 30 minutes to inactivate the complement, and then frozen (-80 °C) until used. The antibody production in response to immunization was assessed by the HA test. The reciprocal of the highest dilution of serum causing complete HA was taken as HA titer and expressed in $\log_2$ values.

2.3.2. Cell-mediated immune (CMI) assay

The CMI response was assessed simultaneously with HI assay through *in vivo* cutaneous delayed-type hypersensitivity (DTH) reaction by measuring the increase in the double skin-fold thickness (SFT) as per Verma et al. (2017) in response to the percutaneous application of 2-4, dinitrochlorobenzene (2-4, DNCB) at the sheep's back site. The sites were clipped, marked, shaved, and thoroughly cleaned with alcohol approximately three hours prior to application of the test chemical. Two areas each of 2 cm diameter were marked at the site, one for the test chemical application and the other as a control. The dose of DNCB application was standardized by conducting prior pilot trials on sheep. For sensitization, 100 µL of 5% solution of DNCB in a vehicle (acetone: olive oil in 4:1 ratio) was used, while 100 µL of 1.5% solution was used for the challenge. The challenge dose was applied 10 days post-sensitization. The solutions were slowly applied percutaneously to cover the whole area and at the same time avoiding its spread beyond the marked region. At the collateral control site, an equal volume of the vehicle only was applied. Thickness (in mm) of double skin-fold at the selected sites was measured at 0, 12, 24, 48, 72 and 96 hours post-challenge with the help of a digital vernier caliper (150 mm with accuracy of ±0.02mm, Qfun, China). All the applications and skin measurements were made by the same person to minimize variations.

2.4. *In vivo* carcass characteristics

After immune assays, the sheep were fasted for 12 h and then weighed to record live slaughter weights. Carcass composition attributes were assessed by ultrasonographic and body biometric measurements on live animals.

2.5. Ultrasonographic (USG) measurements

The ultrasound measurements were performed with a real-time ultrasound device (MyLab40VET, esaote, Italy) with an 18.0 MHz, 12.5 cm linear transducer. The resolution of the scanner calipers was 0.01 cm. The animals were manually immobilized and the acoustic gel was applied at shaved measurement areas to provide good contact between the probe and the skin for image acquisition. Pressure on the transducer head was kept to a minimum to avoid compression of the fat. Following physical palpation and preparation of the examination site, the transducer was placed between the 3rd and 4th lumbar vertebrae (3-4L), lateral to the vertebral column and parallel to it to capture the scanned image of *longissimus dorsi* (rib eye - RE) muscle at that point. Borders of the muscle were drawn on the scan from which the depth, width, and area of the muscle were measured with the electronic callipers of the scanner as per Akdag et al. (2015). The thicknesses of subcutaneous fat was measured between the 12th and 13th thoracic vertebrae (12-13T) as back fat, between 3-4L vertebrae as lumber fat, and over 1st and 2nd coccygeal vertebrae as tail fat in accordance to Teixeira et al.

(2006). All measurements were taken on the left side and 4 cm from the vertebral column. Fat width in between the 12th and 13th ribs as rib flank streaking, and between the scrotal septum and left testicle towards the dorsal section from the caudal surface of the scrotum as scrotal fat were also recorded using electronic calipers of the scanner. All ultrasound scans of animals were performed by the same experienced and well-trained operator.

2.6. Biometric measurements (BM)

Linear body measurements (expressed in cm) on live animals that are related to carcass composition as per Fernandes et al. (2010) were taken with the help of flexible tape and a large calliper while the animals were made to stand on a leveled floor with the head held up. The biometrical measurements recorded were body length (BL), height at withers (HW), heart girth (HG), chest depth (CD), chest width (CW), forearm circumference (FC), thoracic circumference (TC), rump height (RH), paunch girth (PG), pelvic width (PW), hind leg length (HL), buttock width (BW) and posterior buttock circumference (PBC). Also, scrotal length (SL) and scrotal circumference (SC) were measured at greater curvatures as per Mahmood et al. (2018). The largest value for each dimension was recorded. Besides, tail measurements including tail length (TL), tail width (TW), tail thickness (TT), and tail circumference (TC) were recorded as described by Atti & Hamouda (2004).

2.6.1. Statistical analysis

For all results, the individual sheep of the dietary treatments was the experimental unit. Data of the carcass characteristics were analyzed by one-way analysis of variance (ANOVA) test performed using SPSS (2011) statistical software package with the type of diet as the main source of variation. Data of immune responses were analyzed by repeated-measures ANOVA using Proc Mixed procedure of SAS (2009). Differences among treatments were considered significant at $P < 0.05$, whereas $0.05 < P < 0.10$ presented a tendency using Tukey's procedure for multiple comparisons among means.

3. RESULTS

3.1. Bioactive components of WH

A total of 12 components were identified by GC-MS between 16.1 and 44.1 min of retention time (Table 2). Monoterpenes hydrocarbons were the most abundant components found in WH, with Chrysanthenyl acetate and L- β- Pinene representing almost 98% of the total bioactive components. Traces of sesquiterpene were also detected, which form 2% of the remains.

Table 2. Individual bioactive constituents in the experimental *Artemisia absinthium* L. herb (WH) determined by gas chromatography/mass spectrometry

Name of the components	Molecular formula	Molecular weight (g/mol)	Retention time (min)	Proportion of total area	Component (g/kg WH)
Chrysanthenyl acetate; 1-(3,4-Dichlorobenzoyl) proline Q67879791 [(1S,5R)-4,6,6-trimethyl-7-bicyclo[3.1.1]hept-3-enyl] acetate	$C_{12}H_{18}O_2$	194.2	30.73	0.4915	0.7667
L- β- Pinene; 6,6-Dimethyl-2-methylidenebicyclo[3.1.1] heptane	$C_{10}H_{16}$	136.23	16.94	0.3960	0.618
Pin-2(10)-ene					
Sabinyl acetate; 4-methylidene-1-(propan-2-yl)bicyclo[3.1.0]hexan-3-yl acetate	$C_{12}H_{18}O_2$	194.2	32.17	0.0343	0.053
α- Pinene; (1S,5S)-2,6,6-Trimethylbicyclo[3.1.1] hept-2-ene	$C_{10}H_{16}$	136.23	13.98	0.0242	0.037
Cis-Verbenol; (1R,2R,5R)-4,6,6-Trimethylbicyclo[3.1.1] hept-3-en-2-ol	$C_{10}H_{16}O$	152.23	26.23	0.0138	0.022
Sabinene; 4-methylene-1-(1-methylethyl)bicyclo[3.1.0] hexane	$C_{10}H_{16}$	136.23	16.12	0.0128	0.0199
Camphene; 2,2-Dimethyl-3-methylidenebicyclo[2.2.1] heptane	$C_{10}H_{16}$	136.24	14.82	0.0105	0.016
Germacrene; (1E,5E,8S)-1,5-dimethyl-8-(prop-1-en-2-yl) cyclodeca-1,5-diene	$C_{15}H_{24}$	204.35	40.8	0.0073	0.011
Caryophyllene; (1R,4E,9S)-4,11,11-Trimethyl-8-methylidene bicyclo[7.2.0] undec-4-ene	$C_{15}H_{24}$	204.36	38.2	0.0037	0.005
Neryl acetate; (2Z)-3,7-dimethylocta-2,6-dien-1-yl acetate	$C_{12}H_{20}O_2$	196.29	44.09	0.0032	0.0049
β- Pinene; 6,6-Dimethyl-2-methylidenebicyclo[3.1.1] heptane	$C_{10}H_{16}$	136.23	16.44	0.0015	0.002
Pin-2(10)-ene					
β- Bourbonene; 1-methyl-5-methylidene-8-(propan-2-yl) tricyclo[5.3.0.0^{2,6}]decane	$C_{15}H_{24}$	204.35	36.7	0.0012	0.0018
Total sum	-	-	-	1.00	1.55

3.2. Humoral immune response

The results of humoral immune response of the sheep fed diets added with EFE and WH as additives are presented in Table 3. Among the experimental treatments, the antibody titers on different days post-immunization were higher (P < 0.01) in sheep fed wormwood herb added diets (WH and WH + EFE) than the other treatments. Time affected significantly (P < 0.01) the antibody titres for the antigen with a marked increase on the 7th and 14th days of the immunization followed by a gradual declining trend thereafter in all the treatment groups.

Table 3. Humoral immune response in sheep fed total mixed ration (TMR) added with exogenous fibrolytic enzymes (EFE) or wormwood herb (WH) individually and in-combination (EFE + WH) through a 28 days experimental period

‡Dietary treatments	Antibody response to chicken-RBC, log^2 titer					Mean	SEM	*P*-value
	Pre-immunization titer	Post-immunization titer						
	0 d	7th d	14th d	21st d	28th d			
Control	0.00^{c}	3.23^{Ba}	3.05^{Ba}	2.81^{Ba}	2.26^{Bb}	2.27	0.24	<0.001
EFE	0.00^{c}	3.29^{Ba}	3.17^{Ba}	2.81^{Bab}	2.39^{Bb}	2.33	0.25	<0.001
WH	0.00^{c}	3.95^{Aa}	3.83^{Aa}	3.59^{Aab}	3.11^{Ab}	2.90	0.30	<0.001
EFE+WH	0.00^{c}	4.01^{Aa}	3.83^{Aa}	3.65^{Aab}	3.17^{Ab}	2.93	0.31	<0.001
SEM	0.00	0.09	0.10	0.11	0.11	0.14	-	-
P value	-	<0.001	<0.001	<0.001	<0.001	0.187	-	-

‡Control: 0% addition; EFE: TMR added with EFE at 6.0 g/kg DM; WH: TMR added with WH at 45.0 g/kg DM; EFE + WH: TMR added with 6.0 EFE + 45.0 WH g/kg DM, respectively.

^{a,b,c} The means within rows with different lowercase superscripts differ significantly among the days.

^{A,B} The means within columns with different uppercase superscripts differ significantly among the treatments.

SEM= standard error of means.

3.3. Cell mediated immune response

Table 4 and Table 5 present the effects of the dietary treatments on SFT in the experimental sheep in terms of absolute and comparative hypersensitivity response, respectively. The CMI response was similar at 0 h for all groups, while the sheep fed treatment diets had the similar high values of SFT through the first 12 h (P < 0.05) and 24 h (P < 0.01). Sheep of EFE + WH group had the highest (P < 0.01) SFT at each time point post-challenge application, while both WH and EFE+WH groups had higher (P < 0.01) similar overall means. Time also affected significantly (P < 0.01) the SFT values, peak values were observed at 24 h for all groups, after which they had a declining trend reaching to almost normal level after 96 h post-challenge DNCB solution application.

Table 4. Absolute hypersensitivity in sheep fed total mixed ration (TMR) added with exogenous fibrolytic enzymes (EFE) or wormwood herb (WH) individually and in-combination (EFE + WH) through 96 h period

‡Dietary treatments	Skin fold thickness (mm)						Mean	SEM	*P*-value
	0 h	12 h	24 h	48 h	72 h	96 h			
Control	3.96^{c}	6.66^{Bab}	6.94^{Ba}	6.16^{Cab}	5.22^{Cbc}	4.36^{Bc}	5.55^{B}	0.23	<0.001
EFE	4.24^{d}	7.24^{ABa}	7.40^{ABa}	6.54^{BCab}	5.62^{BCbc}	4.80^{Bcd}	5.97^{AB}	0.23	<0.001
WH	4.02^{d}	7.84^{ABab}	8.12^{ABa}	7.48^{ABab}	6.82^{ABbc}	5.94^{Ac}	6.70^{A}	0.27	<0.001
EFE+WH	4.12^{c}	8.06^{Aab}	8.48^{Aa}	7.78^{Aab}	6.98^{Aab}	6.12^{Abc}	6.92^{A}	0.31	<0.001
SEM	0.09	0.20	0.19	0.20	0.23	0.21	0.14	-	-
P value	0.737	0.044	0.009	0.003	0.003	0.001	0.001	-	-

‡Control: 0% addition; EFE: TMR added with EFE at 6.0 g/kg DM; WH: TMR added with WH at 45.0 g/kg DM; EFE + WH: TMR added with 6.0 EFE + 45.0 WH g/kg DM, respectively.

^{a,b,c,d} The means within rows with different lowercase superscripts differ significantly among periods.

^{A,B,C} The means across the columns with different uppercase superscripts differ significantly among treatments.

Table 5. Comparative hypersensitivity in sheep fed total mixed ration (TMR) added with exogenous fibrolytic enzymes (EFE) or wormwood herb (WH) individually and in-combination (EFE + WH) through 96 h period

‡Dietary treatments	Times fold increase to basal value of skin fold thickness						Mean	SEM	*P*-value
	0 h	12 h	24 h	48 h	72 h	96 h			
Control	1.00^{d}	1.69^{Bab}	1.76^{Ba}	1.56^{Bb}	1.31^{Bc}	1.10^{Bd}	1.40^{B}	0.05	<0.001
EFE	1.00^{d}	1.71^{ABab}	1.76^{Ba}	1.55^{Bb}	1.33^{Bc}	1.13^{Bd}	1.41^{B}	0.05	<0.001
WH	1.00^{e}	1.95^{Aab}	2.03^{Aa}	1.86^{Ab}	1.70^{Ac}	1.48^{Ad}	1.67^{A}	0.07	<0.001
EFE+WH	1.00^{d}	1.95^{Aa}	2.05^{Aa}	1.88^{Aab}	1.69^{Abc}	1.48^{Ac}	1.68^{A}	0.07	<0.001
SEM	0.00	0.04	0.04	0.04	0.05	0.04	0.03	-	-
P value	-	0.001	<0.001	<0.001	<0.001	<0.001	0.001	-	-

‡Control: 0% addition; EFE: TMR added with EFE at 6.0 g/kg DM; WH: TMR added with WH at 45.0 g/kg DM; EFE + WH: TMR added with 6.0 EFE + 45.0 WH g/kg DM, respectively.

^{a,b,c,d} The means within rows with different lowercase superscripts differ significantly among periods.

^{A,B,C} The means across the columns with different uppercase superscripts differ significantly among treatments.

3.4. Body ultrasonographic measurements

Table 6 presents the body ultrasonographic measurements of sheep fed the experimental diets. No differences were observed among all the groups of sheep on RE muscle depth and width, back fat, lumbar fat, scrotal fat, and tail fat thicknesses, but larger ($P < 0.05$) RE muscle area and wider ($P < 0.05$) rib flank streaking were observed for sheep fed EFE+WH compared to those fed the control diet.

Table 6. Ultrasonographic measurements of sheep fed total mixed ration (TMR) added with exogenous fibrolytic enzymes (EFE) or wormwood herb (WH) individually and in-combination (EFE + WH)

Attributes	Dietary treatments‡				SEM	*P*-value
	Control	EFE	WH	EFE+WH		
Ribeye (RE) muscle dimensions						
RE muscle width (cm)	2.88	3.06	3.13	3.25	0.06	0.153
RE muscle depth (cm)	1.91	2.04	2.08	2.16	0.04	0.117
RE muscle area (cm2)	8.71^{b}	10.2^{ab}	10.3^{ab}	10.5^{a}	0.27	0.048
Body fat thickness measurements (mm)						
Back fat thickness	2.23	2.54	2.17	2.33	0.08	0.365
Lumbar fat thickness	2.84	3.16	2.77	3.04	0.07	0.239
Rib flank streaking	0.74^{b}	0.81^{ab}	0.83^{ab}	0.89^{a}	0.02	0.045
Scrotal fat thickness	2.71	3.06	3.11	3.18	0.07	0.100
Tail fat thickness	1.78	2.16	2.07	2.23	0.08	0.235

‡Control: 0% addition; EFE: TMR added with EFE at 6.0 g/kg DM; WH: TMR added with WH at 45.0 g/kg DM; EFE + WH: TMR added with 6.0 EFE + 45.0 WH g/kg DM, respectively.

^{a,b} The means within rows with different superscripts differ significantly among the treatments.

SEM = standard error of means.

3.5. Body biometric measurements

The effect of the dietary treatments on live slaughter weight and linear body measurements of the sheep are presented in Table 7. Higher ($P < 0.05$) live slaughter weight was observed in sheep of EFE + WH groups compared to the control. No differences were observed among the groups fed the feed additives individually for all body biometric dimensions, while only the sheep fed EFE + WH diet showed higher ($P < 0.05$) heart girth, height at withers, and thoracic circumference compared to those of the control group. Dietary treatments of EFE and EFE+WH increased ($P < 0.01$) the paunch girth compared to the control group. Scrotal and tail measurements were similar for all the groups of sheep.

4. DISCUSSION

4.1. Bioactive components of WH

The experimental WH consisted of highly bioactive components mostly as monoterpene hydrocarbons predominantly Chrysanthenyl acetate and L-β-Pinene in almost two equal proportions. Similar to our findings, Wani et al. (2014) used the same source of WH and found that the major phytochemical constituents of WH were Chrysanthenyl acetate (49.15%) and L-β-pinene (39.62%) which exhibited a very promising antioxidant profile. The other bioactive constituents normally present in WH includes essential oils, sesquiterpene lactones, phenolic acids, flavonoids, glycosides, carotenoids, coumarins, homo-diterpen peroxides, thiophene, tannins, and lignans (Anibogwu et al., 2021).

4.2. Immuno modulatory response

Ameliorating the immune status of animals is an effective strategy to alleviate the menace of infectious diseases that cause huge losses to animal husbandry and pose serious health risk to consumers. The immune system in an animal body can generally benefit from the nutraceuticals which possess immuno-stimulatory properties (Valenzuela-Grijalva et al., 2017). In the current study, the C-RBC

Table 7. Live slaughter weight and linear body measurements of sheep fed total mixed ration (TMR) added with exogenous fibrolytic enzymes (EFE) or wormwood herb (WH) individually and in-combination (EFE + WH)

Attributes	Dietary treatments‡				SEM	*P*-value
	Control	EFE	WH	EFE+WH		
Live slaughter weight (kg)	19.1[b]	22.5[ab]	23.1[ab]	24.4[a]	0.72	0.026
Linear body measurements (cm)						
Body length	47.6	49.4	50.4	51.5	0.79	0.391
Height at withers	52.8[b]	55.3[ab]	56.6[ab]	59.2[a]	0.86	0.042
Heart girth	67.1[b]	73.3[ab]	74.9[ab]	80.5[a]	1.42	<0.001
Chest depth	22.0	24.3	25.1	25.5	0.54	0.083
Chest width	13.7	14.1	14.8	15.3	0.31	0.314
Forearm circumference	10.9	11.4	11.8	12.4	0.26	0.236
Thoracic circumference	73.1[b]	76.2[ab]	78.3[ab]	80.1[a]	1.00	0.049
Rump height	56.1	57.7	58.2	59.4	0.65	0.365
Paunch girth	91.2[b]	99.6[a]	96.1[ab]	101[a]	1.22	0.005
Pelvic width	12.2	12.8	13.2	14.0	0.31	0.241
Hind leg length	24.0	26.6	27.8	28.5	0.81	0.228
Buttock width	16.6	17.5	18.1	18.6	0.38	0.294
Posterior buttock circumference	41.9	43.8	44.4	46.2	0.79	0.322
Scrotal measurements (cm)						
Scrotal length	11.4	11.9	12.2	12.8	0.26	0.312
Scrotal circumference	23.5	25.0	25.9	26.9	0.63	0.274
Tail measurements (cm)						
Tail length	15.5	16.5	16.6	17.7	0.43	0.379
Tail width	4.97	6.05	5.22	5.92	0.23	0.280
Tail thickness	1.57	1.90	1.67	1.87	0.06	0.202
Tail circumference	8.75	10.7	10.2	10.8	0.35	0.125

‡Control: 0% addition; EFE: TMR added with EFE at 6.0 g/kg DM; WH: TMR added with WH at 45.0 g/kg DM; EFE + WH: TMR added with 6.0 EFE + 45.0 WH g/kg DM, respectively.

[a,b] The means within rows with different superscripts differ significantly among the treatments.

SEM= standard error of means.

haemagglutination titers were influenced by the dietary treatments with time. The similar values of initial immunization titers observed under the current study were likely due to the animals not having been exposed to the antigen prior to that time. After day 7 post-immunization of the experiment, sheep fed WH incorporated diets (WH and EFE+WH) showed higher titers, possibly due to more antibody production by plasma cells in response to antigen by integrated functions of B lymphocytes, helper T lymphocytes, and macrophages. Likewise, the increased SFT of sheep fed WH diets might be the result of increased CMI evaluated in terms of DTH skin response test to 2-4, DNCB which measures the cutaneous swelling mediated by cells of T lymphocyte lineage (natural killer cells and cytotoxic T lymphocytes along with macrophages and their products like lymphokines) and fluid infiltration after percutaneous application of the chemical.

The monoterpenes detected in the experimental WH (Chrysanthenyl acetate and L- β- Pinene) possess significant antioxidant (Canadanovic-Brunet et al., 2005) and anti-inflammatory (Bora &

Sharma, 2011) activities which are related to the suppression of prostaglandin, tumor necrosis factor-alpha and other interleukins synthesis as immune-inhibitory substances, thus resulting in better immune responses. Moreover, the literature reports that WH contains potential immune-promoting agents, which are speculated to be the constituent phytochemicals like tannins (Schreurs et al., 2010), polysaccharides as type-II arabinogalactan (Danilets et al., 2010), and essential oils (Siveen & Kuttan, 2011).

Nutrition modulates the immune functions in animal system through the quality and amount of nutrient intakes (Bobeck, 2020). Improved apparent total tract nutrient digestibility, and greater intake of digestible nutrients with the inclusion of WH in sheep diets, as reported in our previous study (Beigh et al., 2022), indicate enhanced ruminal microbial protein (RMP) synthesis. RMP serves as a major source of high-quality protein entering the animal's small intestine, supplying about 60-85% of the amino acids required for various metabolic functions (Hackmann & Firkins, 2015). This improved microbial protein synthesis might also have contributed to the enhanced immune response observed in the present study. Enhancement of metabolizable proteins supply through improved efficiency of RMP production with increase in nutrient intake elevates the animal health and performance (ARC, 1984). Quantitative improvement of RMP synthesis has been reported to amend the feed conversion efficiency, energy metabolism, nutritional status, immunity, stress tolerance and production in ruminant livestock (Harun & Sali, 2019).

4.3. *In vivo* carcass characteristics

Relationships between USG measurements and body composition and carcass traits in sheep have already been documented (Salazar-Cuytun et al., 2023). In the present study, the individual inclusion of feed additives in the diets did not significantly influence the *in vivo* carcass characteristics determined by USG measurements. The probable reason for this finding may be that the experimental dose of EFE was lower compared to those reported earlier (Gomez-Vazquez et al., 2011); however, the marginal effect may be attributed to increase in energy availability to the animals by EFE inclusion in the diet which has a positive effect on carcass traits. Consistent with our results, many studies reported that carcass characteristics were not affected by commercial fibrolytic enzymes added to the diet of steers (Eun et al., 2009; Vargas et al., 2013) or lambs (Ahmed, 2016). Likewise, the marginal effects of WH added individually in WH diet on carcass composition might be due to some constituent functional ingredients (phytochemicals) such as flavonoids and other antioxidants in WH that would have supported favorable rumen fermentation and richer carcass composition. These results support the earlier findings that carcass characteristics were not altered by inclusion of dried wormwood at 3 and 5% level in the diet of Hanwoo steers (Kim et al., 2002a, 2002b); however, the tendency for higher RE muscle dimensions, and lower back and lumbar fat thicknesses in the present study was in concurrence agreement with findings of Kim et al. (2012) in fattening steers fed diets with WH silage substituting rice straw at 50, 100 or 150 g/kg DM. Dietary inclusion of the two feed additives in-combination resulted in associative enhancement of RE muscle area and rib flank streaking compared to control suggesting the synergistic effects of the EFE + WH in favourable alteration of certain carcass traits in sheep. Significant improvement of apparent total tract nutrient (fibre and ether extract) digestibility by dietary incorporation of the two feed additives in-combination (Beigh et al., 2022) would have increased energy availability from the diet (EFE + WH), and therefore led to better carcass characteristics in sheep of the respective group.

Body biometric measurements (BM) have been used since long time as an indicator to predict carcass composition in animal (Cominotte et al., 2023), and are influenced by various factors among which nutrition is the main governing one (MacGhee et al., 2017). In the current study, dietary inclusion of EFE as well as WH individually did not result in any significant changes in most of the recorded BM. PG was higher in sheep fed EFE incorporated diets (EFE and EFE+WH) probably due to more omental fat, the fact being supported by numerically higher values of fat thicknesses measured at different sites by USG. Hyperlipidemic effect of dietary exogenous enzyme additions has been reported by Hajati et al. (2009), while herbs or their active component(s) may beneficially modify fat metabolism in the body by inhibiting the hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, cholesterol 7α-hydroxylase, and/or fatty acid synthetase activity, which

are the key regulatory enzymes in endogenous lipid synthesis (Lee et al., 2004). The significant effect of the two feed additives added in-combination on few body measurements (HW, TC, HG and PG) might be the result of their synergistic action.

5. CONCLUSIONS

Inclusion of EFE at 6.0 g/kg DM or WH at 45.0 g/kg DM individually in diet of sheep did not affect carcass composition characteristics; however, when the two additives were incorporated in-combination showed promising synergistic effects on the measured parameters. The dietary addition of WH either individually or with EFE improved humoral as well as cell-mediated immune responses with synergism between them. Therefore, the feeding of non-chemical feed additives should preferably be recommended as a sustainable strategy in sheep farming during the most critical periods of the animal life that may attenuate the immunity (e.g. peripartum, onset of lactation, or post-weaning).

Supplementary information ↑

Funding sources

Government of Jammu & Kashmir through the Rashtriya Krishi Vikas Yojana scheme. Project / Grant: SK-RKVY-AN-1.

Supplementary material

Not applicable.

Data availability

Data will be available on request.

Acknowledgements

The authors are grateful to Professor and Head, Division of Veterinary Microbiology and Immunology, and Division of Veterinary Surgery and Radiology, FVSc & AH, Shuhama, SKUAST-Kashmir for providing all the laboratory facilities.

Authorship contribution statement

Yasir A. Beigh: Data curation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing.

Abdul M. Ganai: Conceptualization, Data curation, Investigation, Project administration, Supervision, Validation, Writing – review & editing.

Haidar A. Ahmad: Investigation, Supervision, Validation.

Majid Shafi: Investigation, Software.

Competing interests

The authors have declared that no competing interests exist.

Statement on the use of Artificial Intelligence

Not applicable.

Ethical approval: The study was approved by the Institutional Animal Research and Ethical Committee vide no. AU/FVS/Acad/EC-PG/PF/4814-16 in compliance to the guidelines on the care and use of animals for scientific purposes (NACLAR, 2004).

REFERENCES

Ahmed KD, 2016. Effect of fibrolytic enzymes on serum testosterone level and some of carcass traits in Turkish Awassi male lambs. Indian J Anim Res 50(6): 919-921. https://doi.org/ 10.18805/ijar.11475

Akdag F, Teke B, Meral Y, Arslan S, Ugurlu M, 2015. Prediction of carcass composition by ultrasonic measurement and the effect of region and age on ultrasonic measurements. Small Rumin Res 133: 82-87. https://doi.org/10.1016/j.smallrumres.2015.09.011

Anibogwu R, Jesus KD, Pradhan S, Pashikanti S, Mateen S, Sharma K, 2021. Extraction, isolation and characterization of bioactive compounds from Artemisia and their biological significance: a review. Molecules 26(22): 6995. https://doi.org/10.3390/molecules26226995

Anil, Dutta TK, Chatterjee A, Yadav SK, Mandal DK, Mohammad A, 2023. Effect of exogenous fibrolytic enzymes supplementation to improve voluntary intake, availability of nutrients and growth performance in weaned crossbred calves. Indian J Anim Sci 93(9): 896–902. https://doi.org/10.56093/ijans.v93i9.131419

ARC, 1984. The nutrient requirements of ruminant livestock, Supplement No 1, Agricultural Research Council, Commonwealth Agricultural Bureaus, Farnham Royal, Slough, Berkshire, UK.

Arsenos G, Giannenas I, 2023. Novel ways for animal production. In: Sustainable use of feed additives in livestock. Springer Cham. https://doi.org/10.1007/978-3-031-42855-5.

Atti N, Hamouda MB, 2004. Relationships among carcass composition and tail measurements in fat-tailed Barbarine sheep. Small Rumin Res 53: 151-155. https://doi.org/10.1016/j.smallrumres.2003.08.016

Batiha GES, Olatunde A, El-Mleeh A, Hetta HF, Al-Rejaie S, Alghamdi S, Zahoor M, Beshbishy AM, Murata T, Bastida AZ, Rivero-Perez N, 2020. Bioactive compounds, pharmacological actions, and pharmacokinetics of wormwood (*Artemisia absinthium*). Antibiotics 9: 353. https://doi.org/10.3390/antibiotics9060353

Beigh YA, Ganai AM, Ahmad HA, 2024. *In vitro* fermentation of total mixed ration containing graded levels of Wormwood (*Artemisia absinthium* Linn.) herb. Anim Nutr Feed Techn 24(1): 203-213.

Beigh YA, Ganai AM, Ahmad HA, Farooq J, Sheikh GG, Reshi PA, Haq Z, 2023. Effect of exogenous fibrolytic enzymes cocktail incorporation on *in vitro* degradation and fermentation characteristics of oats straw based total mixed ration. Indian J Anim Sci 93(10): 993-999.

Beigh YA, Ganai AM, Sofi AH, Haq Z, 2022. Effect of feeding wormwood herb (*Artemisia absinthium* Linn.) added total mixed ration on wool production and quality in sheep. J Nat Fibers 19(14): 9741–9753. https://doi.org/10.1080/15440478.2021.1993404

Bobeck EA, 2020. Nutrition and health: companion animal applications: Functional nutrition in livestock and companion animals to modulate the immune response. J Anim Sci 98(3): 1-8. https://doi.org/10.1093/jas/skaa035

Bora KS, Sharma A, 2011. Evaluation of antioxidant and free-radical scavenging potential of *Artemisia absinthium*. Pharm. Biol 49(12): 1216-1223. https://doi.org/10.3109/13880209.2011.578142

Canadanovic-Brunet JM, Dilas SM, Cetkovic G, Tumbas VT, 2005. Free-radical scavenging activity of wormwood (*Artemisia absinthium* L.) extracts. J Sci Food Agric 85: 265-272. https://doi.org/10.1002/jsfa.1950

Cominotte A, Fernandes A, Dorea J, Rosa G, Torres R, Pereira G, Baldassini W, Neto OM, 2023. Use of biometric images to predict body weight and hot carcass weight of Nellore cattle. Animals 13(10): 1679. https://doi.org/10.3390/ani13101679

Danilets MG, Belskii-Iu P, Gurev AM, Belousov MV, Bel'skaia NV, Trofimova ES, Uchasova EG, Alhmedzhanov RR, Ligacheva AA, Iusbov MS, Agefonov VI, 2010. Effect of plant polysaccharides on TH1-dependent immune response: screening investigation. Eksp Klin Farmakol 73: 19-22

Eun JS, ZoBell DR, Dschaak CM, Diaz DE, Tricarico JM, 2009. Effects of supplementing a fibrolytic feed enzyme on the growth performance and carcass characteristics of beef steers. Prof Anim Sci 25(3): 382-387. https://doi.org/10.15232/S1080-7446(15)30729-4

Fernandes HJ, Tedeschi LO, Paulino MF, Paiva LM, 2010. Determination of carcass and body fat compositions of grazing crossbred bulls using body measurements. J Anim Sci 88(4): 1442-1453. https://doi.org/10.2527/jas.2009-1919

Gomez-Vazquez A, Mendoza GD, Aranda E, Perez J, Hernandez A, Pinos-Rodriguez JM, 2011. Influence of fibrolytic enzymes on growth performance and digestion in steers grazing stargrass and supplemented with fermented sugarcane. J Appl Anim Res 39(1): 77-79. https://doi.org/10.1080/09712119.2011.558670

Hackmann TJ, Firkins JL, 2015. Maximizing efficiency of rumen microbial protein production. Front Microbiol 6: 465. https://doi.org/10.3389/fmicb.2015.00465

Hajati H, Rezaei M, Sayyahzadeh H, 2009. The effects of enzyme supplementation on performance, carcass characteristics and some blood parameters of broilers fed on corn-soybean meal-wheat diets. Int J Poult Sci 8(12): 1199-1205. https://doi.org/10.3923/ijps.2009.1199.1205

Harun AY, Sali K, 2019. Factors affecting rumen microbial protein synthesis- a review. Vet. Med. Open J 4(1): 27-35. https://doi.org/10.17140/VMOJ-4-133

Hudson L, Hay FC, 1991. Practical Immunology, 3rd ed. Blackwell Scientific Publications, London.

ICAR, 2013. Nutrient requirements of Sheep, Goat and Rabbit. Indian Council of Agricultural Research, New Delhi, India.

Kholif AE, Patra AK, 2024. Dietary Applications of Exogenous Enzymes to Improve Nutrient Utilization and Performance in Ruminants. In: Mahesh, M.S., Yata, V.K. (eds) Feed Additives and Supplements for Ruminants. Springer, Singapore. https://doi.org/10.1007/978-981-97-0794-2_1

Kim JH, Kim CH, Ko YD, 2002a. Influence of dietary addition of dried wormwood (Artmisia sp.) on the performance and carcass characteristics of Hanwoo steers and the nutrient digestibility of sheep. Asian-Australas J Anim Sci 15(3): 390-395. https://doi.org/10.5713/ajas.2002.390

Kim SC, Adesogan AT, Shin JH, 2012. Effect of dietary addition of wormwood (*Artemisia Montana* Pampan) silage on growth performance, carcass characteristics and muscle fatty acid profiles of beef cattle. Anim Feed Sci Technol 177(1-2): 15-22. https://doi.org/10.1016/j.anifeedsci.2012.06.011

Kim YM, Kim JH, Kim SC, Ha HM, Ko YD, Kim CH, 2002b. Influence of dietary addition of dried wormwood (Artmisia sp.) on the performance, carcass characteristics and fatty acid composition of muscle tissues of Hanwoo heifers. Asian-Australas J Anim Sci 15(4): 549-554. https://doi.org/10.5713/ajas.2002.549

Lakhani N, Kamra DN, Lakhani P, Alhussien MN, 2019. Immune status and haemato-biochemical profile of buffalo calves supplemented with phytogenic feed additives rich in tannins, saponins and essential oils. Trop Anim Health Prod 51: 565-573. https://doi.org/10.1007/s11250-018-1727-z

Lambo MT, Ma H, Liu R, Dai B, Zhang Y, Li Y, 2024. Mechanism, effectiveness, and the prospects of medicinal plants and their bioactive compounds in lowering ruminants' enteric methane emission: a review. Animal 18(4): 101134. https://doi.org/10.1016/j.animal.2024.101134

Lee KW, Everts H, Beynen AC, 2004. Essential oils in broiler nutrition. Int J Poult Sci 3: 738-752. https://doi.org/10.3923/ijps.2004.738.752

Lee SS, Kim DH, Paradhipta DHV, Lee HJ, Yoon H, Joo YH, Adesogan AT, Kim SC, 2020. Effects of Wormwood (*Artemisia montana*) essential oils on digestibility, fermentation indices, and microbial diversity in the rumen. Microorganisms 8: 1605. https://doi.org/10.3390/microorganisms8101605

MacGhee ME, Bradley JS, McCoski SR, Reeg AM, Ealy AD, Johnson SE, 2017. Plane of nutrition affects growth rate, organ size and skeletal muscle satellite cell activity in newborn calves. J Anim Physiol Anim Nutr 101(3): 475-483. https://doi.org/10.1111/jpn.12568

Mahmood S, Kumar A, Singh R, Sarkar M, Singh G, Verma MR, Kumar GVPPSR, 2018. Scrotal circumference: a predictor of testosterone concentration and certain attributes of seminal vesicles influencing buffalo male fertility. Vet World 11(6): 739-747. https://doi.org/10.14202/vetworld.2018.739-747

Martinez OA, Lucey P, Acetoze G, Rossow HA, 2023. Effects of feeding a fibrolytic enzyme to Holstein dairy cows on milk production and reproduction. Appl Anim Sci 39: 306-316. https://doi.org/10.15232/aas.2023-02386

Mohammed HA, 2022. Phytochemical analysis, antioxidant potential, and cytotoxicity evaluation of traditionally used *Artemisia absinthium* L. (Wormwood) growing in the Central Region of Saudi Arabia. Plants 11: 1028. https://doi.org/10.3390/plants11081028

NACLAR, 2004. Guidelines on the care and use of animals for scientific purposes. National Advisory Committee for Laboratory Animal Research.

Pliego AB, Tavakoli M, Khusro A, Seidavi A, Elghandour MMMY, Salem AZM, Marquez-Molina O, Rivas-Caceres RR, 2020. Beneficial and adverse effects of medicinal plants as feed supplements in poultry nutrition: a review. Anim Biotechnol, https://doi.org/10.1080/10495398.2020.1798973

Ramdani D, Yuniarti E, Jayanegara A, Chaudhry AS, 2023. Roles of essential oils, polyphenols, and saponins of medicinal plants as natural additives and anthelmintics in ruminant diets: a systematic review. Animals 13: 767. https://doi.org/10.3390/ani13040767

Roque BM, Reyes GC, Tewoldebrhan TA, Apphuamy JADRN, Lee JJ, Seo S, Kebreab E, 2019. Exogenous β-mannanase supplementation improved immunological and metabolic responses in lactating dairy cows. J Dairy Sci 102: 4198-4204. https://doi.org/10.3168/jds.2018-15568

Salazar-Cuytun R, Herrera-Camacho J, García-Herrera RA, Pozo-Leyva D, Gurgel ALC, Ítavo LCV, Dos Santos GT, Chay-Canul AJ, 2023. Relationships between body mass index, ultrasound measurements and, internal body fat depots in Pelibuey sheep. Biosci J 39: e39062.

SAS, 2009. Statistical Analysis System, version 9.2. SAS Institute, Cary, NC, USA.

Schreurs NM, Pernthaner A, Hein WR, Barry TN, 2010. Condensed tannins for priming innate immunity. Proc N Z Soc Anim Prod 70: 288-290.

Siveen KS, Kuttan G, 2011. Augmentation of humoral and cell mediated immune responses by Thujone. Int. Immunopharmacol 11(12): 1967-1975. https://doi.org/10.1016/j.intimp.2011.08.006

SPSS, 2011. Statistical Package for Social Sciences, version 20.0. Software products, Marketing Department, IBM SPSS Inc., New York, USA

Teixeira A, Matos S, Rodrigues S, Delfa R, Cadavez V, 2006. *In vivo* estimation of lamb carcass composition by real-time ultrasonography. Meat Sci 74(2): 289-295. https://doi.org/10.1016/j.meatsci.2006.03.023

Valdes KI, Salem AZM, Lopez S, Alonso MU, Rivero N, Elghandour MMY, Domínguez IA, Ronquillo MG, Kholif AE, 2015. Influence of exogenous enzymes in presence of *Salix babylonica* extract on digestibility, microbial protein synthesis and performance of lambs fed maize silage. J Agric Sci 153(4): 732-742. https://doi.org/10.1017/S0021859614000975

Valenzuela-Grijalva NV, Pinelli-Saavedra A, Muhlia-Almazan A, Dominguez-Diaz D, Gonzalez-Rios H, 2017. Dietary inclusion effects of phytochemicals as growth promoters in animal production. J Anim Sci Technol 59: 8. https://doi.org/10.1186/s40781-017-0133-9

Vargas JM, Mendoza GD, Rubio-Lozano, De la Salud M, Castrejon FA, 2013. Effect of exogenous fibrolytic enzymes on the carcass characteristics and performance of grain-finished steers. Anim Nutr Feed Technol 13: 435-439.

Verma S, Thakur A, Katoch S, Chander S, Wani AH, Kumar S, Dohroo S, Singh G, Sharma M, 2017. Differences in innate and adaptive immune response traits of Pahari (Indian non-descript indigenous breed) and Jersey crossbred cattle. Vet Immunol Immunopathol 192: 20-27. https://doi.org/10.1016/j.vetimm.2017.09.003

Wani H, Shah SA, Banday JA, 2014. Chemical composition and antioxidant activity of the leaf essential oil of *Artemisia absinthium* growing wild in Kashmir, India. J Phytopharmacol 3: 90-94.