

# Occurrence and protective determinants of ruminal lesions in slaughtered beef cattle: Rumen pH and papilla morphology

## Occurrencia y determinantes protectores de lesiones ruminales en ganado de carne: pH ruminal y la morfología papilas

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### ABSTRACT

Metabolic imbalances and mechanical trauma induced ruminal lesions are major economic and welfare concerns for the cattle industry. The aims were to characterize rumen fermentation and consider feeding duration, presence of foreign bodies, and fermentation products in relation to lesion formation. The aim of this study was to assess the association between morphological risk factors related to nutrition and reticula-rumen lesions as well as foreign bodies by post-mortem examination in 85 slaughtered cattle, with the resulting data analyzed through multiple logistic regression models. Rumen pH was the only variable with one of the lowest likelihood values for lesions (OR = 0.024; P < 0.001). More specifically, when the rumen pH increased by one unit, the likelihood of lesions decreased by roughly 97.6 %. Protection was also significantly associated with rumen acetic acid concentration (OR = 0.95; P < 0.05). On the other hand, a decrease of 1-unit in acetic acid concentration resulted in an odd ratio approximately 1.053-fold higher for the development of lesion. Also, foreign bodies (14.12 % of cattle were positive) were affected by papilla width in the negative direction coefficient (OR = 0.118; P < 0.05). Decreasing the papilla width by 1 cm increased the chance of identifying a foreign body 8.475-fold more than to overrule one. The presence of lesions and macroscopic ventral sac deformations also showed a strong positive correlation. The results of this study emphasize the necessity for a stable rumen pH and maintenance of epithelial integrity, especially papilla width which may play a major role in reducing ruminal health hazards in cattle.

**Key words:** Cattle; fattening; fermentation; foreign bodies; rumen lesions.

### RESUMEN

Las lesiones ruminales derivadas de agentes estresores tanto metabólicos como mecánicos representan un desafío significativo para la economía y el bienestar en la industria ganadera. El objetivo consistió en definir las características de la fermentación ruminal y evaluar factores como la duración del engorde, la presencia de cuerpos extraños y los productos de fermentación en la formación de lesiones. Este estudio investigó y cuantificó los efectos de los factores de riesgo nutricionales y morfológicos sobre las lesiones del retículo-rumen y la presencia de cuerpos extraños en 85 reses sacrificadas, utilizando exámenes post-mortem y análisis de regresión logística múltiple. El pH del rumen fue la única variable con uno de los valores de probabilidad más bajos de lesiones (OR = 0,024; P < 0.001). Más específicamente, cuando el pH del rumen aumentó en una unidad, la probabilidad de lesiones disminuyó aproximadamente en un 97,6 %. La concentración de ácido acético ruminal también mostró un efecto protector significativo (OR = 0,95; P < 0,05). Por el contrario, una disminución de una unidad en la concentración de ácido acético se asoció con un aumento de 1,053 veces en el riesgo de lesiones. Además, los cuerpos extraños (detectados en el 14,12 % del ganado) fueron mitigados significativamente por el ancho de la papila (OR = 0,118; P < 0,05). La disminución de un solo cm del ancho de la papila desencadenó un aumento de la probabilidad de encontrar los cuerpos extraños 8,475 veces y. Además, se observaron fuertes correlaciones positivas entre los cuerpos extraños y las lesiones y deformaciones del saco ventral. Los hallazgos de este estudio establecen que mantener un pH ruminal óptimo y fortalecer la integridad epitelial, especialmente el ancho de la papila, son de importancia crítica para minimizar los riesgos de salud ruminal en el ganado vacuno.

**Palabras clave:** Ganado vacuno; engorde; fermentación; cuerpos extraños; lesiones ruminales.

## INTRODUCTION

The economic sustainability and animal welfare in beef cattle (*Bos taurus*) production are related to the maintenance of optimum rumen health [1]. Rumen serves as an essential fermentation vat in which specific anaerobic microorganism produces volatile fatty acids (VFA) responsible for about 75 % of the animal's metabolic energy [2]. The rumen must maintain a certain stability (38-42 oC, pH in the range of 6-7) and this is achieved through bicarbonate and phosphate buffer systems provided by saliva [3]. This physiological stability is based on the healthy structure of rumen papillae that serves for enhancing VFA absorption and maintaining epithelial integrity [4].

Ruminal disorders are a major costly problem in ruminant production systems, resulting in decreased productivity with high morbidity and mortality [1; 5]. The development of rumen diseases is mainly due to two primary etiological pathways: metabolic disorders, such as acidosis caused by irregular diets and mechanical injuries due to ingesting foreign bodies (FBs) [5]. Metabolic stress is induced by the overconsumption of a highly fermentable concentrate that leads to high levels of accumulated lactic acid and ruminal pH decreasing below the critical level of 5.0 [6]. This marked acidosis leads to irritation, edema and ulceration of the rumen mucosa with resulting higher incidence of lesions. Meanwhile, mechanical factors were often with foreign body syndrome leading to traumatic damage to the rumen and reticulum walls [7]. Penetrating foreign objects may cause severe, even fatal complications such as peritonitis and septicemia [8; 9].

For monitoring and diagnosing of these widespread gastrointestinal pathologies, abattoir monitoring is considered a valuable and cost-effective tool [10]. Although the clinical and macroscopic aspects of lesions in adult cattle have been described extensively in previous literature [11, 12], publications using statistical measures as odds ratios to disengage the interaction between morphological and metabolic factors are scarce. In particular, the simultaneous function of rumen and papilla structure to limit both metabolic and mechanical injury risks is poorly understood in the literature at present [11, 12]. The main aim of this research was to investigate risk and protective factors for development of rumen lesions and the presence of FBs in beef cattle at slaughter, based on quantitative analysis by separate logistic regression modelling.

## MATERIAL AND METHODS

### Animal material

This study was carried out at the Van Lake Slaughterhouse in Van Province, Türkiye, in 2025. The animal material involved 85 beef cattle selected randomly based on different feeding and fattening conditions at the day of slaughter. Owner interview and consultation of official cattle, data on the animals such as breed, age, fattening period, and feeding characteristics were recorded through direct questionnaires to owners and by inspection of official bovine passports delivered by the responsible Veterinarian. The procedure was supervised by recording the sequence of slaughter according to ear tags.

### Postmortem examination and sampling

The postmortem examination process commenced immediately after the slaughter of the animals. Following the opening abdominal cavity, the forestomachs were promptly removed by the slaughterhouse personnel. The omasum and abomasum were separated from the rumen and reticulum. A longitudinal incision was made along the rumen wall to collect rumen fluid. The rumen pH value was determined *in situ* using a pH-meter (Orion™ Pro Star, pH 211 Thermo Scientific) immediately after collecting each animal. Rumen fluid was strained through a four-layer cheesecloth.

For the analysis of VFA, 50 mL strained rumen fluid was acidified with 5 mL of HCl solution and aliquoted into 10 mL falcon tubes. For ammonia nitrogen concentration analysis, 10 mL of rumen fluid was collected separately. All samples were centrifuged at 2000 g for 20 min (Sigma 3-30 K refrigerated high speed centrifuge), and the supernatant was stored at -18 oC (Ultra-low temperature freezer- New Brunswick Scientific) until VFA and NH<sub>3</sub>-N analyses were performed [13].

Before emptying the rumen and reticulum, the contents were meticulously checked for the presence of FBs. Detected FBs (nylon rope, plastic bags, nails, wire pieces, etc.) were collected and classified. Access to the reticulum was gained through the rumen, allowing the structure to be inverted and thoroughly scrutinized for the presence of embedded or free FBs. Lesions were macroscopically evaluated and scored using the criteria established by Johnson *et al.* [10] and Luna-Mendez *et al.* [12] following rapid washing.

### Chemical and morphological analyses

Frozen VFA samples were thawed at room temperature and centrifuged at 12000 g for 10 min. The supernatant was analyzed using high performance liquid chromatography (Shimadzu HPLC Systems). The HPLC conditions were set as follows: 300x7.8 mm HPX-87H column, 0.015 N H<sub>2</sub>SO<sub>4</sub> mobile phase, flow rate of 0.7 mL/min, column temperature of 45 oC, PDA-DAD detector, and an injection volume of 50µL [14]. Ammonia nitrogen concentration was determined according to the distillation method (Buchi steam distillation dist-line) developed by Markham [15].

The photographs (Nikon 105 mm F/2.8 Nano Macro G) for papillae size and area measurements were taken of the ventral right and left sac sections. Papillae morphology (size, color, and shape) was initially evaluated subjectively. Subsequently, the photographs were scaled in the ImageJ software to objectively determine papilla length, width, number and area.

### Statistical analysis

The study utilized data collected from a total of 85 fattening cattle. Statistical analyses were performed using the SAS 9.4 statistical package program [16]. Rumen lesion formation and FBs presence were treated as categorical (binary; presence/absence) dependent variable. Multiple logistic regression analysis was employed to determine the effects of potential risk factors on the occurrence of rumen lesions and FBs presence.

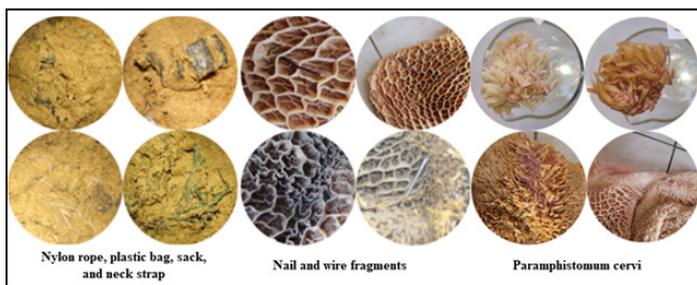
To quantify the association between the risk factors and ruminal outcomes, the statistical analysis derived odds ratios (OR) alongside their corresponding 95 % confidence intervals.

## RESULTS AND DISCUSSION

### Occurrence of foreign bodies and parasites

The investigation revealed that 14.12 % of cattle harbored FBs, showing a distinct anatomical distribution according to the nature of the material. This occurrence rate was significantly lower than those reported in previous studies 73.4 % in İskenderun [17] and 22.27 % in Diyarbakır [18]. This reduced variation may be due to a younger age of the presented cattle for slaughter and stricter fattening management. In addition, the use of magnets as a prophylactic measure for penetrating objects syndromes probably means that the hazardous material load in these subjects is lower. While non-penetrating items, such as nylon ropes and plastic bags, were typically recovered from the rumen, the reticulum predominantly contained hazardous metallic fragments like wires and nails. FBs were classified into two main categories blunt objects (e.g. nylon rope, plastic bags, sacks) found predominantly in the rumen, which can lead to impaction, erosion and hyperplasia [8] and penetrating objects (nails and wire pieces) mostly localized in the reticulum.

These penetrations cause severe, potentially fatal complications including traumatic reticuloperitonitis, pericarditis and hepatitis [9; 18]. In addition to these mechanical stressors, the study identified ruminal parakeratosis in 11 subjects and the presence of the trematode *Paramphistomum cervi* in 5 cattle. These parasites were characterized by their specific localization, either adhering directly to or nestled between the ruminal papillae (FIG. 1).



**FIGURE 1.** Macroscopic visualization of foreign objects and the trematode parasite *Paramphistomum cervi* within the rumen and reticulum of cattle.

The trematode parasite *Paramphistomum cervi* was identified in 5 cattle examined in the study. The parasites were observed on the rumen mucosal surface, located between or adhering to the papillae, and were associated with localized necrosis and epithelial shedding. *P. cervi* known as a tissue-feeding parasite that sustains itself by consuming epithelial cells [19]. The presence of adult *Paramphistomes* in the rumen and reticulum causes ruminitis, irregular rumination, reduced nutrient conversion, and significant economic losses due to compromised efficiency and body condition loss [19; 20]. Supporting the

observed pathology, previous experimental research on *P. cervi* infected goats noted remarkable tissue changes, including epithelial shedding from villus papillae and infiltration in the supporting connective tissue of the rumen 80 days post-infection [21]. A study conducted in Sivas region determined that the prevalence rate of *Paramphistomum* spp. was higher than in the cattle over 3 years of age (12.1 %) compared to young cattle (2.2 %) [20].

### Factors influencing rumen lesions' presence

The results of the multiple logistic regression analysis to explain lesion presence concluded that fermentation parameters such as rumen pH and acetic acid (AA), had a statistically significant protective effect against lesions (TABLE I). The rumen pH was identified as the strongest statistical protective factor against lesion presence (OR = 0.024; P < 0.001). Ruminal pH had a significant negative effect on the risk lesion development. Each unit increase in ruminal pH decreased the likelihood of developing lesions by 97.6 %

The statistical solidity of this finding is also corroborated by the small 95 confidence interval (0.002636-0.130261), which at all times does not encompass the threshold value unity, thus confirming how much the evidence produced about identified protective effect can be classified as reliable. Maintaining pH within the optimal physiological range (typically between 6 and 7) is essential cellulolysis, proteolysis, and deamination [22].

This stability is regulated by endogenous buffering mechanisms, including salivary flow, and the efficient absorption of VFA [23]. Low pH environments, particularly those resulting from the high intake of rapidly fermentable carbohydrates, trigger rapid microbial shifts, leading to increased production of lactic acid by microorganisms like *Streptococcus bovis*.

Lactic acid is ten times stronger than acetic, propionic and butyric acid [24]. When pH drops below 5.0 the resulting caustic environment induces mucosal irritation, edema and erosion, thereby dramatically increasing the risk of lesions [5]. This reaffirms that proper feeding management that utilizes roughage with adequate physically effective NDF is critical for stimulating rumination and preserving pH [25].

Rumen AA concentration also exhibited a statistically significant protective effect against lesion formation (OR = 0.95; P < 0.05). This means a one-unit increase in concentration reduced the lesion occurrence probability by approximately. Conversely, a one-unit decrease in concentration led to an increase in the likelihood of lesion formation ( $1/0,95 = 1,053$ ). Other variables tested, including propionic acid (PA), butyric acid (BA), and ammonia nitrogen (NH<sub>3</sub>-N) did not show a statistically significant effect on lesion occurrence (P > 0,05), although demonstrated a near significant trend (OR = 1.06; 0.0535).

Acetic acid is the major product of roughage fermentation, and its presence typically indicates a healthier, more stable ruminal environment [2]. High concentrations of AA are instrumental in supporting epithelial integrity and energy metabolism, primarily because AA is substantially less acidic than lactic acid, allowing

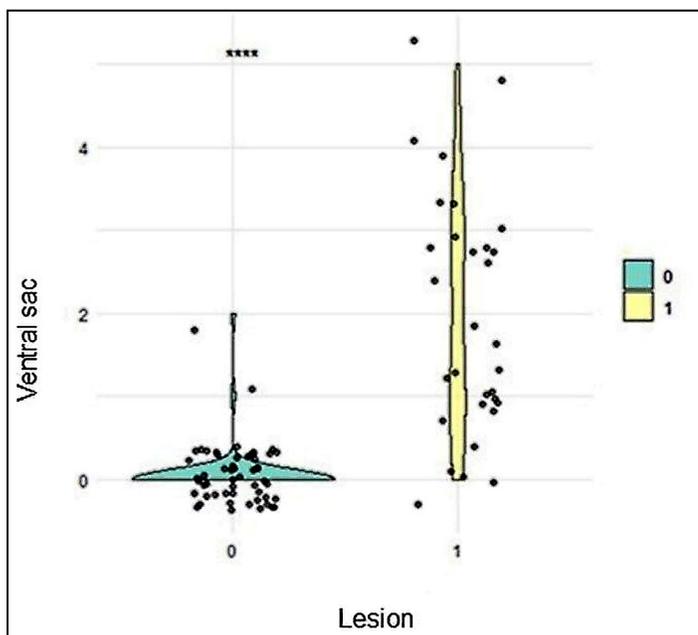
for the effective preservation of the rumen's buffering capacity [24].

This finding is consistent with literature suggesting that VFA's are crucial for epithelial development, are that strategies that promote high AA production may mitigate the negative effects of metabolic stress and pH fluctuations [2].

Independent variables	Coefficient (β)	Standard Error	Odds Ratio (OR)	% 95 Confidence Interval (OR)	P value
AA	-0.052973	0.0261	0.95	0.897839 - 0.996127	0.0421*
PA	-0.005182	0.0798	0.99	0.856375 - 1.176586	0.9482
BA	-0.171601	0.1398	0.84	0.632069 - 1.102143	0.2196
NH <sub>3</sub> -N	0.005897	0.0031	1.06	1.000065 - 1.012338	0.0535
pH	-3.717477	0.9809	0.024	0.002636 - 0.130261	0.000151***

AA: Acetic acid; PA: Propionic acid; BA: Butyric acid; NH<sub>3</sub>-N: Ammonia nitrogen. \*P < 0.05; \*\*\*P < 0.0001.

In FIG. 2, macroscopic descriptions of the ruminal ventral sac were compared for lesion absent (0) and lesion present (1) groups, and a highly significant statistical difference was found in ventral sac values between the lesion groups ( $P < 0,0001$ ). While macroscopic was found in ventral sac in the lesion absent group were considerably low and characterized by a narrow distribution with data points clustered around zero, the values in the lesion present group were distributed across a wider range and the mean values were found to be higher. A strong positive correlation was detected between the presence of lesions and macroscopic deformation of the ventral sac (including papillae free-areas, hemorrhage, ulceration and wart/papilloma) and altered papilla shape. In lesioned cattle, papillae were observed to lose their normal, healthy structure, becoming short, oval, or fragile. Surve data revealed that animals diagnosed with ruminal parakeratosis were primarily fed high-concentrate rations, in conjunction with roughage that was frequently mechanically chopped.



**FIGURE 2.** The relationship between the presence of lesions and ventral sac (Lesion absent (0) and lesion present (1), \*\*\*\* $P < 0.0001$ ).

The physical breakdown of roughage into very small particles reduces chewing and rumination time, which subsequently restricts salivary flow to the rumen [26]. This reduction in salivary buffering contributes to an acidic ruminal environment. The observed shedding of rumen papillae is therefore attributed to the use of finely chopped roughage alongside heavy concentrate use throughout the fattening period. This mechanism aligns with previous findings on parakeratosis etiology [26]. An extensive post-mortem analysis conducted by Viana *et al.* [11], on 1060 male Nellore steers identified a 21,7 % prevalence of ruminal lesions. Among the macroscopic pathological findings, hyperkeratosis emerged as the most frequent condition at 16.8 %, followed by hyperemia at 3,9 %, while ulcerations and tumors were observed in significantly smaller proportions of 0,9 % and 0,2 %, respectively.

These findings highlight that mucosal damage, particularly hyperkeratosis and hyperemia, is a common occurrence in cattle at the finishing stage when fed diets characterized by highly fermentable carbohydrates. Viana *et al.* [11] emphasized that feeding continuous *ad-libitum* high concentrate rations, combined with low roughage inclusion or fine roughage particle size, severely increases the risk of metabolic disorders, culminating in parakeratosis and ruminitis.

## Factors influencing foreign body presence

The logistic regression model, designed to assess the influence of papilla morphology on the presence FBs, demonstrated that papilla width was the sole morphological parameter exhibiting a statistically significant positive effect (OR = 0.118;  $P \leq 0.0298$ ). Quantitatively, a 1 cm increase in papilla width was associated with a reduction in the probability of FBs presence by a factor of 0.118. Conversely, a 1 cm reduction in papilla width dramatically increased the likelihood of finding an FBs by 8.475-fold (1/0.118).

Notably, neither papilla count nor papilla length were identified as statistically significant predictors of foreign body occurrence ( $P > 0.05$ ; TABLE II). The finding supports the hypothesis that wider and thicker papillae may form a more resilient physical barrier against the mechanical trauma induced by ingested foreign objects. This suggests that the chronic irritation or traumatic damage caused by foreign material inherently contributes to an elevated risk of lesion formation in the ruminal mucosa [8; 22].

Independent variables	Coefficient (β)	Standard Error	Odds Ratio (OR)	% 95 Confidence Interval (OR)	P value
Papilla count	-0.2663	0.1786	0.7662	0.5247- 1.0700	0.1360
Papilla length	0.5188	0.3827	1.6800	0.7742- 3.6442	0.1752
Papilla width	-2.1367	0.9835	0.1180	0.0149- 0.7771	0.0298*

\*P < 0.05.

## Effects of age and fattening duration on ruminal characteristics

The influence of animal age and fattening duration on ruminal fermentation and morphological properties was assessed. Age

was not to be a statistically significant determinant for any of the measured fermentation characteristics ( $P > 0.05$ ; TABLE III). In contrast, fattening duration yielded a statistically significant effect on AA concentration ( $P < 0.05$ ).

Specifically, cattle subjected to shorter fattening periods demonstrated statistically higher AA concentration compared to those with longer durations. However, fattening duration significantly influenced papilla length ( $P \leq 0.0128$ ). Cattle with shorter fattening periods exhibited significantly greater papilla length compared to the longer duration group. Furthermore, a trend towards significance was observed for papilla width (TABLE IV). These findings suggest that once ruminants reach the fully functional ruminant phase, the stability of digestive physiology and fermentation becomes more dependent on the administered ration and fattening management rather than age itself [27].

Variables	AA, mmol/L	PA, mmol/L	BA, mmol/L	NH <sub>3</sub> -N, mg/L	pH
Age < 24	55.69 ± 2.23	14.48 ± 0.83	7.16 ± 0.44	452.30 ± 18.24	6.41 ± 0.09
> 24	51.49 ± 2.56	15.87 ± 0.79	6.49 ± 0.47	497.43 ± 20.16	6.30 ± 0.08
P value	0.216	0.234	0.303	0.1	0.371
Fattening < 10	56.93 ± 2.49 <sup>a</sup>	15.81 ± 0.83	6.89 ± 0.40	467.22 ± 21.05	6.45 ± 0.09
duration > 10	50.52 ± 2.20 <sup>b</sup>	14.41 ± 0.79	6.81 ± 0.51	478.93 ± 17.55	6.26 ± 0.08
P value	0.05*	0.225	0.903	0.671	0.109

AA: Acetic acid; PA: Propionic acid; BA: Butyric acid; NH<sub>3</sub>-N: Ammonia nitrogen. <sup>a,b</sup>: Values within a column with different superscripts differ significantly ( $P < 0.05$ ).

Variables	Papilla count, mm <sup>2</sup>	Papilla length, cm	Papilla width, cm
Age < 24	5.02 ± 0.44	4.48 ± 0.22	1.89 ± 0.10
> 24	4.81 ± 0.46	4.62 ± 0.25	1.73 ± 0.10
P value	0.74	0.677	0.249
Fattening < 10	5.11 ± 0.41	4.95 ± 0.26 <sup>a</sup>	1.93 ± 0.11
duration > 10	4.73 ± 0.49	4.14 ± 0.19 <sup>b</sup>	1.70 ± 0.07
P value	0.553	0.0128*	0.0855

Values within a column with different superscripts differ significantly ( $P < 0.05$ ).

Evaluation of the fattening duration demonstrated that AA concentration was higher in cattle subjected to shorter fattening periods compared to those exceeding 10 months. Short fattening regimes typically mandate high-energy, high-concentrate rations to expedite animals reaching slaughter weight, which generally increases total VFA concentration. Although feeding easily digestible carbohydrates often reportedly decreases acetate and elevates propionate, some studies suggest this relationship depends on the specific carbohydrate source within the diet.

Furthermore, experimental work inducing acidosis in sheep reported an increase in acetate concentration concurrent with increased lactic acid accumulation, even as propionate and butyrate levels decreased. Therefore, the elevated AA level observed in the short-term feeding regime suggests either the continued activity of acetate-producing microbial populations or that AA production was sustained due to the absence of a significant difference in rumen pH between the groups.

Fattening duration significantly impacted ruminal wall morphology; papilla length was statistically greater in cattle fattened for 10 months or less. Rumen papillae are essential structures that increase the surface area for nutrient absorption with epithelial development directly correlated with the presence

of VFA [28]. The rumen epithelium is known to exhibit rapid morphological adaptation in response to changes in fermentable organic matter intake, with the speed of adaptation linked to the rate of increase in nutrient load [28]. High energy rations stimulate papilla growth to promote the efficient absorption of VFA. Consequently, the simultaneous finding of high AA concentrations and papilla length in the short-term fattening groups confirms a rapid and strong morphological adaptation of the rumen to high energy feeding.

## CONCLUSION

The study concluded that the feeding regime and fattening duration not only affect cattle performance but also modify rumen fermentation characteristics and ruminal epithelial morphology. The combined presence of metabolic stressors, such as the drop of rumen pH below physiological limits and the reduction of AA concentration, and mechanical stressors FBs and *Paramphistomum cervi* infection resulted in lesions and deformations in the ruminal epithelium, increasing the overall risk of inflammation in the animals.

## Conflict of interest

The authors declare no conflict of interest.

## Use of generative AI and AI-assisted technologies statement

During the preparation of this work the author(s) used [DEEPSEEK V3] in order to [improve language and readability].

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