JOURNAL OF ANIMAL PRODUCTION
Hayvansal Üretim
ISSN 1301-9597
e-ISSN 2645-9043
Published by Ege Animal Science Association
www.journalofanimalproduction.com

Research Article

Received: 09.01.2026 Accepted: 05.06.2026 Final Version: 30.06.2026

Duygu BUDAK [1] * Oktay DEMIRCAN [2] Besime Dogan DAS [3]

[1] Department of Animal Science and Animal Nutrition, Faculty of Veterinary Medicine, Aksaray University, 68100, Aksaray, Türkiye;
[2] Department of Chemistry, Faculty of Arts and Sciences, Bogaziçi University, 34000, Istanbul, Türkiye;
[3] Department of Animal Science and Animal Nutrition, Faculty of Veterinary Medicine, Harran University, 63010, Sanlıurfa, Türkiye;

Effects of Biologically Synthesized Fe_3O_4 Nanoparticles on Nutrient Composition, In Vitro Organic Matter Digestibility, and Methane Production in Dairy Cattle Concentrate Feed

ABSTRACT

Objective: This study evaluated the effects of biologically synthesized iron oxide nanoparticles (Fe_3O_4 NPs) on rumen fermentation, methane (CH_4) production, and in vitro digestibility of a dairy cattle concentrate feed.

Material and Methods: Iron oxide nanoparticles synthesized using Prunus serrulata leaf extract were added to a commercial dairy concentrate at 0, 10, 20, and 30 mg/kg dry matter (DM). Feed samples were analysed for nutrient composition, and in vitro gas production was performed using slaughterhouse-derived rumen fluid with 24 h incubation to estimate CH_4 production, organic matter digestibility, and metabolizable energy using standard gas production equations.

Results: Nanoparticle supplementation did not affect dry matter, ether extract, acid detergent fiber, hemicellulose, organic matter digestibility, or metabolizable energy ($P \geq 0.05$). Crude ash and crude protein increased with Fe_3O_4 NPs ($P < 0.001$), whereas neutral detergent fiber and total carbohydrate values decreased ($P < 0.05$). CH_4 production was significantly reduced at 20 and 30 mg/kg DM supplementation levels, with reductions approaching 9% compared with the control ($P < 0.05$).

Conclusion: Biologically synthesized Fe_3O_4 NPs reduced CH_4 production without impairing feed digestibility or energy value, indicating potential as feed additives for CH_4 mitigation in dairy cattle systems.

Keywords: Iron oxide nanoparticles; rumen fermentation; CH_4 mitigation; trace element bioavailability; in vitro gas production technique.

Biyolojik Olarak Sentezlenmis Fe_3O_4 Nanopartiküllerinin Süt Sıgırı Konsantre Yeminin Besin Madde Bilesimi, İn Vitro Organik Madde Sindirilebilirligi ve Metan Üretimi Üzerindeki Etkileri

ÖZ

Amaç: Bu çalışma, biyolojik olarak sentezlenmiş demir oksit (Fe_3O_4) nanopartiküllerinin süt sığırı konsantre yeminin besin bileşimi, in vitro organik madde sindirilebilirliği, metabolize olabilir enerji ve metan (CH_4) üretimi üzerindeki etkilerini değerlendirmeyi amaçlamıştır.

Materyal ve Yöntem: Prunus serrulata yaprak ekstraktı kullanılarak sentezlenen Fe_3O_4 nanopartikülleri, ticari bir süt sığırı konsantre yemine kuru madde (KM) bazında 0, 10, 20 ve 30 mg/kg düzeylerinde ilave edilmiştir. Yem örneklerinde kuru madde, ham protein, ham kül, ham yağ, nötral deterjan lif, asit deterjan lif, hemiselüloz ve toplam karbonhidrat analizleri yapılmıştır. Organik madde sindirilebilirliği, metabolize olabilir enerji ve CH_4 üretimi ise rumen sıvısı kullanılarak uygulanan in vitro gaz üretim tekniği ile tahmin edilmiştir.

Bulgular: Nanopartikül ilavesi kuru madde, ham yağ, asit deterjan lif, hemiselüloz, organik madde sindirilebilirliği ve metabolize olabilir enerji üzerinde etkili olmamıştır ($P \geq 0.05$). Ham kül ve ham protein düzeyleri Fe_3O_4 NP ilavesi ile artarken ($P < 0.001$), nötral deterjan lif ve toplam karbonhidrat değerleri azalmıştır ($P < 0.05$). CH_4 üretimi, 20 ve 30 mg/kg KM uygulama düzeylerinde kontrol grubuna kıyasla yaklaşık %9 oranında anlamlı şekilde azalmıştır ($P < 0.05$).

Sonuç: Biyolojik olarak sentezlenen Fe_3O_4 nanopartikülleri sindirilebilirlik ve enerji değerini olumsuz etkilemeden CH4 üretimini azaltmıştır. Bu bulgular, Fe_3O_4 nanopartiküllerinin süt sığırı beslemesinde CH_4 azaltımına yönelik yem katkı maddesi olarak potansiyel taşıdığını göstermektedir.

Anahtar Kelime: Demir oksit nanopartikülleri; rumen fermantasyonu; CH_4 üretimi; iz element biyoyararlanımı; in vitro gaz üretim tekniği

J. Anim. Prod., 2026, 67 (1) : 1-9 doi: 10.29185/hayuretim.1860139

How to cite:

BUDAK, D., DEMİRCAN, O. & DAS, B.D. (2026) Effects of Biologically Synthesized Fe_3O_4 Nanoparticles on Nutrient Composition, In Vitro Organic Matter Digestibility, and Methane Production in Dairy Cattle Concentrate Feed. Journal of Animal Production, Vol: 67 (1): 1-9, https://doi.org/10.29185/hayuretim.1860139

* Correspondence: budakduygu@aksaray.edu.tr

INTRODUCTION

Trace mineral nanoparticles, particularly iron-based nanomaterials, are increasingly investigated as feed additives because of their potential to enhance nutrient bioavailability and influence feed functionality. Recent studies have reported increasing interest in green-synthesized metallic nanoparticles as sustainable feed additives in animal nutrition systems (Kaya 2025). Manipulation of materials at the nanoscale offers opportunities to modify the physicochemical and biological behavior of feed nutrients, thereby improving nutrient utilization and reducing the environmental footprint of livestock production (Dumlu, 2024). Due to their high surface area and reactivity, Fe_3O_4 nanoparticles may interact with biological matrices and influence microbial processes within the rumen (Kaya, 2025). Unlike conventional mineral supplements, these nanoparticles can participate in surface-mediated reactions that influence microbial metabolism and substrate utilization in anaerobic fermentation systems. In the present study, Prunus serrulata leaf extract was used for green synthesis of Fe_3O_4 nanoparticles due to its rich phenolic and flavonoid content, which can function as natural reducing and stabilizing agents during nanoparticle formation (Singh and Onuegbu, 2020; Bhagat and Singh, 2022).

Iron-based nanoparticles have received considerable attention due to their strong redox activity and ability to interact with proteins, carbohydrates, and lipids through surface-driven adsorption and complexation processes (Schwaminger et al., 2019). In non-ruminant animal models, nano-iron supplementation has been shown to improve growth performance and feed efficiency, demonstrating that iron nanoparticles can function as highly bioavailable mineral sources (Basharat et al., 2023; Almeldin et al., 2024). However, in ruminant systems, the primary site of interaction between dietary additives and microorganisms is the rumen, where microbial fermentation regulates nutrient degradation, hydrogen metabolism, and gaseous end-product formation, including CH_4. Therefore, understanding how Fe_3O_4 nanoparticles influence rumen fermentation is essential for evaluating their potential role in CH_4 mitigation strategies.

CH_4 production in the rumen represents a major energetic loss for the host animal and a significant source of greenhouse gas emissions. Methanogenesis is driven by hydrogen (H2) generated during microbial fermentation, and effective mitigation strategies may involve redirection of reducing equivalents away from methanogenic archaea toward alternative electron sinks. Iron oxides, particularly Fe_3O_4, possess electron-accepting and surface-reactive properties that enable them to participate in anaerobic redox reactions and influence microbial electron flow. In other anaerobic biological systems, Fe_3O_4 nanoparticles have been reported to influence fermentation characteristics and gas production by altering hydrogen availability and microbial activity (El-Nemr et al., 2021; Alkhrissat et al., 2023). These properties suggest that Fe_3O_4 nanoparticles may influence rumen fermentation and CH_4 formation; however, previous studies have reported variable responses depending on nanoparticle characteristics, dose, and experimental conditions.

Despite increasing interest in Fe_3O_4 nanoparticles, most existing studies have focused on intestinal absorption, systemic bioavailability, or non-ruminant species (Tolkien et al., 2015; Alphandéry, 2019; Garcia-Fernandez et al., 2020), while their potential role in rumen fermentation and enteric CH_4 production remains insufficiently characterized. In particular, the effects of biologically synthesized Fe_3O_4 nanoparticles on nutrient composition and fermentation gas output in ruminant feeds have not been adequately characterized under in vitro conditions.

For the development of sustainable animal feeding strategies, it is important that novel feed additives such as nanoparticles do not adversely affect feed quality, nutrient composition, or digestibility; therefore, evaluating their effects on feed characteristics is of critical importance (Doğan and Kırkpınar 2025). To the best of our knowledge, limited studies have evaluated biologically synthesized Fe_3O_4 nanoparticles for influencing rumen fermentation characteristics and CH_4 production. Therefore, the objective of this study was to evaluate the effects of biologically synthesized Fe_3O_4 nanoparticles added to a dairy concentrate feed on nutrient composition, in vitro organic matter digestibility, and CH_4 production. Specifically, this study aimed to determine whether increasing inclusion levels of Fe_3O_4 nanoparticles may influence rumen fermentation characteristics and CH_4 output in an in vitro system.

MATERIAL AND METHODS

Bio-synthesis and characterization of Fe_3O_4 NPs

Fe_3O_4 NPs were synthesized using Prunus Serrulata (Japanese cherry blossom) leaf extract. The leaves were collected from Boğaziçi University North Campus. The synthesis was conducted in the Chemistry Department Laboratories following the green synthesis protocol described by Şengönül and Demircan (2023). The leaf extract was added to the FeCl3 solution under magnetic stirring, and the pH of the reaction mixture was adjusted using 0.25 M NaOH. The mixture was stirred for 2 h to ensure completion of the reaction. A black precipitate formed immediately after the addition of Prunus serrulata leaf extract, indicating the formation of Fe_3O_4 nanoparticles. The resulting Fe_3O_4 nanoparticles were centrifuged at 6000 rpm for 10 min, washed twice with distilled water and once with ethanol, and dried at 80 °C overnight.

The morphology and elemental composition of the synthesized nanoparticles were characterized using SEM, STEM, and EDX analyses (Thermo Scientific Quattro S). UV–Vis spectroscopy (Varian Cary-100), FT-IR analysis with ATR attachment (Thermo Scientific Nicolet 380), LC-MS analysis (Shimadzu LC20AD), and 1H NMR analysis (Varian Mercury-VX 400 MHz) were performed as described by Şengönül and Demircan (2023).

Feed material and in vitro gas production technique

A commercially purchased dairy cattle concentrate feed was used in the study, consisting of 20.17% corn grain, 16.63% soybean meal, 12.00% wheat, 7.30% wheat bran, 12.00% barley, 5.50% sunflower seed meal, 15.80% distiller's dried grains with solubles, 5.00% molasses, 5.00% limestone, and 20 mg/kg Fe. Total Fe in the concentrate feed was determined by inductively coupled plasma mass spectrometry (ICP-MS, Thermo-X Series 2) using the method reported by Nuttall et al. (1995). In the study, Fe_3O_4 NPs inclusion levels were selected with reference to the dietary iron requirements reported by NRC (2021).

The experimental treatment consisted of supplementation of the concentrate feed with Fe_3O_4 nanoparticles at 0, 10, 20, and 30 mg/kg DM. The selected supplementation levels were determined based on previously reported nano-mineral and rumen fermentation studies, taking into consideration biologically relevant concentrations and the need to avoid excessive mineral inclusion that could adversely affect microbial activity under in vitro conditions. Proximate nutrient analyses (DM, crude protein (CP), crude ash (CA), and ether extract (EE)) analyses of the feeds were performed according to AOAC (2005) standard procedure and acid detergent fiber (ADF) and neutral detergent fiber (NDF) analyses were performed according to Van Soest (1991) using ANKOM 220 Fiber Analyzer device. Total carbohydrate (TC) (%) = 100 – (CP + CA + EE) and hemicellulose (HCL, %) = NDF (%) – ADF (%) values were calculated by using the formulations reported by Filik (2020).

Fresh rumen fluid collected from a local slaughterhouse was used as the inoculum source for the gas production technique applied to estimate organic matter digestibility (OMD) and metabolizable energy (ME). Each treatment was conducted with three replicates, and the experiment was performed in two separate runs. Fresh rumen fluid was obtained from clinically healthy cattle and pooled prior to incubation to minimize individual variation. The rumen fluid was immediately transferred into pre-warmed, CO2-flushed thermos containers and transported to the laboratory within 1 h. Throughout preparation, continuous CO2 flushing was applied to maintain anaerobic conditions. The gas production procedure and estimation of OMD and ME values from 24 h total gas production were performed according to the method and regression equations described by Menke and Steingass (1988). CH_4 production values were estimated from total gas production measurements using the calculation approach integrated into the in vitro gas production technique and were not directly measured using gas chromatography or CH_4 sensors.

In the application of the materials, prepared with pure water; macromineral solution (containing 5.7 g Na2HPO4, 6.2 g KH2PO4, 0.6 g MgSO4 (7H2O)), micromineral solution (containing 13.2 g CaCl2 (2H2O), 10 g MnCl2 (4H2O), 1.0 g CoCl2 (6H2O), 8.0 g FeCl3 (6 H2O)) , buffer solution (containing 39 g NaHCO3, 4 g (NH4)HCO3) , 100 mg resazurin, reduction solution (containing 2 ml 1 N NaOH and 285 mg Na2S (7H2O)) were used. 500 ml of filtered rumen fluid was added to the mixture formed by these solutions taken into the Woulf bottle. While CO2 gas was continuously introduced into this mixture through a thin hose, the color change was checked (about 15 minutes). All procedures up to this stage were performed at 39ºC. Previously, 200-220 mg of the feed samples were placed in glass syringes (Model Fortuna, Häberle Labortechnik, Lonsee-Ettlenschie�, Germany) and kept in the incubator at 39ºC. After 30 ml of rumen fluid buffer solution mixture was poured into the glass syringes with the help of a dispenser, the air bubbles were removed and the clamp at the tip was squeezed. Initial syringe volume was recorded prior to incubation. After gas production was recorded, the results obtained were used to calculate the in vitro OMD and ME content of the feed using the following equations.

$$OMD\ (\%) = 14.88 + 0.889 \times GP + 0.45 \times CP + 0.0651 \times CA$$

$$ME\ (MJ/kg\ DM) = 1.06 + 0.157 \times GP + 0.084 \times CP + 0.22 \times EE - 0.081 \times CA$$

Where:
GP = 24 h net total gas production (mL/200 mg DM)
CP = crude protein (% DM)
EE = ether extract (% DM)
CA = crude ash (% DM)

Statistical analysis

The experiment was conducted as a completely randomized design with four dietary treatments. All statistical analyses were performed using SPSS software (version 20.0; IBM Corp., Armonk, NY, USA). Data were analyzed by one-way analysis of variance (ANOVA) to test the effect of treatment. When a significant treatment effect was detected, differences among means were evaluated using Duncan's multiple range test. Statistical significance was declared at $P < 0.05$.

The statistical model used was:

$$Y_{ij} = \mu + T_i + e_{ij}$$

where Yij is the observed value of the dependent variable, μ is the overall mean, Ti is the fixed effect of the ith treatment, and eij is the random error term.

RESULTS

The effects of Fe_3O_4 NPs supplementation on the nutrient composition of the concentrate feed are shown in Table 1. According to the results, Fe_3O_4 NP supplementation did not significantly affect DM, EE, ADF, or HCL contents of the concentrate feed ($P \geq 0.05$). In contrast, CA and CP values increased significantly with increasing Fe3O4 NP supplementation levels ($P \leq 0.001$), whereas NDF and TC decreased compared with the control group ($P \leq 0.05$).

Tablo 1. Fe3O4 NP ilavesinin süt sığırı konsantre yeminin besin madde bileşimi üzerindeki etkileri

Table 1. Effects of Fe3O4 NP supplementation on nutrient composition of dairy concentrate feed

Nutrient composition (%)	Fe_3O_4 NP levels (mg/kg DM)				SEM*	P Value
	0	10	20	30		
DM	90.412	90.405	90.334	90.211	0.105	0.925
CA	8.525c	9.369b	10.538a	11.145a	0.321	< 0.001
EE	4.586	4.526	4.334	4.323	0.091	0.722
CP	16.995c	17.586b	17.849b	18.461a	0.170	0.001
NDF	23.993a	23.199b	22.934b	22.613b	0.180	0.011
ADF	11.558	11.446	11.233	11.229	0.101	0.651
TC	69.894a	68.519b	67.279c	66.071d	0.438	< 0.001
HCL	12.435	11.753	11.701	11.384	0.190	0.273

SEM: Standard error of the mean.
n = 6 observations per treatment.
DM: Dry matter; CA: Crude ash; EE: Ether extract; CP: Crude protein; NDF: Neutral detergent fiber; ADF: Acid detergent fiber; TC: Total carbohydrate; HCL: Hemicellulose.
Different lowercase letters within the same row indicate significant differences (P < 0.05).

The effects of Fe_3O_4 NPs supplementation on in vitro OMD, CH_4 production, and ME values are presented in Table 2. OMD and ME values were not significantly affected by Fe_3O_4 NP supplementation ($P \geq 0.05$). CH_4 production was significantly reduced in the groups supplemented with 20 and 30 mg/kg DM Fe_3O_4 NPs compared with the control group ($P \leq 0.05$), corresponding to reductions of 9.2% and 8.9%, respectively. No significant difference was observed between the 10 mg/kg DM treatment and the control group.

Tablo 2. Fe_3O_4 NP ilavesinin OMD, CH_4 üretimi ve ME değerleri üzerindeki etkileri
Table 2. Effects of Fe_3O_4 NP supplementation on OMD, CH_4 production, and ME values

Parameters	Fe_3O_4 NP levels (mg/kg DM)				SEM*	P Value
	0	10	20	30		
OMD, %	52.080	53.153	53.143	53.697	0.397	0.600
ME, MJ/kg DM	7.873	7.921	8.049	8.103	0.046	0.272
CH_4 production, %	13.637a	12.839ab	12.384b	12.426b	0.193	0.044

SEM: Standard error of the mean.
n = 6 observations per treatment.
OMD: Estimated organic matter digestibility; ME: Estimated metabolizable energy; CH_4: Methane production.
Different lowercase letters within the same row indicate significant differences ($P < 0.05$).

DISCUSSION and CONCLUSION

In the present study, the absence of significant differences among treatments in DM, EE, ADF, and HCL following Fe_3O_4 NPs supplementation at 0–30 mg/kg DM indicates that this additive did not measurably alter the major compositional characteristics of the concentrate feed. Considering that Fe_3O_4 NPs were included at milligram-per-kilogram levels and do not provide organic matter, lipids, or structural carbohydrates, the lack of changes in DM, EE, ADF, and HCL may be interpreted as being consistent with basic feed composition principles.

The high surface area and surface reactivity of Fe_3O_4 NPs are important physicochemical features that generally determine their capacity to interact with feed components and rumen microbial populations. These surface characteristics may influence nutrient adsorption, bioavailability, and interactions with the rumen microbial populations; however, there is currently no direct evidence in the literature that trace-level (mg/kg) mineral supplementation can directly modify the structural dry matter or fiber fractions of feeds. Therefore, the absence of changes in DM, EE, ADF, and HCL observed in the present study suggests that the nanoparticles did not induce measurable alterations in the chemical composition of these feed components.

The significant increase in CA content with increasing Fe_3O_4 NPs supplementation directly reflects the inorganic nature of the nanomineral additive. Because Fe_3O_4 is an inorganic mineral compound, its inclusion in the feed inevitably increases the ash fraction measured after combustion. Accordingly, the higher CA values observed at 20 and 30 mg/kg DM are consistent with the greater amount of mineral material added to the concentrate, rather than with changes in the organic composition of the feed.

In addition, the reduction in NDF and TC, together with unchanged ADF, suggests that the observed differences were more evident in the relatively labile and analytically extractable carbohydrate-associated fractions rather than the recalcitrant cellulose–lignin complex. Fe_3O_4 NPs are known to interact strongly with organic macromolecules, including proteins and polysaccharides, through surface-driven mechanisms such as adsorption, ion exchange, and chemical complexation (Xu et al., 2014).

The concurrent increase in CP and decrease in NDF and TC should be interpreted cautiously. Although Fe_3O_4 nanoparticles may interact with organic molecules and influence analytical fractionation processes, the present study did not directly evaluate these mechanisms. Therefore, the observed differences cannot be conclusively attributed to nanoparticle-induced changes in nutrient distribution or analytical fractionation. Fe_3O_4 NPs are widely reported to bind and interact with biomolecules in complex biological and organic matrices, which can influence how these compounds are recovered and quantified during chemical analysis (Abarca-Cabrera et al., 2021). Recent studies further suggest that nanoparticles can act not only as antimicrobials but also as factors influencing rumen fermentation characteristics and microbial community structure, with implications for nutrient utilization and gas production (Kholif et al., 2025).

Surface-dependent interactions of Fe_3O_4 nanoparticles have also been reported in anaerobic fermentation systems, where surface-modified Fe3O4 NPs influenced substrate degradation and biogas production depending on their physicochemical properties (El-Nemr et al., 2021). In the present study, these observations may help explain some of the analytical differences observed among CP, NDF, and TC fractions without changes in ADF; however, the underlying mechanisms were not directly evaluated.

Evaluation of nutrient composition was included in the present study to provide complementary information for interpreting potential interactions between Fe_3O_4 nanoparticles and the concentrate feed components under in vitro conditions. Since nutrient fractions are commonly used in the interpretation of ruminal fermentation characteristics, assessment of proximate composition was considered relevant for supporting the evaluation of OMD and CH_4 responses. Differences observed among treatment groups in certain nutrient fractions should be interpreted within the methodological context of proximate feed analysis. Since Fe_3O_4 nanoparticles were incorporated at relatively low inclusion levels and do not represent conventional organic nutrient components, part of the variation may be related to analytical distribution and calculated nutrient fractions rather than direct modification of the intrinsic nutritive structure of the concentrate feed.

In this study, as shown in Table 2, Fe_3O_4 NPs supplementation did not significantly affect OMD or ME across treatments, indicating that the overall capacity of rumen microbial populations to degrade organic matter and generate utilizable energy was maintained under the batch fermentation conditions applied. This outcome is consistent with the known properties of in vitro batch systems, which are sensitive to treatment effects on gas end products but may be less sensitive to subtle changes in degradability because of end-product accumulation and the absence of passage and absorption processes that occur in vivo (Vinyard and Faciola, 2022).

Recent reviews highlight that CH_4 reduction remains a priority for reducing ruminant greenhouse gas emissions, with a range of nutritional and additive strategies under investigation (Roques, 2024). In contrast, CH_4 production was significantly reduced at 20 and 30 mg/kg DM Fe_3O_4 NPs, while OMD and ME remained unchanged. This response pattern is consistent with the general objective of methane-mitigation strategies in ruminant nutrition: decreasing CH_4 formation without impairing digestion or energy yield. In the rumen, CH_4 is produced primarily by methanogenic archaea using hydrogen (H2) and CO2 (or formate) generated during microbial fermentation, and effective mitigation strategies generally aim to redirect reducing equivalents away from methanogenesis rather than to suppress overall fermentation (Beauchemin et al., 2020; Palangi et al., 2022). The maintenance of OMD and ME alongside reduced CH_4 may suggest a possible shift in fermentation gas partitioning rather than a general inhibition of substrate degradation. However, because volatile fatty acid profiles, hydrogen balance, microbial populations, and detailed total gas production kinetics were not evaluated, this interpretation remains hypothetical and should be considered cautiously. Accordingly, enteric CH_4 mitigation strategies encompass a range of dietary and additive interventions, including approaches aimed at influencing fermentation characteristics and the use of specific inhibitors, which have been reviewed extensively in recent studies (Tseten et al., 2022; Hristov et al., 2022; Palangi et al., 2022). A comprehensive review of dietary reduction of enteric CH_4 underscores the diverse effects of additives and highlights the need for further mechanistic and practical validation (Lileikis et al., 2023). Similar effects on gas and CH_4 production by Fe_3O_4 nanoparticles have also been reported in other biological fermentation systems, where Fe_3O_4 supplementation influenced gas yields and biodegradation during anaerobic co-digestion (Alkhrissat et al., 2023).

Although direct rumen-specific evidence for Fe_3O_4 NPs as a methane-mitigating feed additive remains limited, mineral-based and nano-structured materials have been shown in rumen inoculum systems to reduce CH_4 without necessarily decreasing digestibility. For example, iron-rich kaolin minerals reduced enteric CH_4 generation in vitro while maintaining fermentation performance (Islam et al., 2025). Similarly, modified nano-clays have been reported to decrease CH_4 production in vitro with no consistent negative effects on total gas production or degradability, illustrating that surface-active mineral materials can influence rumen gas outcomes without broadly suppressing microbial activity (Abo-Sherif et al., 2025). These observations are consistent with broader in vitro meta-analyses showing that multiple additive classes can reduce CH_4 while leaving digestibility largely unchanged, depending on dose and experimental context (Martins et al., 2024).

The absence of a statistically significant CH_4 reduction at the lowest Fe_3O_4 NPs level (10 mg/kg DM) suggests that, under the present in vitro conditions, higher inclusion rates were required for the CH_4 response to become detectable. However, because batch systems differ from the in vivo rumen in buffering capacity, end-product accumulation, and microbial dynamics, this pattern should be interpreted conservatively and verified with additional endpoints (e.g., VFA profiles, dissolved H2, archaeal abundance) and in vivo validation (Vinyard and Faciola, 2022).

Overall, Table 2 indicates that Fe_3O_4 NPs supplementation at 20–30 mg/kg DM was associated with a modest reduction in CH_4 production in vitro while maintaining OMD and ME. However, in the absence of detailed gas kinetics and additional fermentation parameters, the underlying mechanisms and practical biological relevance of this response require further investigation. Confirmation of this effect in vivo and in continuous-culture systems will be necessary to establish its practical relevance for dairy cattle production.

The present study was conducted using an in vitro batch fermentation system, which provides a controlled and repeatable environment for screening dietary treatments but does not fully replicate the dynamic conditions of the rumen in vivo. In particular, processes such as feed intake regulation, digesta passage, absorption of fermentation end-products, and host–microbe interactions are not captured in batch systems. Consequently, although Fe_3O_4 NPs supplementation reduced CH_4 production without impairing organic matter digestibility in vitro, the magnitude and persistence of this effect must be confirmed under in vivo conditions.

In addition, the present study did not include measurements of volatile fatty acid profiles, hydrogen balance, microbial community composition, total gas production kinetics, or derived nutritional parameters such as total digestible nutrients (TDN), net energy for lactation (NEL), relative feed value (RFV), and short-chain fatty acids (SCFA), which are important for comprehensive interpretation of rumen fermentation characteristics and CH_4 production responses. Future studies incorporating these endpoints would allow a more comprehensive evaluation of how Fe_3O_4 NPs influence rumen fermentation pathways, nutrient utilization, and CH_4 mitigation under both in vitro and in vivo conditions.

Because nanoparticle behavior in biological systems depends on physicochemical properties such as particle size, surface chemistry, and aggregation state, further research should also evaluate how these characteristics affect rumen fermentation and CH_4 outcomes. Such work would help identify formulations that maximize CH_4 reduction while maintaining digestive efficiency.

Overall, the present findings indicate that Fe_3O_4 NPs have potential as feed additives for reducing CH_4 production in vitro, but comprehensive in vivo validation and safety assessments are required before practical application in dairy production systems.

The present findings indicate that Fe_3O_4 NPs can be added to dairy concentrate feeds at low levels (up to 30 mg/kg DM) without impairing feed composition, organic matter digestibility, or metabolizable energy. Importantly, Fe_3O_4 NPs supplementation at 20–30 mg/kg DM significantly reduced CH_4 production, whereas 10 mg/kg DM had no measurable effect, indicating a dose-related response.

The maintenance of digestibility and energy supply alongside reduced CH_4 may indicate differences in fermentation gas outcomes rather than suppression of overall microbial activity. Together with the observed changes in analytical nutrient fractions, these results suggest that Fe_3O_4 NPs may interact with the feed components and rumen environment.

Based on these findings, Fe_3O_4 NPs may have potential as feed additives for reducing CH_4 production in dairy cattle; however, further in vivo validation is required before practical application can be considered. Future in vivo studies are now required to confirm their efficacy, safety, and optimal inclusion level under practical feeding conditions.

Data availability: Data will be made available upon reasonable request.

Author contributions*: All authors contributed equally to the preparation of the article.

Competing interests.: There is no conflict of interest between the authors in this study

Ethical statement: All researchers declared it that "all animal procedures were conducted in accordance with EU Directive for animal experiments (European Union, 2010), ARRIVE guidelines (Kilkenny et al., 2010) and national regulation on the protection of experimental animals used for experimental "

Financial support.: -

Article description: This article was edited by Editor Çağri KANDEMİR.

REFERENCES

Abarca-Cabrera L, Fraga-García P, Berensmeier S. 2021. Bio-nano interactions: binding proteins, polysaccharides, lipids and nucleic acids onto magnetic nanoparticles. Biomaterials Research, 25(1): 12.

Abo-Sherif S, Sallam S, Allam AM, El-Adawy M, Soltan Y. 2025. In vitro evaluation of ruminal fermentation and methane production in response to the addition of modified nano-bentonite with or without saccharomyces cerevisiae to a forage-based diet. Animals, 15(14): 2081.

Alkhrissat T, Kassab G, Abdel-Jaber MT. 2023. Impact of iron oxide nanoparticles on anaerobic co-digestion of cow manure and sewage sludge. Energies, 16(15): 5844.

Almeldin YA, Eldlebshany AE, Elkhalek EA, Lohakare J, Abdel-Wareth AA. 2024. Assessment of dietary supplementation of green iron oxide nanoparticles: impact on growth performance, ammonia emissions, carcass criteria, tissue iron content, and meat quality in broiler chickens under hot climate conditions. Frontiers in Veterinary Science, 11: 1393335.

Alphandéry E. 2019. Biodistribution and targeting properties of iron oxide nanoparticles for treatments of cancer and iron anemia disease. Nanotoxicology, 13(5): 573-596.

AOAC 2005. Association of Official Analytical Chemists: Official Methods of Analysis. 18th ed., USA, Arlington, VA.

Basharat S, Tahir SK, Majeed KA, Yousaf MS, Hussain KK, Rashid MA, Zaneb H, Rehman H. 2023. Effects of iron oxide nanoparticle supplementation on the growth performance, serum metabolites, meat quality, and jejunal basal morphology in broilers. Animals, 14(1): 99.

Beauchemin KA, Ungerfeld EM, Eckard RJ, Wang M. 2020. Review: Fifty years of research on rumen methanogenesis and mitigation. Animal, 14(1): 2–16.

Bhagat S, Singh S. 2022. Nanominerals in nutrition: Recent developments, present burning issues and future perspectives. Food Research International, 160: 111703.

Doğan ES, Kırkpınar F. 2025. Bazı alternatif yem kaynaklarının besin maddesi kompozisyonları ve potansiyel yem değerlerinin belirlenmesi. Journal of Animal Production, 66(2): 178-189.

Dumlu B. 2024. Importance of Nano-Sized Feed Additives in Animal Nutrition. Journal of Agricultural Production, 5(1): 55-72.

El-Nemr A, Hassaan MA, Elkatory MR, Ragab S, Pantaleo A. 2021. Efficiency of Fe3O4 nanoparticles with different pretreatments for enhancing biogas yield of macroalgae Ulva intestinalis linnaeus. Molecules, 26(16): 5105.

Filik G. 2020. Biodegradability of quinoa stalks: The potential of quinoa stalks as a forage source or as biomass for energy production. Fuel, 266: 117064.

Garcia-Fernandez J, Turiel D, Bettmer J, Jakubowski N, Panne U, Rivas García L, Llopis J, González CS, Montes-Bayón M. 2020. In vitro and in situ experiments to evaluate the biodistribution and cellular toxicity of ultrasmall iron oxide nanoparticles potentially used as oral iron supplements. Nanotoxicology, 14(3): 388-403.

Hristov AN, Melgar A, Wasson D, Arndt C. 2022. Symposium review: Effective nutritional strategies to mitigate enteric methane in dairy cattle. Journal of Dairy Science, 105(10): 8543-8557.

Islam MR, Naidu R, Biswas B. 2025. Natural kaolin minerals with varying iron contents reduced cattle’s enteric methane generation in vitro. Applied Geochemistry, 189: 106459.

Kaya H. 2025. Effects of green-synthesized metallic nanoparticles in animal feeds. Gümüşhane University Journal of Science, 15(1): 298–320.

Kholif AE, Anele A, Chahine M, Anele UY. 2025. Applications of nanotechnology in ruminant animal production: Advances, challenges, and future prospects. Nanomaterials, 15(23): 1773.

Lileikis T, Nainienė R, Bliznikas S, Uchockis V. 2023. Dietary ruminant enteric methane mitigation strategies: current findings, potential risks and applicability. Animals, 13(16): 2586.

Martins LF, Cueva SF, Lage CFA, Ramin M, Silvestre T, Tricarico J, Hristov AN. 2024. A meta-analysis of methane-mitigation potential of feed additives evaluated in vitro. Journal of Dairy Science, 107(1): 288-300.

Menke KH, Steingass H. 1988. Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. Animal Research and Development, 28(1): 7-55.

NRC 2021. National Research Council: Nutrient requirements of dairy cattle. 8th revised ed., National Academies Press, Washington (DC).

Nuttall KL, Gordon WH, Ash KO. 1995. Inductively coupled plasma mass spectrometry for trace element analysis in the clinical laboratory. Annals of Clinical & Laboratory Science, 25(3): 264–271.

Palangi V, Lackner M. 2022. Management of enteric methane emissions in ruminants using feed additives: A review. Animals, 12(24): 3452.

Roques S, Martinez-Fernandez G, Ramayo-Caldas Y, Popova M, Denman S, Meale SJ, Morgavi DP. 2024. Recent advances in enteric methane mitigation and the long road to sustainable ruminant production. Annual Review of Animal Biosciences, 12(1): 321-343.

Schwaminger SP, Blank-Shim SA, Scheifele I, Pipich V, Fraga-García P, Berensmeier S. 2019. Design of interactions between nanomaterials and proteins: a highly affine peptide tag to bare iron oxide nanoparticles for magnetic protein separation. Biotechnology Journal, 14(3): 1800055.

Singh NB, Onuegbu CU. 2020. Nano-minerals as livestock feed additives. In 21st Century Nanoscience–A Handbook (pp. 20-1). CRC Press.

Şengönül H, Demircan O. 2023. Synthesis and characterization of Fe3O4 nanoparticles using Prunus serrulata leaf extract. BioNanoScience, 13(4): 1944-1954.

Tolkien Z, Stecher L, Mander AP, Pereira DIA, Powell JJ. 2015. Ferrous sulfate supplementation causes significant gastrointestinal side-effects in adults: A systematic review and meta-analysis. PLoS ONE, 10(2): e0117383.

Tseten T, Sanjorjo RA, Kwon M, Kim SW. 2022. Strategies to mitigate enteric methane emissions from ruminant animals. Journal of Microbiology and Biotechnology, 32(3): 269.

Xu JK, Zhang FF, Sun JJ, Sheng J, Wang F, Sun M. 2014. Bio and nanomaterials based on Fe3O4. Molecules, 19(12): 21506-21528.

Van Soest PJ, Robertson JB, Lewis BA. 1991. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. Journal of Dairy Science, 74(10): 3583-3597.

Vinyard JR, Faciola AP. 2022. Unraveling the pros and cons of various in vitro methodologies for ruminant nutrition. Translational Animal Science, 6(4): txac130.