ScientificScholar® Knowledge is power
Publisher of Scientific Journals

https://jksus.org

Journal of King Saud University – Science

2025 37 (1) 2852024

Original Article

Effects of carob (*Ceratonia siliqua* L.) pod supplementation of lamb diets on *in vitro* methane production, digestion, and microbial yield

Özer Kurt[a,*]

[a]*Department of Animal Production and Technology, Muş Alparslan University, Mus, Turkey*

A R T I C L E I N F O

Keywords:
Carob pod
CH_4 production
Digestibility
Gas production
Lamb diet

A B S T R A C T

Enteric fermentation in ruminants produces methane (CH_4), which is a major gas that contributes to global warming. The pods of the carob tree, *Ceratonia siliqua* L., which are abundant in tannins and water-soluble carbohydrates (WSC), could improve microbial protein yield (MPY) and reduce emissions of CH_4 without influencing digestion. The effects of carob pod supplementation in lamb diets on CH_4 emissions, digestibility, partitioning factor (PF), MPY, and EMPY (efficiency of MPY) were examined in this study. Four iso-caloric and iso-nitrogenous lamb diets (17% crude protein, 2650 kcal/kg dry matter) with differing quantities of carob pods were developed and evaluated using the Menke *in vitro* gas generation technique. Gas production (GP), CH_4 emissions, digestibility, PF, MPY, and EMPY were evaluated during a 24-hour fermentation using buffered rumen fluid from 'Awassi' sheep. The incorporation of carob pods considerably decreased gas and CH_4 production ($p < 0.001$), with a decrease of up to 15% in CH_4 emission at higher levels of supplementation. The increase in PF, MPY, and EMPY was accompanied by no change in digestibility. At 30% supplementation, MPY increased by 35.32 mg, while EMPY increased to 31.5% from 23.14% in the control. There was a linear reduction in gas and CH_4 emission as the amount of carob supplementation increased. Lamb diets supplemented with 30% carob pods had a 15% decrease in CH_4 emissions and an increase in MPY without any change in digestibility. Additional *in vivo* research is needed to validate the long-term impacts on performance and health, but the results show promise for carob pods to improve ruminant production and decrease emissions of greenhouse gases.

1. Introduction

Methane (CH_4) emissions from ruminant animals have emerged as a significant concern owing to their contribution to global greenhouse gases and their detrimental effects on global warming and climate change (FAO, 2023). Enteric fermentation (the digestive process in ruminants) is the main source of CH_4 emissions (Zhao et al., 2020). Enteric fermentation not only results in global warming but also loss of energy (Johnson and Johnson, 1995). Various strategies could be utilized to mitigate CH_4 emissions from ruminant animals, including improved management practices, superior genetics, nutritional interventions, and innovative technology (Cottle et al., 2011; Hristov et al., 2013; Kumar et al., 2014; Patra and Yu, 2013; Beauchemin et al., 2022). One of the most important dietary interventions is the use of feedstuffs with high water-soluble carbohydrates (WSC) and condensed tannins (CT). The WSC and CT in diets can mitigate CH_4 production through a variety of ways, including promoting the production of propionate, which acts as a hydrogen sink, reducing the hydrogen available for methanogens, thus decreasing CH_4 production, altering the rumen microbial community to favor propionate-producing bacteria over CH_4-producing archaea, shifting fermentation patterns from fiber to more efficient carbohydrate fermentation, which generates less CH_4 (Johnson and Johnson, 1995; Newbold et al., 2005; Patra and Yu, 2013; Ramin and Huntanen, 2013).

The carob tree (*Ceratonia siliqua* L.) is a legume native to or widely grown in Mediterranean nations. Its abundant pods are used by humans and animals equally. (Karabulut et al., 2006; Kotrotsios et al., 2012; Bulca, 2016; Saratsis et al., 2016; Al-Nawass and Al-Saady, 2019; Richane et al., 2022; Basharat et al., 2023; Kurt, 2023). It is an evergreen legume tree indigenous to Southern Europe, widely cultivated for its edible pods known as carob pods. Carob pods have gained importance as a potential source of animal feed, especially for ruminants, due to their unique nutritional properties (Ikram et al., 2023). The distribution of carob pods the Mediterranean region is considerable, as the tree is well adapted to the climate of the region and has been a staple food for centuries (Winer, 1980). Carob pods have been traditionally used in the past as a sweetener and to treat various diseases, and more recently, they have been explored as an inexpensive food source for animals and humans (Naghmouchi et al., 2012). Ruminants find carob pods appealing as a food source because of their high sugar content (Naghmouchi et al., 2012). The availability of high-quality fodder is minimal throughout the summer months, constraining the performance of ruminants in most regions of Türkiye due to insufficient calorie and protein intake. Small ruminants depend on the leaves and pods of trees and bushes for sustenance during this critical summer phase. Carob pods could improve the productivity of sheep and goats and increase farmers' profits in Southern Europe during periods of traditional feed

***Corresponding author:**
E-mail address: o.kurt@alparslan.edu.tr (Ö. Kurt)

Received: 04 November, 2024 **Accepted:** 19 January, 2025 **Epub Ahead of Print:** 28 February, 2025 **Published:** 22 March, 2025

DOI: 10.25259/JKSUS_285_2024

shortages (Aloueedat et al., 2019). Ruminant nutrition and performance issues are prevalent in Türkiye throughout the summer due to a lack of high-quality feed. Proper planning and use of different feed sources are necessary to guarantee sufficient livestock nourishment throughout the year.

Results from both *in vitro* and *in vivo* investigations show that increasing WSC may decrease CH_4 production by decreasing the acetate-to-propionate ratio (Rivero et al., 2020; Lovett et al., 2006). Tannins may limit methanogens by reducing the population of protozoa that symbiotically harbor archaea and by directly suppressing some archaea like WSC (Aboagye and Beauchemin, 2019). The use of tannins in animal diets may effectively reduce CH_4 emissions by directly targeting protozoal populations and methanogenic archaea. This combined action not only mitigates greenhouse gas emissions but also underscores the promise of tannin-rich forages as a sustainable method for enhancing ruminant nutrition and environmental outcomes. Recently, Kurt (2023) has shown that carob pods from different growing regions are rich in WSC and moderate in CT.

While there is some literature regarding the impact of supplementing sheep diets with carob pods on feed intake, milk yield, and some blood parameters is available, there is currently no evidence concerning the effects of carob pod supplementation in lamb diets on CH_4 emissions, digestibility, and MPY. Therefore, it was hypothesized that supplementation lamb diets with carob pods, which contain WSC and CT, would reduce CH_4 emission and promote microbial protein production by altering fermentation patterns without compromising digestibility. The present study aimed to determine the impact of including carob pods in lamb diets on digestibility, PF, MPY, EMPY, gas production (GP), and CH_4 emissions.

2. Materials and Methods

Carob pod sample used in the current study was acquired from Mersin (36° 48' 43.5708" N, 34° 38' 29.3244" E) provinces, Türkiye. The diet consisted of barley grain, carob pods, wheat bran, sunflower seed meal, and alfalfa hay, which are commercially available and widely used in sheep diets. The dietary components were pulverized to pass through a one-mm filter and kept in nylon bags for further *in vitro* gas generation and chemical analysis.

2.1 Chemical analysis of diet ingredients

The dry matter (DM) and crude protein (CP) were measured by the Kjeldahl technique (N×6.25), whereas ether extract (EE) and crude ash (CA) were assessed using the AOAC protocol (AOAC, 1990). The carob pod's CT content was assessed using the butanol-HCL technique (Makkar et al., 1995). The water-soluble carbohydrate content of carob pod was assessed using the methodology outlined by Lane and Eynon (1934). All analyses were conducted in duplicate. The metabolizable energy (ME) values of the components were determined using equation (1) established by Menke and Steingass (1988). ME was computed as follows

$$ME\left(MJ\frac{g}{kg}DM\ basis\right) = 1.06 + 0.1570GP + 0.084CP + 0.2220EE - 0.081CA\ldots \quad (1)$$

Here, GP = gas production (mL per 0.200 g sample), CP = crude protein (%), EE = ether extract (%), and CA = crude ash (%).

The chemical composition and ME values of the diet ingredients are given in Table 1.

2.2 Preparation of lamb diets

The four iso-caloric and iso-nitrogenous diets (with 17% CP/kg DM basis and 2650 metabolic energy kcal/kg DM basis) were formulated with graded carob pod content according to the NRC (2007) requirements for lambs. The compositions of the diets for lambs are shown in Table 2. Tannic acid or WSC were not included in diet 1 (control).

Table 1.
Chemical compositions (g/kg DM basis) and metabolizable energy (kcal/kg DM basis) values of diet ingredients.

Ingredients	DM	CA	CP	EE	WSC	CT	ME
Alfalfa hay	944.1	91.0	186.4	15.3	40.2	N.D	2370
Barley grain	907.8	21.2	112.5	30.3	117.7	N.D	2793
Carob pod	919.8	31.0	91.4	33.8	260.3	24.9	2009
Wheat bran	911.2	34.1	155.3	26.9	80.2	N.D	3047
Sunflower meal	924.4	71.8	390.7	14.5	66.4	N.D	2478

DM: Dry matter (%), CA: Crude ash (%), CP: Crude protein (%), EE: Ether extract (%), WSC: Water soluble carbohydrate (%), CT: Condensed tannin (%), ME: Metabolizable energy (kcal/kg DM), N.D: Not detected

Table 2.
The composition of lamb diets containing carob pods.

Diets ingredients	Diets			
	I	II	III	IV
Barley grain	500	400	300	200
Carob pod	0.0	100	200	300
Oil	6	22	39	55
Wheat bran	120	84	47	11
Sunflower seed meal	148	168	188	208
Alfalfa hay	200	200	200	200
Salt	10	10	10	10
$CaCO_3$	15	15	15	15
Min-Vit mixture	1	1	1	1
Total (g)	1000	1000	1000	1000
ME (kcal/kg DM)	2652.1	2645.0	2642.9	2635.7
CP (g/kg DM	170.0	170.1	170.1	170.3
CA (g/kg DM)	43.5	44.5	45.5	47
EE (g/kg DM)	29.5	44.8	61.1	77.5
CT (g/kg DM)	0	2.5	5.0	7.5
WSC (g/kg DM)	86.39	99.08	111.69	124.39

ME: Metabolizable energy, DM: Dry matter, CP: Crude protein, CA: Crude ash, EE: Ether extract, CT: Condensed tannin, WSC: Water soluble carbohydrate

2.3 Determining of gas and methane production of lamb diets including graded level of carob pod

Approval for the *in vitro* experiments conducted in this study was obtained from the Animal Ethics Committee of Kahramanmaraş Sütçü Imam University. (Protocol No.: 2024/2-3). GP, CH_4 production, true DM digestibility, and MPY of the lamb diets were determined using the *in vitro* Gp technique proposed by Menke et al. (1979). Approximately 500 mg lamb diet samples in 100 mL glass syringes were fermented in triplicates with 40 mL buffered rumen liquid (1:2 V/V) in a thermal bathing at 39°C incubation 24 hours. Rumen liquids were collected from three fistulated 'Awassi' sheep (55–60 kg live weight, 1–1.5 years old) fed a diet containing alfalfa (60%) and barley (40%) at 1.2 times the ME requirement pre-morning feeding (NRC, 2007) and sieved through 4 sheets of gauze. In addition, 3 glass syringes without substrate were used to obtain blanks. The GP of diets was determined after 24 hours of fermentation. The percentage of CH_4 in the gas of the diets was analyzed using an infrared CH_4 analyzer (Sensor Europe GmbH, Erkrath, Germany (Goel et al., 2008). CH_4 (mL) production of diets was calculated by using equation 2.

$$CH4\ production(mL) = Percentage\ of\ CH4(\%) \times Total\ gas\ production(mL) \quad (2)$$

2.4 Determining of truly degraded substrate of lamb diets including graded level of carob pod

After a 24-hour fermentation period, the residues in the glass syringes were transferred to a beaker containing 50 mL of neutral detergent fiber solution. The residue was filtered through a pre-weighed sintered glass crucible after one hour of boiling. The glass crucibles with the non-fermented diet samples were positioned and maintained in an oven at 65°C overnight to ascertain the true digestible substrate (TDS), PF, MPY, and EMPY of the diet samples.

The TDS, PF, EMPY, and MPY of diet specimens were estimated using equations 3-6 suggested by Blümmel et al. (1997a) as the following.

$$DS(mg) = Substrate\ incubated(mg) - \ the\ residue(mg)\ldots \tag{3}$$

$$PF = (TDS / GP)\ldots \tag{4}$$

$$MPY(mg) = [TDS - (gas\ production \times 2.2)]\ldots \tag{5}$$

$$EMPY(\%) = ((TDS - [gas\ production \times 2.2])/TDS) \times 100\ldots \tag{6}$$

2.5. Statistical analysis

One-way analysis of variance (ANOVA) was carried out to determine the effects of supplementation of the lamb diet with carob pods on GP, CH_4, PF, TDS, MPY, EMPY, and true digestibility of diets. The data's normality was assessed before the analysis, revealing a normal distribution. The data fulfilled the normality assumption for ANOVA; hence, the original data was analyzed. Mean differences were considered significant at $p<0.05$. Tukey tests were used to determine the difference among the means.

3. Result

3.1 Effect of supplementation of lamb diet with carob pod on gas, methane, digestibility, and microbial yield

The addition of carob pods to the lamb diet significantly affected GP (mL) and CH4 production (mL), while not impacting the percentage of CH_4 in the gas produced (Table 3). The GP and CH_4 production ranged from 107.75 - 120.75 mL and 15.88 - 18.58 mL, respectively. Gas and CH_4 production were significantly lower in diets III and IV than I and II. The relationship between the ratio of carob pod supplementation and GP in the diet is shown in Fig. 1. Lambs that consumed a higher percentage of carob pods exhibited reduced gas emissions. GP decreased by 0.0485 units for each milligram of carob pod inclusion

Fig. 2 demonstrates the correlation between the supplementation ratio of carob pods in the diet and CH_4 produced. CH_4 production reduced as the ratio of carob pods in the lambs' diet increased. The mean decrease in CH_4 production (mL) per mg of carob pod supplement was 0.0104 units.

On the other hand, the supplementation of carob pods had no effect on TDS and true digestibility, while the PF increased with the supplementation of carob pods. The PF of the diets was between 2.87 and 3.21, with the highest values determined for diets III and IV. The relationship between the PF and the supplementation ratio of carob pods in the diet is shown in Fig. 3. The PF improved with increasing levels of carob pod in the lamb diets.

The supplementation of carob pods increased MPY and EMPY. The MPY and EMPY ranged from 80.00 - 109.06 mg and 23.14 - 31.50%, respectively (Table 3). The highest values were recorded for diets III and IV. The relationship between MPY and carob pod supplementation ratio is shown in Fig. 4. The MPY improved with an increasing ratio of carob pods in lamb diets. The mean increase in MPY per mg of carob pod supplement was 0.111 units.

Table 3.
Effect of supplementation of lamb diet with carob pod on gas, methane, digestibility and microbial yield.

Parameters	Diets				SEM	*p*
	I	II	III	IV		
Gas (mL)	120.00[a]	120.75[a]	109.00[b]	107.75[b]	2.561	<0.001
CH_4 (mL)	18.54[a]	18.58[a]	16.13[b]	15.88[b]	0.522	<0.001
CH_4 (%)	15.45	15.39	14.83	14.74	0.468	0.333
Truly degraded substrate (mg)	344.59	345.65	345.00	346.11	3.916	0.980
Partitioning factor	2.87[b]	2.86[b]	3.17[a]	3.21[a]	0.073	<0.001
MPY (mg)	80.59[b]	80.00[b]	105.20[a]	109.06[a]	5.940	<0.001
EMPY (%)	23.37[b]	23.14[b]	30.49[a]	31.50[a]	1.629	<0.001
True digestibility (%)	74.03	74.21	74.22	74.23	0.789	0.993
CH_4 (mL/ per gram digested substrate)	53.83[a]	53.76[a]	46.79[b]	45.92[b]	1.588	<0.001

[ab] Row means with common superscripts do not differ at p<0.05. SEM: Standard error mean, MPY: Microbial protein yield, EMPY: Efficiency of microbial protein yield.

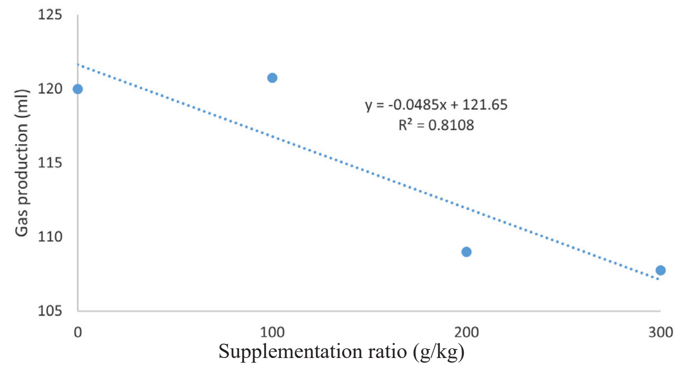

Fig. 1. Relationship between gas production and supplementation ratio.

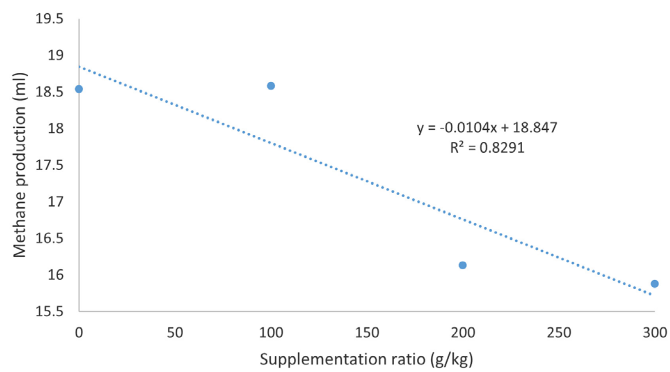

Fig. 2. Relationship between methane production and supplementation ratio.

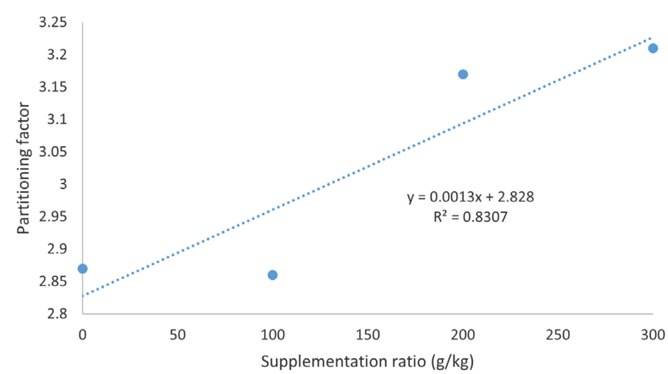

Fig. 3. Relationship between partitioning factor and supplementation ratio.

Fig. 5 shows the correlation between the production of gases and the MPY ratio. A negative association exists between GP and microbial yield.

The relationship between CH_4 (mL/digested DM) and the supplementation ratio is shown in Fig. 6. The CH_4 (mL/digested DM) decreased as the degree of carob pods in the lamb diets increased. The mean decrease in CH_4 (mL/digested DM) per mg of carob pods supplement was 0.0307 units.

4. Discussion

The essential process of carbohydrate fermentation in the digestive tracts of ruminants significantly influences their energy metabolism and overall health. The fermentation of carbohydrates *in vitro* using buffered rumen fluid yields many products, including microbial biomass, short-chain fatty acids (SCFAs), and gases, primarily carbon dioxide (CO_2) and CH_4 (Beuvink and Spoelstra, 1992; Blummel and Orskov, 1993). Therefore, it is essential to understand the dynamics of carbohydrate

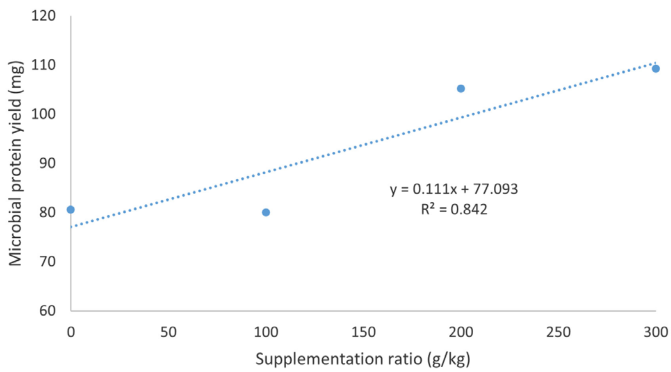

Fig. 4. Relationship between microbial protein yield and supplementation ratio.

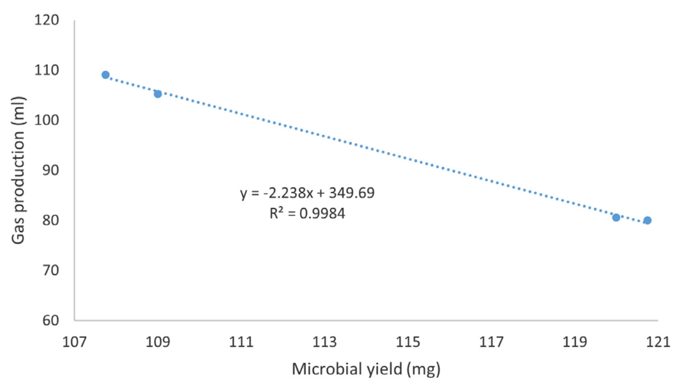

Fig. 5. The relationship between gas production and microbial yield.

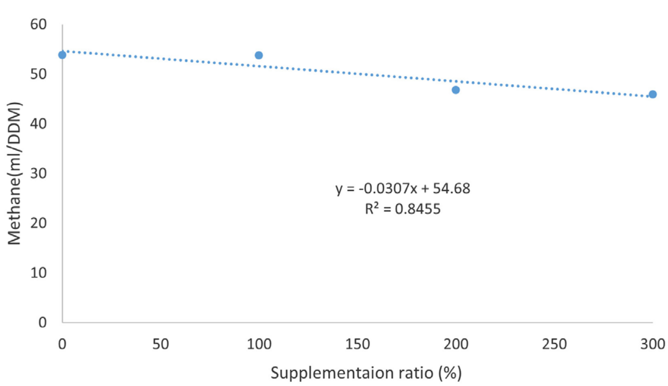

Fig. 6. The relationship between methane (mL/DDM) and supplementation ratio. DDM: digested dry matter.

fermentation in rumen fluid to enhance ruminant nutrition and reduce CH_4 emissions. Producers may attain energy efficiency and reduce greenhouse gas emissions by careful regulation of the types and amounts of fermentable substrates.

The supplementation of diet with carob pod decreased the gas and CH_4 production without compromising the digestibility of diets (Table 3). The mean decreases in gas (mL) and CH_4 (mL) per mg of carob pods supplement were 0.0485 and 0.0104 units, respectively. Therefore, the decrease in gas and CH_4 production might be associated with a shift in the composition of volatile fatty acids (VFAs) produced during fermentation. It would be more informative to obtain sufficient details about VFAs composition to adequately interpret these results. This is one of the limitations of the current experiment. However, Lee et al. (2003) showed that a linear increase in the proportion of propionic acids was obtained at the expense of acetate when WSC contents increased. Such a shift in the VFA profile of rumen will decrease the amount of H_2 and thus, gas and CH_4 production. This is also in agreement with the findings of Mills et al. (2001). On the other hand, the mean decrease in CH_4 production per mg carob pod supplementation was 0.0307 units when the CH_4 production was expressed as CH_4 mL/ per gram digested substrate. This result agrees with the findings of Purcell et al. (2014), who showed that there was a linear decrease in CH_4 production (mL) per gram of DMD with increasing WSC content of the incubated substrate.

Ruminant nutrition and metabolism are significantly influenced by VFAs, which are generated during the fermentation of carbohydrates in the rumen. The kind of fermentation substrate used significantly influences the molar ratios of these VFAs. Enhancing ruminant diets and reducing greenhouse gas emissions necessitates comprehension of the effects of different substrates on VFA profiles and gas emissions. Unlike substrates that promote elevated levels of acetate and butyrate, those abundant in water-soluble carbohydrates facilitate the production of propionate, reducing gas and CH_4 emissions. (Newbold et al., 2005; Ramin et al., 2013; Patra and Yu, 2013) This is because the fermentation of substrate to acetate and butyrate may yield more hydrogen, depending on the metabolic pathways and micro-organisms involved. By promoting propionate formation, the hydrogen that would have been used by methanogens to form CH_4 is instead used for propionate production, thereby reducing CH_4 production.

The supplementation of diets with carob pod increased the portioning factor. The mean increase in partitioning factor per mg carob pod supplementation was 0.0013 units. The partitioning factor is one of the important fermentation parameters and ranges from 2.87 to 3.21. The partitioning factors obtained in the current study fell into the theoretical range (2.75 to 4.41) indicated by Blümmel et al. (1997b). Blümmel et al. (1997a) suggested that roughages with a high partitioning factor had a higher intake. Therefore, the increase in partitioning factors due to carob pod supplementation might improve feed intake, thereby milk production, or growth performance of lambs.

As the quantity of carob pod in the lamb meal enhanced, the MPY correspondingly increased. The MPY improved by an average of 0.111 units per mg of carob pod supplementation. An inverse relationship exists between the two variables to some extent since the partitioning factor of fermented matter between microbial cells and SCFAs (gas) is not uniform (Blümmel et al., 1997b).

The dietary WSC and CT content enhanced with the increase of carob pod supplementation (Table 2). The presence of WSC and tannin may positively influence nutrient partitioning, favoring enhanced microbial output over the synthesis of SCFAs (gas) (Baba et al., 2002). The inclusion of tannins in the diet is predicted to positively influence the reduction of CH_4 emissions by directly suppressing some archaea and indirectly decreasing protozoa populations (Aboagye and Beauchemin, 2019). The previously proposed hypothesis was supported by the fact that supplementation lamb diet with carob pods containing WSC and tannin reduced CH_4 emissions and improved microbial protein production without affecting digestibility.

The decrease in gas and CH_4 production without compromising digestibility is desirable because the fermentable substrate has been diverted into microbial protein production rather than VFA production. The reason for shifting the fermented substrate into MPY instead of VFA production (GP) could be related to the WSC and tannin content of the carob pods.

Microbial protein plays an important role in providing a rich source of high-quality and digestible protein to ruminant animals for growth, milk production, reproduction and health. As can be seen from Table 3, the supplementation of lamb diets with carob pod increased the microbial protein. This result might have some practical implication since microbial protein plays an important role in providing a rich source of high-quality and digestible protein for ruminant animals for growth, milk production, reproduction, and health.

The comparison of *in vitro* and *in vivo* assessments of energy metabolism, specifically the EMPY, shows significant variations in study approaches. Although it is often expected that these estimations may vary owing to the absence of product flow in *in vitro* systems, research has shown substantial correlations between the two methodologies. Blümmel et al. (1999) indicated a correlation between *in vitro* GP estimates and *in vivo* EMPY estimations derived from renal allantoin excretion in steers. It was proposed that assessing gas volume and substrate degradability at substrate-specific incubation intervals rather than a standard 24 hours might enhance the correlation.

The current *in vitro* experiment has some limitations in terms of correlating with *in vivo* experiments due to differences in rumen dynamics. Although the current experiment is very important in providing possible insights on the effects of supplementation of diets with carobs, the long-term effects of carob pod supplementation on production and health remain unexplained.

Therefore, before large implications this should be tested *in vivo* animal experiments to evaluate the long-term effect of the carob pod supplementation on animal performance and health.

5. Conclusions

The carob pod has the potential to improve microbial protein production and reduce CH_4 emissions from lambs. It was found that carob pods can be used ~30% in the diet for lambs to increase microbial protein production by ~35.32 mg and reduce CH_4 emissions by 15% without compromising the digestibility of the diet. Carob pods deserve further investigation to explore the impact on CH_4 emissions per unit of animal product.

CRediT authorship contribution statement

Ö. Kurt: Writing review & editing, Writing original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Declaration of Generative AI and AI-assisted technologies in the writing process

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

References

Aboagye, I., Beauchemin, K., 2019. Potential of molecular weight and structure of tannins to reduce methane emissions from ruminants: A review. Animals (Basel) 9, 856. https://doi.org/10.3390/ani9110856

Al-Nawass, K.J., Al-Saady, H.A., 2019. Effect of adding two levels of Cerotonia siliqua L. to the diets on the specific qualities of milk and its correlation with some of the vital blood characteristics of local Awaasi lambs. In Journal of Physics: Conference Series, IOP Publishing, September 1294, pp. 092026. https://doi.org/10.1088/1742-6596/1294/9/092026

Aloueedat, M., Obeidat, B., Awawdeh, M., 2019. Effects of partial replacement of conventional with alternative feeds on nutrient intake, digestibility, milk yield and composition of awassi ewes and lambs. Animals (Basel) 9, 684. https://doi.org/10.3390/ani9090684

AOAC. 1990. Official Method of Analysis Association of Official Analytical Chemists, 15th Edition, Washington, DC. USA.

Baba, A.S.H., Castro, F.B., Ørskov, E.R., 2002. Partitioning of energy and degradability of browse plants in vitro and the implications of blocking the effects of tannin by the addition of polyethylene glycol. J. Animal Feed Sci. Technol. 95, 93-104. https://doi.org/10.1016/S0377-8401(01)00283-8

Basharat, Z., Afzaal, M., Saeed, F., Islam, F., Hussain, M., Ikram, A., Pervaiz M.U., Awuchi, C.G., 2023. Nutritional and functional profile of carob bean (Ceratonia siliqua): A comprehensive review. Int. J. Food Prop. 26, 389-413. https://doi.org/10.1080/10942912.2022.2164590

Beauchemin, K., Ungerfeld, E., Abdalla, A., Alvarez, C., Arndt, C., Becquet, P., Benchaar, C., Berndt, A., Mauricio, R., McAllister, T., Oyhantçabal, W., Salami, S., Shalloo, L., Sun, Y., Tricarico, J., Uwizeye, A., De Camillis, C., Bernoux, M., Robinson, T., Kebreab, E., 2022. Invited review: Current enteric methane mitigation options. J. Dairy Sci. 105, 9297-9326. https://doi.org/10.3168/jds.2022-22091

Beuvink, J.M.W., Spoelstra, S.F., 1992. Interactions between substrate, fermentation end-products, buffering systems and gas production upon fermentation of different carbohydrates by mixed rumen microorganisms in vitro. Appl. Microbiol. Biotechnol. 37, 505-509. https://doi.org/10.1007/BF00180978

Blümmel, M., Makkar, H.P.S., Becker, K., 1997b. In vitro gas production: a technique revisited. Journal of Animal Physiology and Animal Nutrition 77, 24-34. https://doi.org/10.1111/j.1439-0396.1997.tb00734.x

Blummel, M., Orskov, E.R., 1993. Comparison of gas production and nylon bag degradability of roughages in ¨ predicting feed intake in cattle. Anim. Feed Sci. Technol. 40, 109-119.

Blümmel, M., Schröder, A., Südekum, K.H., Becker, K., 1999. Estimating ruminal microbial efficiencies in silage-fed cattle: comparison of an in vitro method with a combination of in situ and in vivo measurements. J. Anim. Physiol. Anim. Nutr. 81, 57-67. https://onlinelibrary.wiley.com/doi/pdf/10.1046/j.1439-0396.1999.812198.x

Blümmel, M., Steingass, H., Becker, K., 1997a. The relationship between in vitro gas production, in vitro microbial biomass yield and 15N incorporation and its implications for the prediction of voluntary feed intake of roughages. Br. J. Nutr. 77, 911-921. https://doi.org/10.1079/bjn19970089

Bulca, S., 2016. Some properties of carob pod and its use in different areas including food technology. Scientific Bulletin Series F. Biotechnologies 20, 142-147. https://biotechnologyjournal.usamv.ro/pdf/2016/Art24.pdf

Cottle, D.J., Nolan, J.V., Wiedemann, S.G., 2011. Ruminant enteric methane mitigation: a review. Anim. Prod. Sci. 51, 491-514. https://doi.org/10.1071/AN10163

Food and Agriculture Organization (FAO), 2023. Methane emissions in livestock and rice systems, Rome: Food and Agriculture Organization of the United Nations. p. 41-44 [accessed 2024 Aug 02]. Available from: https://doi.org/10.4060/cc7607en

Goel, G., Makkar, H.P.S., Becker, K., 2008. Effect of Sesbania sesban and Carduus pycnocephalus leaves and Fenugreek (Trigonella foenum-graecum L.) seeds and their extract on partitioning of nutrients from roughage and concentrate-based feeds to methane. J. Animal Feed Sci. Technol. 147, 72-89. https://doi.org/10.1016/j.anifeedsci.2007.09.010

Hristov, A.N., Ott, T., Tricarico, J., Rotz, A., Waghorn, G., Adesogan, A., Firkins, J.L., 2013. Special topics—Mitigation of methane and nitrous oxide emissions from animal operations: III. A review of animal management mitigation options. J. Anim. Sci. 91, 5095-5113. https://doi.org/10.2527/jas.2013-6585

Ikram, A., Khalid, W., Wajeeha Zafar, K., Ali, A., Afzal, M., Aziz, A., Faiz Ul Rasool, I., Al-Farga, A., Aqlan, F., Koraqi, H., 2023. Nutritional, biochemical, and clinical applications of carob: A review. Food Sci. Nutr. 11, 3641-54. https://doi.org/10.1002/fsn3.3367

Johnson, K., Johnson, D., 1995. Methane emissions from cattle. J. Anim. Sci. 73, 2483-92. https://doi.org/10.2527/1995.7382483x

Karabulut, A., Canbolat, O., Kamalak, A., 2006. Evaluation of carob, Ceratonia siliqua pods as a feed for sheep. Livest. Res. Rural. Dev. 18, 104. https://lrrd.cipav.org.co/lrrd18/7/kara18104.htm

Kotrotsios, N., Christaki, E., Bonos, E., Florou-Paneri, P., 2012. Dietary carob pods on growth performance and meat quality of fattening pigs. Asian-Australas J. Anim. Sci. 25, 880-5. https://doi.org/10.5713/ajas.2011.11521

Kumar, S., Choudhury, P., Carro, M., Griffith, G., Dagar, S., Puniya, M., Calabro, S., Ravella, S., Dhewa, T., Upadhyay, R., Sirohi, S., Kundu, S., Wanapat, M., Puniya, A., 2014. New aspects and strategies for methane mitigation from ruminants. Appl. Microbiol. Biotechnol. 98, 31-44. https://doi.org/10.1007/s00253-013-5365-0

Kurt, Ö., 2023. Nutritional value of carob (Ceratonica siliqua) pods from different growing sites for sheep. Pak. J. Agric. Sci. 60, 649-656. https://doi.org/10.21162/PAKJAS/2 3.52

Lane, J.H., Eynon, L., 1934. Determination of reducing sugars by Fehling's solution with methylene blue indicator. London: Norman Rodger. p. 1-8.

Lee, M.R., Merry, R.J., Davies, D.R., Moorby, J.M., Humphreys, M.O., Theodorou, M.K., Scollan, N.D., 2003. Effect of increasing availability of water-soluble carbohydrates on in vitro rumen fermentation. Animal Feed Sci. Technol. 104, 59-70. https://doi.org/10.1016/S0377-8401(02)00319-X

Lovett, D.K., McGilloway, D., Bortolozzo, A., Hawkins, M., Callan, J., Flynn, B., O'Mara, F.P., 2006. In vitro fermentation patterns and methane production as influenced by cultivar and season of harvest of Lolium perenne L. Grass Forage Sci. 61, 9-21. https://doi.org/10.1111/j.1365-2494.2006.00500.x

Makkar, H.P.S., Blummel, M., Becker, K., 1995. Formation of complexes between polyvinyl pyrrolidones or polyethylene glycols and their implication in gas production and true digestibility in vitro techniques. British Journal of Nutrition 73, 897-913. https://doi.org/10.1079/BJN19950095

Menke, H.H., 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, 7-55.

Menke, K.H., Raab, L., Salewski, A., Steingass, H., Fritz, D., Schneider, W., 1979. The estimation of digestibility and metabolizable energy content of ruminant feedstuffs from the gas production when they incubated with rumen liquor in vitro. Journal of Agricultural Science (Cambridge) 92, 217-222. https://doi.org/10.1017/S0021859600086305

Mills, J., Dijkstra, J., Bannink, A., Cammell, S., Kebreab, E., France, J., 2001. A mechanistic model of whole-tract digestion and methanogenesis in the lactating dairy cow: Model development, evaluation, and application. J. Anim. Sci. 79, 1584-97. https://doi.org/10.2527/2001.7961584x

Naghmouchi, S., Khouja, M.L., Khaldi, A., Rejeb, M.N., Zgoulli, S., Thonart, P., Boussaid, M., 2012. Biochemical diversity of wild carob tree populations and its economic value. Edited by Tony Povilitis pp. 27-42. https://doi.org/10.5772/47929

Newbold, C., López, S., Nelson, N., Ouda, J., Wallace, R., Moss, A., 2005. Propionate precursors and other metabolic intermediates as possible alternative electron acceptors to methanogenesis in ruminal fermentation in vitro. Br. J. Nutr. 94, 27-35. https://doi.org/10.1079/bjn20051445

NRC, 2007. Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids, and New World Camelids, 1st ed. National Academy Press, Washington, DC.

Patra, A., 2012. Enteric methane mitigation technologies for ruminant livestock: A synthesis of current research and future directions. Environ. Monit. Assess. 184, 1929-52. https://doi.org/10.1007/s10661-011-2090-y

Patra, A., Yu, Z., 2013. Effects of gas composition in headspace and bicarbonate concentrations in media on gas and methane production, degradability, and rumen fermentation using in vitro gas production techniques. J. Dairy Sci. 96, 4592-600. https://doi.org/10.3168/jds.2013-6606

Purcell, P.J., Boland, T.M., O'Kiely, P., 2014. The effect of water-soluble carbohydrate concentration and type on in vitro rumen methane output of perennial ryegrass determined using a 24-hour batch-culture gas production technique. Ir. J. Agric. Food Res. 53, 21-36. https://www.jstor.org/stable/24369733

Ramin, M., Krizsan, S.J., Jančík, F., Huhtanen, P., 2013. Measurements of methane emissions from feed samples in filter bags or dispersed in the medium in an in vitro gas production system. J. Dairy Sci. 96, 4643-4646. https://doi.org/10.3168/jds.2013-6556

Richane, A., Ismail, H., Darej, C., Attia, K., Moujahed, N., 2022. Potential of tunisian carob pulp as feed for ruminants: Chemical composition and in vitro assessment. Trop. Anim. Health Prod. 54, 58. https://doi.org/10.1007/s11250-022-03071-4

Rivero, M., Keim, J., Balocchi, O., Lee, M., 2020. In vitro fermentation patterns and methane output of perennial ryegrass differing in water-Soluble carbohydrate and nitrogen concentrations. Animals (Basel) 10, 1076. https://doi.org/10.3390/ani10061076

Saratsis, A., Voutzourakis, N., Theodosiou, T., Stefanakis, A., Sotiraki, S., 2016. The effect of sainfoin (Onobrychis viciifolia) and carob pods (Ceratonia siliqua) feeding regimes on the control of lamb coccidiosis. Parasitol. Res. 115, 2233-42. https://doi.org/10.1007/s00436-016-4966-9

Winer, N., 1980. The potential of the carob (Ceratonia siliqua). Int. Tree Crops J. 1, 15-26. https://doi.org/10.1080/01435698.1980.9752711

Zhao, Y., Nan, X., Yang, L., Zheng, S., Jiang, L., Xiong, B., 2020. A review of enteric methane emission measurement techniques in ruminants. Animals (Basel) 10, 1004. https://doi.org/10.3390/ani10061004