

Nutritional value of carob (*Ceratonica siliqua*) pods from different growing sites for sheep

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The current experiment aimed to determine the effects of the growing site on the chemical composition and fermentation parameters of carob pods. Carob pods from ten different trees were hand harvested from seven different growing sites namely; Silifke, Akdeniz, Tarsus, Aydıncık, Taşucu, Toroslar and Gülnar in Mersin, Turkey in 2019 and oven dried at 50°C for three days. After drying, the carob pods were ground through a 1 mm-wide sieve for analysis and *in vitro* gas production (GP) testing. The growing site had a significant effect on the chemical composition, GP, and digestion values of carob pods. The crude ash (CA), crude protein (CP), ether extracts (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF), and condensed tannin (CT) contents of carob pods ranged from 2.49 to 3.51%, 5.58 to 9.14%, 2.88 to 3.38%, 17.67 to 28.03%, 11.17 to 17.94%, and 1.98 to 5.05%, respectively. The GP, methane (CH₄) (mL), percentage of CH₄, metabolizable energy (ME), and organic matter digestibility (OMD) ranged from 95.66 to 123.0 mL g⁻¹ DM, 16.23 to 20.12 mL g⁻¹ DM, 15.69 to 16.96%, 8.24 to 9.72 MJ kg⁻¹ DM, and 54.53 to 63.40%, respectively. The GP, CH₄ production, and ME contents of carob pods obtained from Silifke, Akdeniz, Tarsus, and Taşucu were significantly higher than those from the other growing sites, whereas the OMD of carob pods from Silifke and Tarsus was significantly higher than that from the other sites. The true digestible substrate (TDS), microbial protein yield (MPY), efficiency of microbial protein yield (EMPY), and true substrate digestibility (TSD) of carob pods ranged from 319.54 to 469.62 mg, 109.07 to 207.09 mg, 32.34 to 44.10%, and 68.43 to 96.11%, respectively. The TDS, MPY, EMPY, and TSD of carob pods from Taşucu were significantly higher than those from the other sites. The current study revealed valuable insight regarding the chemical composition and fermentation parameters of carob pods grown at different sites.

Carob pods can be used in sheep diets to meet the energy requirement of mature sheep for maintenance and lactation whereas protein supplementation is essential when incorporating carob pods into the diets of lactating sheep to fulfill their dietary requirements.

Keywords: Carob, pods, nutrition, digestion, gas production.

INTRODUCTION

Carob, which is a species with high temperature and light requirements, drought-resistant, and sensitive to frost, is a characteristic component of the maquis vegetation found in areas where the true Mediterranean climate prevails in our country (Günel, 2013). It generally thrives in regions where the annual average temperature does not drop below 17°C, the coldest monthly average is above 7°C, the warmest monthly average is below 25°C, and the annual rainfall exceeds 600 mm. The predominant soil types in its distribution areas are red Mediterranean soils and red-brown Mediterranean soils (Günel, 2013). Carob trees are cultivated or naturally grown mainly in the Mediterranean and provide nutrients not only for animals but also for humans (Karabulut *et al.*, 2006;

Obeidat *et al.*, 2012; Youssef *et al.*, 2013). Carob trees produce a considerable number of pods in most parts of the South of Turkey (Karabulut *et al.*, 2006). The carob pod yield per tree ranged from 16.6 to 93 kg depending on soil type (FAOSTAT 2021). Annual carob production of was approximately 14.469 tons (FAOSTAT 2021). Tous *et al* (2008) also reported that the carob pod yield per tree ranged from 13 to 48 kg depending on cultivar. The crude protein (CP), ether extracts (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF), and condensed tannin (CT) contents of carob pods have been reported as 5.9%, 3.5%, 29.2%, 14.4%, and 1.6%, respectively (Karabulut *et al.*, 2006). It was also reported that the crude ash (CA), CP, NDF, and ADF contents of Spanish carob pods range from 2.25 to 5.0%, 1.9 to 7.2%,



27.43 to 50.1%, and 24.13 to 49.47%, respectively (Albanell *et al.*, 1991).

It is well known that food shortages have a detrimental effect on the animal production in Turkey during the drought period as in the most parts of world (Danandeh Mehr *et al.*, 2020). Carob pods may be used in conventional feedstuffs during food shortages to improve the productivity of small ruminants and farmers' profits in the Mediterranean basin since carob pods is one of the locally available feedstuffs (Aloueedat *et al.*, 2019).

Although there are several studies regarding the nutritional value of carob pods, the lack of information regarding pods grown at different sites is one of the limiting factors when considering whether to use pods in sheep rations. There is also limited information concerning the fermentation parameters of carob pods grown at different sites, including CH₄ production, microbial protein yield (MPY), partitioning factor (PF) and true substrate digestibility (TSD). The estimates for the CH₄ production and MPY are very important parameters to evaluate nutritive value of feedstuffs since CH₄ production during fermentation results in loss of 2-12 gross energy intake of animal (Johnson and Johnson 1995)

Kamalak *et al.* (2012) showed that growing site had a significant effect on the chemical composition and fermentation parameters of honey locusts pods. Therefore, it was hypothesized that growing sites may affect the chemical composition and fermentation parameters of carob pods (Kamalak *et al.* 2012).

Generally *in vitro* GP techniques along with chemical composition has been used to evaluate potential nutritive value and fermentation parameters of the uninvestigated feedstuffs used in ruminant animals (Getachew *et al.*, 2002; Salem *et al.*, 2013; Liu *et al.*, 2016; Lopez *et al.*, 2016; Zhang *et al.*, 2016; Tian *et al.*, 2018; Marius *et al.*, 2021; Choudhary *et al.*, 2022). The CH₄ production during fermentation results

in loss of 2-12 % of gross energy intake of animal (Johnson and Johnson 1995).

The current study aimed to determine the effect of the growing site on the chemical composition, *in vitro* GP, MP, TSD, PF, MPY, and efficiency of microbial protein yield (EMPY) of carob pods.

MATERIALS AND METHODS

Collection of carob pods: Carob pods from seventy trees (ten different trees per site) were hand harvested from seven different sites namely; Silifke, Akdeniz, Tarsus, Aydıncık, Taşucu, Toroslar and Gülnar in Mersin, Turkey in 2019 and dried at 50°C for three days in oven. After drying, the carob pods were ground through a 1 mm sieve for analysis and *in vitro* GP test.

Samples were collected from different sites in the research conducted in Mersin, which is located in the Mediterranean Region. Soil structure, climate and rainfall amounts are given in Table 1.

Chemical analysis of carob pods: The current study was conducted at the Department of Animal Science, Faculty of Agriculture University, of Kahramanmaraş Sutcu Imam, Kahramanmaraş, Türkiye. Dry matter (DM), CA, (CP) and EE contents of carob pods were analyzed according to AOAC (1990).

The NDF and ADF contents of carob pods were analyzed by using the method proposed by Van Soest (1991). The water-soluble sugar contents of carob pods were analyzed using Fehling's solution with methylene blue indicator (Lane and Eynon 1934). The CT contents of carob pods were determined using the *Butanol-HCl* method (Makkar *et al.*, 1995).

Determination of gas and methane production: The GP, CH₄ production, true DM digestibility, and MPY were determined using the *in vitro* GP technique (Menke *et al.*1979).

Table 1. Climate and soil properties of the regions sample collected.

Sites	Climate (Average temperature and precipitation °C)	Soil properties	Reference
Slifke-Taşucu	A semi-arid climate within the Mediterranean region; hot and drought in summers and warm and rainy in winters. The mean annual precipitation is around 572 mm.	Holocene alluvial soils	Anonymous, 2008; Anonymous, 2008; Saribas <i>et al.</i> 2013
Akdeniz	Mediterranean (hot and dry summers and mild and rainy winters), the Mean annual temperature is 16°C, and the mean precipitation is 540 mm.	Sand and clay	Anonymous, 2023a
Tarsus	Mediterranean (hot and dry summers and mild and rainy winters) with a mean annual temperature of 18°C, and a mean precipitation value of 610 mm.	Clay, silt, sand and gravel	Kurt <i>et al.</i> 2012
Aydıncık	Mediterranean climate with hot, dry summers and mild, wet winters. The mean annual precipitation 701 mm and average temperature is 19.1°C.	Sand and clay	Koc, 2003; Hatipoglu-Bagci and Sazan, 2014
Toroslar	Mediterranean (hot and dry summers and mild and rainy winters), the Mean annual temperature is 18°C, and the mean precipitation is 596.2 mm.	Zonal, intrazonal and azonal, alluvial	Akiş and Bozlak, 2008
Gülnar	Summers are warm, dry and clear, and winters are very cold, and rainy. the Mean annual temperature is 16°C, and the mean precipitation is 627 mm.	Terra-Rossa	Anonymous, 2023b

Approximately 0.5g of carob pod sample was fermented with 40 mL of buffered rumen fluid in 100 mL glass syringes in triplicate in a water bath set at 39°C for a 24 h. The rumen fluid used in the experiment was sourced from three fistulated three year old male Awassi sheep that were fed a diet composed of grass hay (800 g d⁻¹) and barley (500 g d⁻¹) in one daily meal. Rumen fluid was collected from *each sheep before morning feeding through fistula and pooled* into prewarmed thermos. *The rumen fluid was strained through four layers of cheese cloth before use.*

Three glass syringes without substrate were also included to obtain the blank control samples. The GP was determined after 24 h of fermentation. The proportion of CH₄ in the total GP was measured using an infrared methane analyzer manufactured by Sensor Europe GmbH, Erkrath, Germany, as described by Goel *et al.* (2008). The amount of CH₄ (mL) was computed as follows.

$$\text{CH}_4 \text{ production (mL)} = \text{total GP (mL)} \times \text{percentage of CH}_4 (\%)$$

To estimate the metabolizable energy (ME) and organic matter digestibility (OMD) values, equations proposed by Menke and Steingass (1988) were utilized.

$$\text{ME (MJ kg}^{-1} \text{ DM)} = 1.06 + 0.1570\text{GP} + 0.084\text{CP} + 0.2220\text{EE} - 0.081\text{CA}$$

$$\text{OMD (\%)} = 14.88 + 0.8893\text{GP} + 0.448\text{CP} + 0.651\text{CA}$$

GP: Gas production (mL) of a 0.2 g sample of carob pods after 24 hours of incubation.

Determination of truly degraded substrate: Following a 24-hour fermentation period, the residues from the glass syringes were collected and transferred into a beaker along with 50 mL of NDF solution. This mixture was boiled for an hour and then passed through a pre-weighed sintered glass crucible. The crucibles containing unfermented carob pod samples were then placed in an oven set at 65°C for 12 h to calculate the values for true digestible substrate (TDS), PF, MPY, and EMPY of the carob pod samples.

The TDS, PF, MPY, and EMPY were estimated by using the equations suggested by Blümmel *et al.* (1997), as follows:

True Digestible Substrate (mg) = substrate incubated (mg) – the residue (mg).

$$\text{Partitioning Factor} = (\text{TDS}/\text{GP})$$

$$\text{Microbial Protein Yield (mg)} = (\text{TDS} - (2.2 \times \text{GP}))$$

$$\text{Efficiency of Microbial Protein Yield} = ((\text{TDS} - (2.2 \times \text{GP}))/\text{TDS}) \times 100$$

The experimental protocols conducted in this study were reviewed and approved by the Animal Experimentation Ethics Committee of Kahramanmaraş Sutcu Imam University, Faculty of Agriculture, Türkiye and were conducted in accordance with ethical standards. The approval for the protocols was granted under the reference number 2020/10-01.

Statistical analyses: One-way analysis of variance was performed to examine the data. Statistical significance of the mean differences was assessed by Duncan's test with a significance level of P < 0.05. Statistical analyses were performed IBM SPSS Statistic Version 20.0 (2011).

RESULTS

The effect of the growing districts on chemical composition of carob pods: The chemical composition of carob pods from each growing site is listed (Table 2). The growing site had a substantial effect on the chemical composition of carob pods. The DM contents of carob pods varied between 91.81 and 96.48%, with the highest content observed for Tasucu and the lowest observed for Aydıncık and Toroslar (P < 0.001). The CA contents of carob pod ranged from 2.49 to 3.51%, with the highest values obtained for Tarsus (P < 0.001). The CP contents of carob pods ranged from 5.58 to 9.14%, with the highest values obtained for Toroslar. The EE of carob pods varied between 2.88 to 3.38%, with the highest values obtained for Toroslar (P < 0.001). The NDF and ADF contents of carob pods varied between 17.67 to 28.03% and 11.17 to 17.94%, respectively, with the highest values obtained from Akdeniz (P < 0.001). The CT content ranged from 1.98 to 5.05%, and the highest values were obtained from Tasucu (P < 0.001).

Effect of the growing site on GP, CH₄, ME, digestibility, PF, and MPY of carob pods: The effect of the growing site on GP, CH₄, ME, OMD, TSD, PF, MPY, and EMPY of carob

Table 2. Effect of the growing site on the chemical composition of carob pods.

Sites	Rate of carob pods (%)							
	DM (%)	CA (%)	CP (%)	EE (%)	NDF (%)	ADF (%)	WSC (%)	CT (%)
Silifke	95.39 ^b	3.07 ^b	7.10 ^d	2.98 ^d	22.62 ^c	14.99 ^b	27.79 ^c	2.29 ^{bc}
Akdeniz	95.82 ^b	3.24 ^b	7.19 ^d	3.03 ^d	28.03 ^a	17.94 ^a	25.19 ^d	1.98 ^c
Tarsus	95.44 ^b	3.51 ^a	7.61 ^c	3.28 ^b	22.95 ^{bc}	15.76 ^b	28.04 ^c	2.90 ^b
Aydıncık	91.81 ^d	3.03 ^b	6.31 ^e	2.88 ^e	19.28 ^d	11.68 ^c	30.69 ^a	2.85 ^b
Tasucu	96.48 ^a	2.49 ^c	5.58 ^f	3.05 ^d	18.28 ^{de}	11.17 ^c	31.66 ^a	5.05 ^a
Toroslar	91.98 ^d	3.15 ^b	9.14 ^a	3.38 ^a	23.78 ^b	16.00 ^b	26.03 ^d	2.49 ^{bc}
Gülнар	92.38 ^b	3.07 ^b	8.02 ^b	3.18 ^c	17.67 ^e	11.89 ^c	29.40 ^b	2.03 ^c
SEM	0.196	0.112	0.133	0.040	0.510	0.521	0.456	0.275
<i>p</i>	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Sig.	***	***	***	***	***	***	***	***

^{a b c} Column means with common superscripts do not differ (P > 0.05), *** P < 0.001. SEM: Standard error mean. Sig: Significant

Table 3. Effect of the growing site on GP, CH₄, ME, digestibility, PF, and MPY of carob pods.

Sites	Parameters									
	GP (mL)	CH ₄ (mL)	CH ₄ (%)	ME (MJ kg ⁻¹ DM)	OMD (%)	TDS (mg)	PF	MPY (mg)	EMPY (%)	TSD (%)
Silifke	123.00 ^a	20.12 ^a	16.35 ^{ab}	9.72 ^a	63.40 ^a	399.95 ^{bc}	3.25 ^e	129.35 ^c	32.34 ^e	83.07 ^b
Akdeniz	115.33 ^b	19.32 ^a	16.75 ^a	9.22 ^b	60.73 ^b	412.63 ^b	3.58 ^c	158.89 ^b	41.28 ^{ab}	85.06 ^b
Tarsus	122.00 ^{ab}	19.25 ^a	15.78 ^b	9.68 ^a	63.30 ^a	405.15 ^b	3.32 ^{de}	136.75 ^c	33.75 ^{de}	83.55 ^b
Aydıncık	104.66 ^c	16.42 ^b	15.69 ^b	8.47 ^c	56.40 ^c	392.19 ^c	3.75 ^b	161.93 ^b	41.28 ^{ab}	84.25 ^b
Tasucu	119.33 ^{ab}	20.09 ^a	16.83 ^a	9.40 ^b	60.90 ^b	469.62 ^a	3.93 ^a	207.09 ^a	44.10 ^a	96.11 ^a
Toroslar	95.66 ^d	16.23 ^b	16.96 ^a	8.24 ^c	54.53 ^d	319.54 ^c	3.34 ^{de}	109.07 ^d	34.12 ^{de}	68.43 ^d
Gülнар	100.00 ^{cd}	16.84 ^b	16.84 ^a	8.47 ^c	56.03 ^c	346.34 ^d	3.46 ^{cd}	126.34 ^c	36.48 ^{cd}	74.96 ^c
SEM	1.984	0.516	0.260	0.118	0.679	4.800	0.052	3.910	0.922	0.954
<i>p</i>	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Sig.	***	***	NS	***	***	***	***	***	***	***

^{a b c} Column means with common superscripts do not differ ($P > 0.05$), *** $P < 0.001$. SEM: standard error mean, Sig: Significant, NS: Non-significant. The relationships among estimated fermentation parameters were shown in Figure 1-5.

pods is characterized (Table 3). The growing site had a significant effect on GP, CH₄, ME, OMD, TSD, PF, MPY, and EMPY ($P < 0.001$).

The GP, CH₄ production, percentage of CH₄, ME, and OMD ranged from 95.66 to 123.0 mL g⁻¹ DM, 16.23 to 20.12 mL g⁻¹ DM, 15.69 and 16.96%, 8.24 to 9.72 MJ kg⁻¹ DM, 54.53 to 63.40%, respectively. The GP, CH₄ production, and ME contents of carob pods obtained from Silifke, Akdeniz, Tarsus, and Tasucu were significantly higher than those from the other sites, whereas the OMD values of carob pods obtained from Silifke and Tarsus were significantly higher than that from the other sites. The TDS, MPY, EMPY, and TSD of carob pods ranged from 319.54 to 469.62 mg, 109.07 to 207.09 mg, 32.34 to 44.10%, and 68.43 to 96.11%, respectively. The TDS, MPY, EMPY, and TSD of carob pods from Tasucu were significantly higher than those from the other sites ($P < 0.001$).

DISCUSSION

The hypothesis that the growing site affects the chemical composition and fermentation parameters of carob pods was proven. There are significant variations among carob pods growing in different sites in terms of chemical composition. In their study, Kamalak *et al.* (2012) observed notable disparities in the chemical composition of carob pods, which were determined to be influenced by the specific growth location. They suggested that these differences in composition could be attributed to the varying climatic conditions and soil types present at each growing site. Considering the information given in Table 1, it can be thought that it may have an effect on Soil structure, climate and rainfall differences in chemical composition and fermentation parameters.

The CA, CP, and EE contents of carob pods studied in the current report were consistent with the findings obtained by Youssef *et al.* (2013), Karabulut *et al.* (2006), and Medjekal *et al.* (2018), whereas the NDF and ADF contents of carob

pods, except for those from Akdeniz, were significantly lower than those obtained by Karabulut *et al.* (2006) and Medjekal *et al.* (2018). Furthermore, the CT contents of carob pods were considerably lower than those obtained by Karabulut *et al.* (2006). Differences in NDF, ADF and CT contents are probably related to plant variety, growing location conditions like dry or semi-dry region, and differences in early or late harvest stage. According to El-kamShatnawi and Mohawesh (2000), ewes necessitate 7-9% CP for maintenance and 10-12% for lactation. The CP contents of carob pods, with the exception of those from Aydıncık and Tasucu, met the minimum maintenance protein requirements but fell short of those needed for lactation (Table 2). Moreover, Norton (2003) reported that feedstuffs containing less than 8% CP fail to provide the necessary levels of ammonia required by rumen microorganisms for optimal activity. Thus, protein supplementation is essential when incorporating carob pods into the diets of lactating sheep to fulfill their dietary requirements.

It was reported that low levels of CT in diets may form complexes with protein and prevent the extensive degradation of protein in the rumen (Singleton VL 1981; Lohan *et al.*, 1983; Barry and Duncan 1984). The CT contents of carob pods are within the optimal range for rumen escape protein according to Barry *et al.* (1984), except for the samples from Tasucu, where the carob pod content was 5.05% of the DM. Therefore, the low level of CT in carob pods may have a beneficial effect on bypass protein.

NRC (2007) suggests that the energy concentration of diets should be 7958 MJ kg⁻¹ to meet the requirement of mature sheep with 0.71-1.32 kg of milk yield for maintenance and lactation. As can be seen from Table 3 the energy contents of carob pods were considerably higher than those required by mature sheep with 0.71-1.32 kg of milk yield for maintenance and lactation.

The differences in fermentation parameters were attributed to differences in the chemical compositions of carob pods grown at different sites. The GPs of carob pods obtained in the

current work were in line with the findings of Karabulut *et al.* (2006) and Medjekal *et al.* (2018). According to Lopez *et al.* (2010), it has been suggested that certain feedstuffs may possess an ability to reduce CH₄ production. This is indicated by a CH₄ percentage of less than 14% in the total gas produced during a 24-hour incubation period. The findings of this study suggest that carob pods are unlikely to possess anti-methanogenic potential, as the percentage of CH₄ in the total GP was high. The PFs of carob pods ranged from 3.25 to 3.93, which was significantly higher in carob pods from Tasucu compared with the other sites. In this study, the PF of carob pods were found to be within the theoretical range of 2.74 to 4.65, as reported by Blümmel *et al.* (1997).

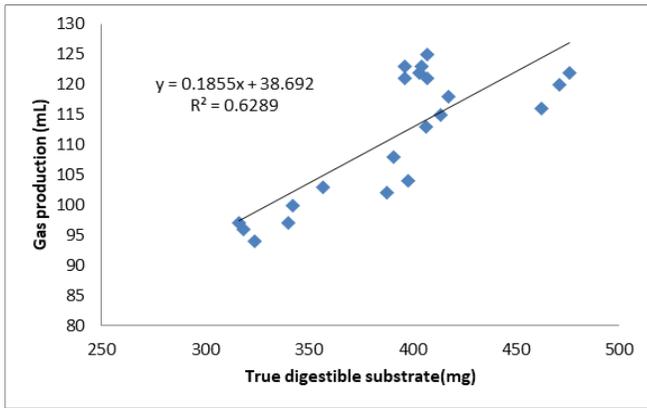


Figure 1. Relationship between Gas production (GP) and True digestible substrate (TDS). The X-axis is the GP (mL) measured by Sensor Europe GmbH, Erkrath, Germany. The Y-axis is TDS (mg) = substrate incubated (mg) – the residue (mg).

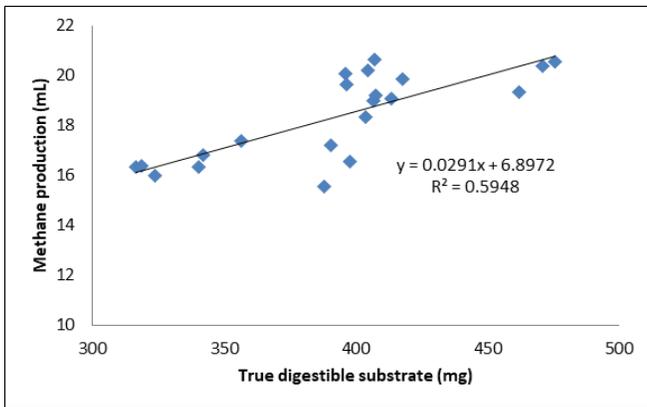


Figure 2. Relationship between CH₄ production and True digestible substrate (TDS). The X-axis is the CH₄ (mL) measured by Sensor Europe GmbH, Erkrath, Germany. The Y-axis is TDS (mg) = substrate incubated (mg) – the residue (mg).

The relationships between gas or CH₄ production and TDS are presented in Figures 1 and Figures 2. There is a linear correlation between GP and TDS. The more substrate is available to microorganisms, the more gas and CH₄ are produced. The amount of energy loss as gas and CH₄ depends on the extent of fermentable substrate.

The relationships between MPY or EMPY and PF are shown in Figures 3 and 4. The PF represents the percentage of nutrients that are allocated to the GP and MPY. Higher PF values indicate a greater proportion of the substrate being directed towards microbial mass, resulting in higher EMPY as well.

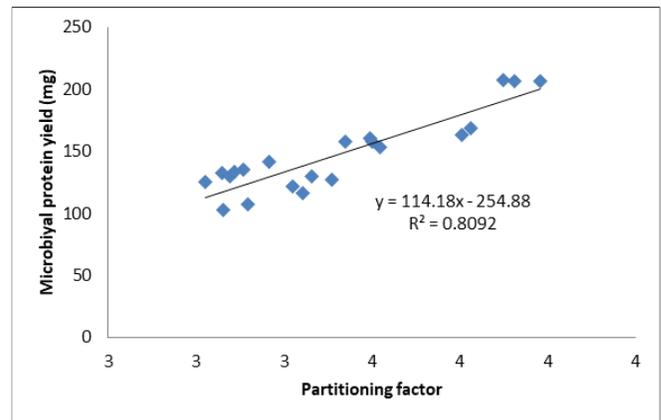


Figure 3. Relationship between Partitioning factor (PF) and Microbial protein yield (MPY). PF values indicate a greater proportion of the substrate being directed towards microbial mass. MPY (mg) were determined using the in vitro GP technique suggested by Menke *et al.* (1979).

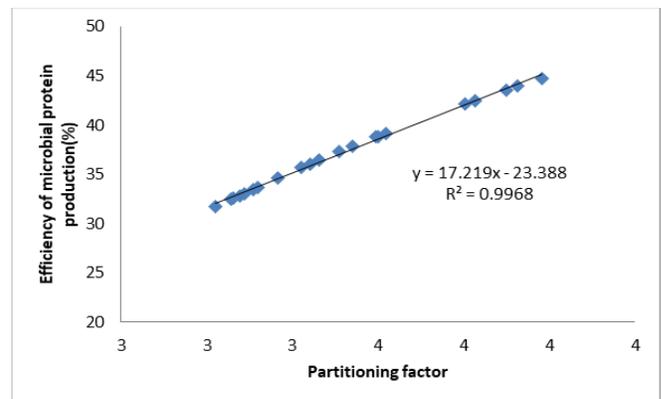


Figure 4. Relationship between Partitioning factor (PF) and Efficiency of microbial protein production (EMPY). PF values indicate a greater proportion of the substrate being directed towards microbial mass. EMPY (%) were determined using the in vitro GP technique suggested by Menke *et al.* (1979).

The relationships between the TDS and MPY or EMPY are shown in Figures 5 and 6.

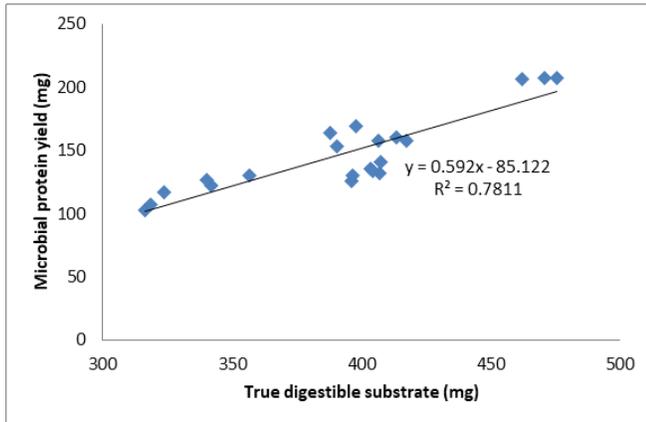


Figure 5. Relationship between True digestible substrate (TDS) and Microbial protein yield (MPY). The X-axis is TDS (mg) = substrate incubated (mg) – the residue (mg). The Y-axis is MPY (mg) were determined using the *in vitro* GP technique suggested by Menke *et al.* (1979).

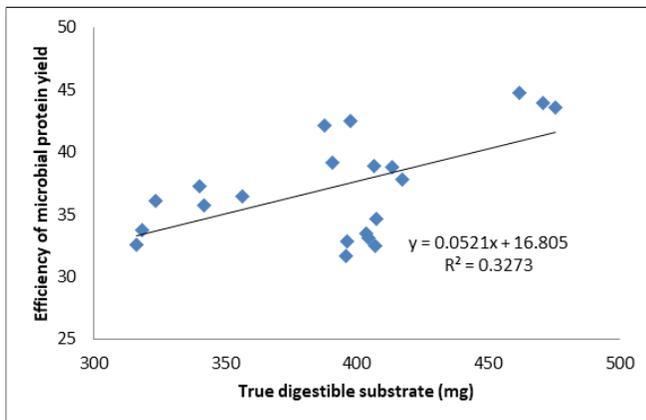


Figure 6. Relationship between True digestible substrate (TDS) and Efficiency of microbial protein production (EMPY). The X-axis is TDS (mg) = substrate incubated (mg) – the residue (mg). The Y-axis is EMPY were estimated by using the equations suggested by Blümmel *et al.* (1997).

Conclusion: The current study provided valuable insight into the chemical composition and fermentation parameters of carob pods grown at different sites. The growing site had a substantial effect on the chemical composition including GP, CH₄ production, ME, OMD, TSD, PF, MPY, and EMPY. Carob pods can be used in sheep diets to meet the energy requirement of mature sheep for maintenance and lactation whereas protein supplementation is essential when incorporating carob pods into the diets of lactating sheep to

fulfill their dietary requirements. However further *in vivo* trials are required to determine the optimum inclusion level of carob pods in sheep diets.

Conflicts of interest: The Authors declare that there is no conflict of interest.

Authors' Contribution Statements: Özer Kurt conceived the idea and executed the field research with laboratory analyses.

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