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The Effect of Safflower on the *In Vitro* Digestion Parameters and Methane Production in Horse and Ruminant

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Abstract

The aim of this study was to determine the effects of using 5%, 10%, and 20% of safflower (*Carthamus tinctorius* L. Dinçer; without thorns) grain, and its hay and straw, on the *in vitro* fermentation parameters in the diets of horses and ruminants. The addition of up to 5% of crushed safflower grain to a horse's diet had no negative effect on the *in vitro* total gas production, true dry matter digestion (T-DMD), metabolic energy (ME), gas yield at 24 h (GY_{24}), partial factor (PF_{24}), microbial crude protein production (MCP) and short chain fatty acid composition (SCFA) of digestion fluid; however, increasing the grain content negatively affected certain parameters ($p<0.05$). The hay and straw at 5%–20% ratio in a horse's diet had a positive effect on *in vitro* gas production, ME, SCFA, and GY_{24}. We observed that 5% safflower grain in ruminants' diets did not negatively affect the *in vitro* cumulative gas production up to 96 h, T-DMD, true organic matter digestion (T-OMD), ME, net energy lactation (NEL), GY_{24}, PF_{24}, and MCP values and SCFA compositions; but 10% and 20% levels negatively affected the *in vitro* gas production, ME, NEL, and SCFA values ($p<0.05$). The use of up to 20% hay and straw had no negative effect on the parameters ($p>0.05$). Using safflower grain, hay and straw in horse and ruminants' diets did not affect the *in vitro* methane production ($p>0.05$). Consequently, using up to 5% safflower grain, and 20% hay and straw has the potential as a feed source in the diets of horses and ruminants.

Keywords: Digestion, horse, *in vitro* gas production, ruminant, safflower

Introduction

Safflower is *Carthamus tinctorious* L species in the *Compositae (*or *Asteraceae*) family of the *Campanulatae (Asterales*) order. The gene centre of safflower is known as Africa, the Middle- East and Asian continents; it can be planted in winter or summer, or as a crop rotation plant. This plant, which can be grown in different environmental and soil conditions, is one of the earliest crops used by humans. It is an annual and is stake rooted; and there are thorny and thorn-less species and it is an oil-seed plant which can include 70-80% linoleic acid or 80% oleic acid in oils (Baumler et al., 2006; Gilbert, 2008; Gumus and Kucukersan, 2016; Landau et al., 2004; Sahebi et al., 2011). This oil-seed plant can adapt more easily to different soil conditions than those of other oil-seed plants. Safflower, which has attracted attention as a food crop resistant to drought, is of extreme importance today due to the effects of global warm (Altin et al., 2012).

The grain of safflower is described as a hulled seed (*achene*) due to its covering with a hull layer. This seed is shaped like a sunflower seed, but is white-coloured and is smaller and harder than a sunflower seed. Previous studies have found the hull ratio in safflower grains to be 33-60% (Gumus and Kucukersan, 2016). The safflower grain contains 13-19% crude protein (CP), 24-28% ether extract (EE), 42% neutral detergent fibre (NDF), 32% acid detergent fibre (ADF) (Bozan and Temelli, 2008; Dschaak et al., 2011). Recently, safflower varieties have been developed with high oil levels (47% EE) and low fibre (25% NDF, 18% ADF) (Dschaak et al., 2011). The safflower herbage includes 9.5-13.8% CP, 37.2-42.1% NDF, 0.4-0.7% tannin and 0.2-0.4% non-protein nitrogen in total nitrogen (Asgharzadeh et al., 2013). Quality

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herbage can be obtained from safflower and despite its thorny leaves can be consumed by sheep and goats. In previous study, the preference for and rejection of safflower herbage (especially stems) by sheep and dairy cattle was determined to be very close to that of wheat straw (Landau et al., 2005). The herbage or hay of safflower has been used as forage in the diets of cows in Australia and sheep in Italy (Landau et al., 2004 and 2005).

The drought in the Mediterranean Levant has reached its the highest level for the last 900 years, and semi-arid soils have turned to arid; and arid soils have turned to desert (Altin et al., 2012; Cook et al., 2016; IPCC. 2014). The importance of safflower, which is an oilseed plant cultivated on moorland and arid/semi-arid lands, has increased in arid and semi-arid countries. In countries which experience drought safflower stands out as an alternative culture plant in terms of oil for human nutrition and feed (grain and forage) for herbivorous nutrition. Recently, the plantation areas and amount of production in the harvest of the safflower plant in areas which have arid and semi-arid climatic conditions of Turkey, have also increased (Gumus and Kucukersan, 2016; TSI, 2016). In 2015, the safflower plant was cultivated on 0.43 million decares and a total of 70 thousand tonnes (162 kg/decare) was harvested in Turkey (TSI, 2016). The planting area of safflower in Turkey is increasing day by day. In the study, the use of up to 5%, 10% and 20% of safflower grain, safflower herbage and safflower straw in horse and ruminant total mix ration (TMR) aimed to determine the effect on *in vitro* digestion parameters.

Materials and Methods

The scientific procedures of the study were conducted according to research protocol approved (Date: January 14, 2015; Decision number: 15/10) by the Local Ethics Committee for Animal Experiments of Erciyes University.

The samples of safflower herbage, safflower straw and safflower grain

The safflower (*Carthamus tinctorius* L. Dinçer) samples used in the present study were collected from the province of Kırşehir, Turkey. Kırşehir is located (38°49″-39°48″ north latitudes, 33°25″ - 34°43″ east longitudes) in Turkey's Central Anatolia region. The Dinçer type safflower is without thorns and grows 985 m above sea level. Steppe and dry forests are the dominant vegetation in this location. Arid conditions and desert-like steppe vegetation are dominant in the Kırşehir province due to temperature and rainfall (Altin et al., 2012). Samples of safflower herbage obtained by cutting the green safflower plant in the pre-flowering stage (Figure 1) using scissors. The herbage samples were cut 1 cm above the soil, included the aerial parts (leaf, stem, preflowering bud) of the plants. Fresh-wet herbage was dried and then used in chemical and digestion analyses of the study. Safflower grain and straw were mature grains obtained after the safflower plant was harvested (Figure 2, 3).

Chemical analysis

The samples of safflower herbage were dried in an oven (Binder, Germany) for 24 hours at 55°C and then 8 hours at 105°C. The grain and straw samples of safflower which were ground to size to pass through a 1 mm sieve (IKA Werke, Staufen im Breisgau, Germany), were also dried in an oven for 24 hours at 105°C. The safflower herbage was ground to size to pass through a 1 mm sieve (IKA Werke, Staufen im Breisgau, Germany) and then dried for 24 hours at 105°C. After this procedure, the dry matter (DM) values of these samples were calculated. The ash, crude protein (CP) and ether extract (EE) contents were detected according to the Association of Official Analytical Chemists (AOAC 1995; method 920.39; method 942.05; method 942.01). The neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL) contents, were analysed using a glass crucible on an FIWE3 fibre analyser (Velp, Italy) (Van-Soest et al., 1991). The NDF was detected using sodium sulphite and thermo-stable α-amylase (Megazyme, Ireland) (called as "aNDF"). The aNDF, ADF, and ADL contents were corrected for ash residue (called as "aNDFom, ADFom, and ADL", respectively). Analyses were carried out in triplicate.

The metabolic energy (ME) values were calculated using the following formula (MAFF, 1984) by the nutrient contents determined by analysis of the grain, herbage and straw of the safflower.

ME (kcal/kg DM): 3227 + 62.86 * EE% - 31.79 * ash% - 32.50 * ADFom% (MAFF, 1984).

In vitro digestion

Ruminant and horse rations used in the study and added rations, including 5%, 10% and 20% of the safflower grain, safflower herbage and safflower straw are given in Table 1 and 2.

In vitro digestion technique for ruminants

As inoculum, fresh rumen fluid was used. Rumen fluid (approximately 1.0 L) was obtained from two beef cattle (*Hereford*) fed with a diet containing an 80% concentrated mix feed and 20% forage in DM applied in intensive fattening. Rumen fluid was obtained via a stomach tube into two hours after the morning feeding and collected in a thermos including water at 39°C using CO_2 gas, and filtered with four layers of cheesecloth in the laboratory. The total mix ration (TMR) for beef cattle evaluated to determine *in vitro* digestion in ruminants is given in Table 1. This TMR was prepared for fattening cattle which are 12 months of age, with 400 kg of live weight and 1.4 kg of live weight gain.

The *in vitro* digestion technique performed in the current study is the Hohenheim *in vitro* gas production technique (Menke et al., 1988), which incubated filtered rumen fluid (10 mL), buffer mixture (20 mL) and substrate (milled feed sample, 200±10 mg). This buffer mixture includes 474 mL of bi-distilled water, 237.33 mL of macro-mineral solution, 237.33 mL of buffer solution, 0.12 mL of trace-mineral solution, 1.22 mL of resazurin solution and 50 mL of reducing solution in one litre. Dried samples were incubated in rumen fluid and buffer mixture in 100 mL glass syringes (Model For-

Figure 1. Safflower (*Carthamus tinctorius* L. Dinçer) herbage

Figure 2. Safflower (*Carthamus tinctorius* L. Dinçer) grain

Figure 3. Safflower (*Carthamus tinctorius* L. Dinçer) straw

tuna, Germany) (n=6) (triplicate; cumulative gas production plus triplicate; dry matter-organic matter loss). The three blank syringes (no template; rumen fluid plus buffer mixture) were incubated to calculate the total gas production. The syringes were incubated in a water bath with a thermostat (Special Waterbath, Yapar Stainless Steel Ltd., Kahramanmaraş, Turkey), which has a stainless reservoir, at 39°C for up to 96 h.

Table 1. The supplementation of safflower to beef cattle total mix ration

		Supplementation of safflower								
	Feed kg/day	Safflower grain			Safflower herbage			Safflower straw		
Feeds	**(as DM)**	**5%**	**10%**	**20%**	**5%**	**10%**	**20%**	**5%**	**10%**	**20%**
Safflower	-	0.50	1.00	2.00	0.5	1.00	2.00	0.50	1.00	2.00
Corn silage	1.20	1.20	1.20	1.20	0.70	0.20	-	1.20	1.20	1.00
Wheat straw	1.80	1.80	1.80	2.25	1.80	1.80	1.00	1.30	0.80	-
Barley grain	3.15	2.65	2.15	0.00	3.15	3.15	3.15	3.15	3.15	3.15
Concentrated feed mix*	3.60	3.60	3.10	2.80	3.60	3.60	3.60	3.60	3.60	3.60
Cotton seed meal	-	-	0.50	1.50	-	-	-	-	-	-
Total feed kg/day (as DM)	9.75	9.75	9.75	9.75	9.75	9.75	9.75	9.75	9.75	9.75
Crude protein and energy composition (Calculated)										
CP (% DM)	12.00	11.58	11.66	11.96	12.03	12.06	12.45	12.01	12.02	11.98
ME (kcal/kg DM)	2457.00	2498.00	2515.00	2488.00	2449.00	2441.00	2483.00	2465.00	2472.00	2473.00
Nutrient composition analysed										
Ash	7.25									
CP	11.50									
EE	2.64									
aNDFom	36.15									
ADFom	20.58									
ADL	3.29									

CP: crude protein as %; aNDFom: assayed with a heat stable amylase and expressed exclusive of residual ash as %; ADFom: ADF expressed exclusive of residual ash as %; ADL: acid detergent lignin as %; EE: diethyl ether extract as %.
*: Beef cattle concentrated feed mixture included 15%CP and 2700 kcal/kg ME.

***In vitro* digestion technique for horses**

The *in vitro* digestion technique in horses was carried out according to Sunvold et al. (1995) and Sweney (2012), which incubated feed sample in faeces inoculum and fermentation medium, which included solution A, solution B, trace mineral solution, water-soluble vitamins, folate:biotin solution, riboflavin solution, hemin solution, short-chain fatty acids, rezurine, yeast extract, trypticase, Na_2CO_3 and Cystein HCl*H_2O (Table 3). The faeces samples used as an inoculum in the current study were obtained from two thoroughbred horses (6-7 years of age, 480-500 kg in body weight) that were fed with a diet containing 70% forage and 30% concentrate feed, in DM basis. Faeces samples were collected soon after defecation and transferred into a thermos containing water at 39°C under CO_2 gas and transferred to the laboratory. Faeces samples were diluted at a 1:10 ratio with 0.9% sterile serum physiologic solution (Polifleks, Polifarma, Turkey) using a laboratory type blender (Waring Products Division, Torrington C.T., USA). Diluted faeces inoculum was filtered through four layers of cheesecloth under constant CO_2 gas (anaerobically) and used in the *in vitro* digestion technique.

The horse *in vitro* digestion technique was carried out in glass syringes with 100 ml volume (Model Fortuna, Haberle Labortechnik, Germany). The samples (500±10 mg as DM) were incubated with a medium mixture (30 mL) and faeces inoculum (5 mL) in glass syringes (n=6). The syringes were closed using clips and then the initial volume recorded and incubated in a water bath with a thermostat (Special Waterbath, Yapar Stainless Steel Ltd., Kahramanmaraş, Turkey), which has a stainless reservoir, at 39.0±0.2°C for up to 48h. In addition, six blank syringes (no template; medium mixture plus faeces inoculum) were used to calculate the total gas production.

Determination of cumulative gas production

In *in vitro* incubations, the total gas volume was recorded from the calibrated scale on the syringe at 3, 6, 12, 24, 48, 72, and 96 hours for ruminants and at 6, 12, 18, 24, 36, and 48 hours for horses.

Determination of methane production

After measuring the total gas volume at 24 h, the tubing of the plastic syringe outlet was inserted into the inlet of the methane analyser (Sensor, Europe GmbH, Erkrath, Germany) and the piston was pushed to insert the accumulated gas into the analyser.

Determination of *in vitro* true dry matter disappearance and *in vitro* true organic matter disappearance values

Three of the *in vitro* fermentation syringes for both ruminants

Table 2. The supplementation of safflower to horse total mix ration

		Supplementation of safflower								
	Feed kg/day	Safflower grain			Safflower herbage			Safflower straw		
Feeds	**(as DM)**	**5%**	**10%**	**20%**	**5%**	**10%**	**20%**	**5%**	**10%**	**20%**
Safflower		0.50	0.90	1.80	0.50	0.90	1.80	0.50	0.90	1.80
Wheat straw	3.00	3.00	3.00	3.50	3.00	2.65	2.30	2.50	2.30	2.20
Grass hay, mature	3.00	3.00	2.70	2.00	2.50	2.50	2.50	3.00	2.80	2.00
Alfalfa hay, mid maturity	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Barley grain	0.50	-	-	-	0.50	0.50	-	0.50	0.50	0.50
Vegetable oil	0.10	0.10	-	-	0.10	0.05	-	0.10	0.10	0.10
Oat grain	0.50	0.50	0.50	-	0.50	0.50	0.50	0.50	0.50	0.50
Cotton seed meal	0.50	0.50	0.50	0.30	0.50	0.50	0.50	0.50	0.50	0.50
Total feed kg/day (as DM)	9.10	9.10	9.10	9.10	9.10	9.10	9.10	9.10	9.10	9.10
Crude protein and energy composition (Calculated)										
CP (% DM)	12.31	12.30	12.48	11.57	12.16	12.36	12.34	12.30	12.15	12.00
DE (kcal/kg DM)	2043.00	2022.00	2048.00	2088.00	2069.00	2069.00	2012.00	2065.00	2074.00	2078.00
Nutrient composition analysed										
Ash	10.69									
CP	10.62									
EE	3.34									
aNDFom	30.26									
ADFom	23.81									
ADL	4.68									

CP: crude protein as %; aNDFom: assayed with a heat stable amylase and expressed exclusive of residual ash as %; ADFom: ADF expressed exclusive of residual ash as %; ADL: acid detergent lignin as %; EE: diethyl ether extract as %; DE: digestible energy; DE=ME/0.80.

and horses were stopped after 24 h. The *in vitro* true dry matter disappearance (T-DMd) and the *in vitro* true organic matter disappearance (T-OMd) values of substrates were calculated at 24 h of incubations.

The *in vitro* dry matter- and organic matter - disappearance was determined by filtering the fermentation residues using a vacuum unit (Velp Dietary Fibre Analyzer, Italy) on pre-weighed glass crucibles (Velp, porosity #2, Italy) the fermentation residues, which was dried at 105°C and burning the residual at 550°C. *In vitro* T-DMd was calculated as 1 - [(DM residue - DM blank)/initial DM)] x 100. *In vitro* T-OMd was calculated as 1 - [(OM residue - OM blank)/ initial OM)] x 100.

Determination of estimated digestion values and end-products

The ME and OMD contents of the samples were calculated using the equations of Menke and Steingass (1988).

The gas yields (GY_{24}), partial factor (PF_{24}), and microbial crude protein production levels (MCP) of the samples at 24 h were calculated using the equations:

$GY_{24} = [(GP_{24} \times 10^3) : T\text{-}DMd]$

$PF_{24} = T\text{-}DMd : GP_{24}$

MCP (mg/g DM) = mg T-DMd - (mL gas × 2.2 mg/mL)

T-DMd: *in vitro* dry matter disappearance (mg) for g DM at 24 h (mg/g DM)

GP_{24}: volume (mL) of total gas produced by g DM at 24 h (mL/g DM)

The molarities of estimated short chain fatty acid (SCFA) produced by substrate at 24 hours of *in vitro* fermentations were calculated using the following formula of Getachew et al. (2008):

SCFA (mmol/0.2 g DM) = 0.0222 GP - 0.00425

The GP is net gas production at 24 h (mL/0.2 g DM)

Statistical analysis

The experiment data were first subjected to Levene's test to detect the variance homogeneity. One-way variance analyses (ANOVA) were implemented for homogeneous variances by General Linear Model procedures to test treatment differences.

Table 3. Composition of *in vitro* fermentation medium

Component	mL/L	Amount
Solution A[a]		330.0
Solution B[b]		330.0
Trace mineral solution[c]		10.0
Water-soluble vitamins[d]		20.0
Folate: biotin solution[e]		5.0
Riboflavin solution[f]		5.0
Hemin solution[g]		2.5
Short chain fatty acids[h]		0.4
Resazurine[i]		1.0
Distilled H_2O		296.0
	g/L	
Yeast extract		0.5
Trypticase		0.5
Na_2CO_3		4.0
Cystein HCl*H_2O		0.5

[a]Composition (g/L): NaCl, 5.4; KH_2PO_4, 2.7; CaCl2*H_2O, 0.16; $MgCl_2$*$6H_2O$, 0.12; $MnCl_2$*$4H_2O$, 0.06; $CoCl_2$*$6H_2O$, 0.06; (NH4)2SO_4, 5.4.
[b]Composition: K_2HPO_4, 2.7 g/L.
[c]Composition (mg/L): ethylene diamine tetraacetic acid (disodium salt), 500; $FeSO_4$*$7H_2O$, 200; $ZnSO_4$*$7H_2O$, 10; $MnCl_2$*$4H_2O$, 3;H_3PO_4, 30; $CoCl_2$*$6H_2O$, 20; $CuCl_2$*$2H_2O$, 1; $NiCl_2$*$6H_2O$, 2; Na_2MoO_4*$2H_2O$, 3.
[d]Composition (mg/L): thiamin-HCl, 100; d-pantothenic acid, 100; niacin, 100; pyridoxine, 100; p- aminobenzoic acid, 5; vitamin B12, 0.25.
[e]Composition (mg/L): folic acid, 10; d-biotin, 2; NH_4HCO_3, 100.
[f]Composition: riboflavin, 10 mg/L in 5 mmol/L of Hepes.
[g]Hemin: Hemin 500 mg/L of 10 mmol/L NaOH
[h]Composition: n-valerate, isovalerate, isobutyrate and DL alpha- methylbutyrate, 250 mL/L
[i]Composition: 1 g resazurine/L distilled water

Data was analyzed based on the statistical model: Y*ij* = µ*ij* +S*i* + e*i*. Where, Y*ij* = the general mean common for each parameter under investigation. S_i = the *i*th effect of the safflower grain, safflower herbage or safflower straw on the observed parameters, and e*i* = the standard error term. The means were separated by Tukey's multiple range test at p<0.05. Analyses were performed using Statistical Package for the Social Sciences (SPSS) 17.0 software (IBM Corp.; Armonk, NY, USA).

Results

The nutrient compositions of safflower grain, safflower herbage and safflower straw are given in Table 4.

In vitro digestion parameters in horse

Supplementation of up to 20% crushed safflower grain in horse ration did not have a linear effect on cumulative total gas production for the first 12 hours (p>0.05). *In vitro* total gas production during the 12th and 18th hours of incubation was higher than that of 0% safflower grain (p<0.05; quadratic); but the increasing dose of safflower grain was negatively affected

Table 4. The nutrient matter and energy composition of safflower grain, safflower herbage and safflower straw used in study

	Safflower grain	Safflower herbage	Safflower straw
CP	12.30	8.10	3.74
Ash	2.02	8.82	7.71
EE	27.42	2.13	1.37
aNDFom	49.33	39.05	49.98
ADFom	40.53	31.99	44.29
ADL	13.24	4.75	6.67
ME	3569.18	2040.83	1628.59

CP: crude protein as % in dry matter; aNDFom: assayed with a heat stable amylase and expressed exclusive of residual ash as % in dry matter; ADFom: ADF expressed exclusive of residual ash as % in dry matter; ADL: acid detergent lignin as % in dry matter; EE: diethyl ether extract as % in dry matter; ME: metabolisable energy as kcal/kg in dry matter

(p<0.05; linear). Although *in vitro* total gas produced by 5% safflower grain supplementation to horse TMR was similar to the control ration (0% safflower grain) at 24 - 48 hours of incubation, those produced by 10% and 20% safflower grain were low linearly than that of control ration (p<0.05) (Table 5).

Safflower herbage supplementation to horse TMR positively affected *in vitro* cumulative total gas production during the all incubation (6, 12, 18, 24, 36 and 48 hours) (p<0.05).

In horses, at 24 hours of incubation, *in vitro* total gas production of TMR with safflower herbage reached 183-195 mL/g DM, and this production level reached 229-253 mL/g DM at 48 hours (Table 5).

Up to 20% safflower straw was used in horse TMR and increased linearly the *in vitro* cumulative gas production at 6, 12, 18 and 24 hours of incubation (p<0.05). At the 36th and 48th hours of incubation, the horse TMR with up to 20% safflower straw did not negatively affect *in vitro* cumulative gas production (p>0.05) (Table 5).

Supplementation of up to 20% of crushed safflower grain, safflower herbage and safflower straw to horse TMR did not have a significant effect on methane production at 24 hours of *in vitro* gas production (p>0.05). The *in vitro* methane production ranged from 0.22 to 0.42 mL/g DM (Table 5).

Up to 20% safflower in horse TMR decreased linearly the *in vitro* T-DMd, T-OMd, ME and SCFA values (p<0.05). On the other hand, the *in vitro* GY_{24}, PF_{24}, and MCP values of horse TMR did not change with the use of up to 20% safflower grain (p>0.05). It was determined that the use of 5%, 10% and 20% of safflower herbage in horse TMR decreased *in vitro* T-DMd, PF_{24} and MCP values (p<0.05). The *in vitro* T-OMd value of TMR were not affected by the use of safflower herbage in horses (p>0.05) (Table 6). The *in vitro* GY_{24}, ME and SCFA values of horse TMR showed

Table 5. Effect of safflower in horse TMR on *in vitro* cumulative gas production and methane production

			***In vitro* cumulative gas production (mL/g DM)**					
Supplementation to TMR		**Methane**	**6 h**	**12 h**	**18 h**	**24 h**	**36 h**	**48 h**
0% Safflower grain		0.29	3.94	43.37	73.94	183.11	221.39	229.29
5% Safflower grain		0.36	6.58	53.25	87.78	184.08	222.09	231.92
10% Safflower grain		0.27	5.27	43.49	72.16	161.45	198.10	200.08
20% Safflower grain		0.22	5.26	41.08	66.52	158.37	172.18	174.13
	SEM	0.06	0.51	1.70	2.71	3.86	6.17	7.12
p value	L	0.640	0.380	0.136	0.029	0.002	<0.001	<0.001
	Q	0.709	0.210	0.026	0.015	0.445	<0.001	<0.001
0% Safflower herbage		0.29	3.94	43.37	73.94	183.11	220.92	229.43
5% Safflower herbage		0.38	5.94	58.17	92.22	194.35	243.96	247.94
10% Safflower herbage		0.39	6.60	56.81	91.17	195.57	251.18	253.50
20% Safflower herbage		0.37	7.30	53.13	90.65	189.94	243.50	251.16
	SEM	0.07	0.54	1.91	2.45	2.17	3.80	4.47
p value	L	0.771	0.033	0.010	0.001	0.038	0.003	<0.001
	Q	0.799	0.507	0.001	0.002	0.007	0.004	0.004
0% Safflower straw		0.29	3.94	43.37	73.94	183.11	220.92	229.43
5% Safflower straw		0.33	4.64	59.18	93.74	195.51	233.34	232.03
10% Safflower straw		0.40	5.93	61.94	99.85	204.32	237.78	241.46
20% Safflower straw		0.42	7.84	66.71	108.89	214.52	240.37	245.98
	SEM	0.08	0.52	2.74	3.96	4.02	4.21	4.04
p value	L	0.604	0.002	<0.001	<0.001	<0.001	0.132	0.145
	Q	0.976	0.379	0.014	0.030	0.427	0.572	0.909

L: linear; Q: quadratic; Methane: *In vitro* methane production as mL/g DM at 24; SEM: standard error of means

an increase in all levels of safflower herbage ($p<0.05$). It was observed that this increase slightly decreased with the use of 20% (Table 6). It was determined that the use of safflower straw in horse TMR decreased linearly the *in vitro* T-DMd, PF_{24} and MCP values ($p<0.05$). The *in vitro* GY_{24}, ME and SCFA values of horse TMR were increased in linear contrast ($p<0.001$) depending on the increase in the level of safflower straw. In contrast, *in vitro* T-OMd were not affected by up to 20% safflower straw ($p>0.05$) (Table 6).

***In vitro* digestion parameters in ruminant**

Safflower grain supplementation of up to 20% to beef cattle TMR decreased linearly *in vitro* cumulative gas production at 3, 6, 12, 24, 48, 72, and 96 hours ($p<0.05$). The in vitro methane production (mL/0.2 g DM) of beef cattle TMR reduced linearly by safflower grain supplementation level ($p<0.05$) (Table 7).

The safflower herbage and safflower straw (5-20% in DM) used in beef cattle TMR did not change *in vitro* cumulative total gas production up to 96 hours and methane production at 24 hours of incubation ($p<0.05$). In the beef cattle TMR, the use of crushed safflower grain did not change *in vitro* T-DMd, T-OMd, GY_{24} and PF_{24} values ($p>0.05$). In relation to the increase of safflower grain in beef cattle TMR, the ME and NEL values of TMR and the SCFA concentration of digestion fluid decreased linearly ($p<0.001$) (Table 8). The *in vitro* estimated T-DMd, T-OMd, GY24, PF24, ME, NEL and SCFA values of beef cattle TMR were not affected by up to 20% safflower herbage and safflower straw ($p<0.05$) (Table 8).

Discussion

Nutrient composition in the grain, herbage and straw of safflower

Similar to our present study findings, Oğuz et al. (2014) reported that safflower grain grown in Turkey contained about 12% CP, 33% EE, 33% ADF and 44% NDF in DM. In another study, Ingale and Shrivastava (2011) stated that safflower grain (*C. tinctorius* PBNS-12 and PBNS-40) grown in India contained approximately 16% CP, 25-29% EE and 3.5% ash. Paya et al. (2014) (arid climate, Iran) found CP values (16%) of safflower grain was high than that of our findings, in line with the findings of Ingale and Shrivastava (2011). Stanford et al. (2001) reported that safflower straw, which containing seed-bound plant heads, contained about 13% CP, 13% EE, 40% ADF and 50% NDF in DM. The aNDFom and ADFom

Table 6. Effect of safflower in horse TMR on *in vitro* fermentation parameters

Supplementation to TMR		T-DMd	T-OMd	GY_{24}	PF_{24}	MCP	ME	SCFA
0% Safflower grain		494.46	560.36	371.18	2.69	90.70	8.12	0.81
5% Safflower grain		480.26	527.98	383.64	2.60	75.27	8.13	0.81
10% Safflower grain		439.79	449.37	367.15	2.72	84.60	7.52	0.71
20% Safflower grain		438.12	503.83	361.56	2.78	89.70	7.43	0.69
	SEM	7.79	15.46	5.12	0.03	4.96	0.10	0.01
p value	L	<0.001	0.037	0.365	0.330	0.899	0.001	0.001
	Q	0.274	0.086	0.418	0.418	0.370	0.661	0.661
0% Safflower herbage		494.46	560.36	371.18	2.69	90.70	8.12	0.81
5% Safflower herbage		457.47	512.99	425.25	2.35	29.89	8.41	0.85
10% Safflower herbage		462.41	517.88	422.91	2.36	32.15	8.44	0.86
20% Safflower herbage		465.46	529.51	408.81	2.45	47.59	8.29	0.83
	SEM	5.58	12.65	7.59	0.04	8.47	0.04	0.01
p value	L	0.056	0.483	0.026	0.016	0.020	0.057	0.057
	Q	0.041	0.301	0.006	0.003	0.005	0.004	0.004
0% Safflower straw		494.46	560.36	371.18	2.69	90.70	8.12	0.81
5% Safflower straw		504.25	542.39	387.66	2.58	74.12	8.44	0.86
10% Safflower straw		480.15	521.02	425.54	2.35	30.65	8.68	0.90
20% Safflower straw		478.33	510.60	448.50	2.23	6.38	8.96	0.94
	SEM	3.90	9.56	9.25	0.05	10.19	0.09	0.01
p value	L	0.014	0.065	<0.001	<0.001	<0.001	<0.001	<0.001
	Q	0.296	0.838	0.242	0.821	0.189	0.731	0.731

GY_{24}: gas yield is total gas volume (mL) produced for g T-DMd at 24 h; MCP: microbial crude protein is produced at 24 h (mg/g DM); ME: metabolic energy as MJ/kg DM; PF_{24}: partial factor is ratio T-DMd to GP24 at 24 h; SFCA: molarities of short chain fatty acid in fermentation fluid at 24 h; T-DMd: *in vitro* true-dry matter disappearance (mg) for g DM at 24 h (mg/g DM); T-OMd: *in vitro* true-organic matter disappearance; SEM: standard error of means; L: linear; Q: quadratic

contents of the safflower grain and safflower straw in the present study were similar to the findings of previous researchers (Asgharzadeh et al., 2013; Sahebi et al., 2011; Stanford et al., 2001). In another study, although aNDFom and ADFom values of safflower herbage grown in arid climatic conditions (in Jordan), were found to be similar to the results of the present study, CP (13.4%), ash (10.8%) values of it were high than those of the present study (Landau et al., 2004). In addition, the calculated ME value of safflower herbage was parallel to that of Asgharzadeh et al. (2013).

Generally, the CP content of safflower herbage, harvested at the pre-flowering stage in the current study, was similar to some meadow-pasture grass (*Dactylis Glomerata*, *Lolium multiflorum* at the end of vegetative). The safflower straw contains the lowest plant cell wall substances than those (70-78% aNDFom and 50-55% ADFom) of wheat straw and similar CP and EE to wheat straw (NRC, 1989).

The differences among the findings of the present study and previous studies can be attributed to variables in the safflower species used and the soil and climatic conditions grown. When evaluated in terms of nutrient content, it can be seen that safflower herbage and safflower straw have potential as alternative forages sources. In addition, the safflower grain may be a good source energy and moderate protein source due to its EE and CP content. The effect of the safflower grain, straw and herbage on the digestibility must be determined.

The *in vitro* fermentation values in horse TMR

In the present study, *in vitro* methane volume produced by aspiration grain, herbage and straw at different levels in horse TMR was 0.22-0.42 mL/g DM at 24 hours of *in vitro* incubation. The *in vitro* methane production of horse TMR was diverse in range from 0.43 to 0.59 mL/g DM by Kara and Baytok (2017). As it is understood from these values, methane is not produced (Ellis et al., 2007) in the digestive tract of horses as much as in ruminants, and it is observed that the contribution of horses to global warming is not as high as ruminants.

The 5% safflower seeds in horse TMR did not affect the *in vitro* total gas production, ME, SCFA, T-DMd and T-OMd during the 48-hour of *in vitro* incubation period. This demonstrates that up to 5% crushed safflower grain can be used in the horse TMR without affecting digestion parameters. However, the use of 10% and 20% crushed safflower grain in horse TMR cannot be recommended due to adverse effects on the *in vitro* total gas production. Neg-

Table 7. Effect of safflower in ruminant TMR on *in vitro* cumulative gas production and methane production

			***In vitro* cumulative gas production (mL/0.2 g DM)**						
Supplementation to TMR		**Methane**	**3 h**	**6 h**	**12 h**	**24 h**	**48 h**	**72 h**	**96 h**
0% Safflower grain		9.35	16.45	32.73	47.39	59.72	70.99	72.41	73.83
5% Safflower grain		9.48	12.09	25.44	43.59	57.83	69.94	71.72	72.43
10% Safflower grain		9.10	12.03	25.31	40.90	53.65	63.21	65.68	67.46
20% Safflower grain		8.29	10.54	23.72	38.47	50.77	61.31	63.41	64.83
	SEM	0.51	2.32	3.65	3.64	3.71	4.51	4.60	4.75
p value	L	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	0.006
	Q	0.004	<0.001	<0.001	0.400	0.306	0.610	0.613	0.756
0% Safflower herbage		9.35	16.45	32.73	47.39	59.72	70.99	72.41	73.83
5% Safflower herbage		9.83	15.32	30.11	46.87	60.06	71.82	72.89	73.61
10% Safflower herbage		9.30	15.08	28.92	45.39	58.01	69.58	71.32	72.39
20% Safflower herbage		9.30	15.83	33.24	49.41	61.39	72.64	74.74	75.79
	SEM	0.14	0.22	0.55	0.54	0.56	0.71	0.72	0.89
p value	L	0.634	0.252	0.819	0.213	0.528	0.694	0.429	0.603
	Q	0.454	0.390	0.201	0.017	0.167	0.476	0.344	0.373
0% Safflower straw		9.35	16.45	32.73	47.39	59.72	70.99	72.41	73.83
5% Safflower straw		9.00	17.30	31.96	46.44	57.74	69.74	71.15	72.21
10% Safflower straw		9.14	16.85	31.77	47.57	61.26	72.50	73.55	73.91
20% Safflower straw		9.71	16.47	32.06	47.83	60.80	71.67	73.07	73.78
	SEM	0.15	0.15	0.24	0.60	0.62	0.54	0.57	0.73
p value	L	0.391	0.774	0.377	0.699	0.208	0.343	0.430	0.840
	Q	0.163	0.060	0.338	0.668	0.511	0.851	0.752	0.663

L: linear; Q: quadratic; Methane: *In vitro* methane production as ml/g DM at 24; SEM: standard error of means

ative effects of high safflower grain (10% and 20%) on digestion parameters (*in vitro* total gas, ME, SCFA, T-DMd and T-OMd) can be attributed to the husk content and high ADL levels (Blümmel and Orskov, 1993; Getachew et al., 2008; Menke and Steinbgass, 1988). Previous studies have also shown that *in vitro* digestion parameters are negatively correlated with the structural carbohydrate content of the plant (Kara et al., 2016; Kara, 2016).

The *in vitro* digestion parameters (*in vitro* total gas, T-DMd, T-OMd, $GY_{24,}$ ME, SCFA and ruminal pH) show that the safflower herbage can be used up to 20% in instead of some forage (meadow hay and wheat straw) and concentrate (barley and vegetable oil) feeds of DM in horse TMR. Similarly, *in vitro* digestion parameter applies to safflower straw and up to 20% can be used as forage in DM in horse TMR. These effects of safflower herbage and straw could be caused by the lower levels of aNDFom, ADF and ADL that can be included in grass herbage or hay (forages) which is harvested after the seed-binding stage (Kara, 2016; NRC, 1989 and 2001). These results indicate that the herbage and straw of safflower without thorns are the preferred forage for horse TMR.

The *in vitro* fermentation values in beef cattle TMR

Research on the *in vitro* digestion of safflower plant in ruminants is still very limited. In the present study, *the in vitro* cumulative gas production of safflower in ruminants was lower than those of safflower herbage and straw, which is compatible with the findings of Sahebi et al (2011). The present study indicated that the addition of 5% safflower grain to beef TMR does not have a negative effect on the *in vitro* gas production, ME, NE_{L}, SCFA, *in vitro* T-DMd, T-OMd, GY_{24} and PF_{24} values and the safflower grain at this level can be used in ruminant TMR suggesting that studies on safflower grain should be carried out. However, the use of safflower grain at 10% and 20% levels in ruminant TMR does not affect the *in vitro* T-DMd, T-OMd, GY_{24} and PF_{24} values, despite the linear reduction of *in vitro* gas production, ME, NE_{L} and SCFA values. These fermentation results will not reveal a problem on ruminal digestion of beef TMR.

The *in vitro* methane (mL/0.2 g DM) produced by unit DM of beef TMR decreased linearly with increasing rates of safflower grain in beef TMR and is an expected result due to the reduce *in vitro* gas production (Ellis et al., 2007; Kara et al., 2015).

The use of up to 20% safflower herbage in beef TMR did not adversely affect *in vitro* cumulative total gas and methane production levels and *in vitro* T-DMd, T-OMd, GY_{24}, PF_{24}, ME,

Table 8. Effect of safflower in ruminant TMR on *in vitro* fermentation parameters

		T-DMd	T-OMd	GY_{24}	PF_{24}	ME	NEL	SCFA
0% Safflower grain		516.78	549.27	588.06	1.73	11.19	6.95	1.32
5% Safflower grain		654.20	745.24	446.17	2.26	10.94	6.73	1.27
10% Safflower grain		468.84	550.85	572.49	1.74	10.37	6.25	1.18
20% Safflower grain		526.68	577.44	488.84	2.07	9.98	5.92	1.12
	SEM	27.11	28.81	23.62	0.08	0.14	0.12	0.02
p value	L	0.422	0.508	0.328	0.428	<0.001	<0.001	<0.001
	Q	0.362	0.044	0.451	0.459	0.306	<0.001	0.306
0% Safflower herbage		516.78	549.27	588.06	1.73	11.19	6.95	1.32
5% Safflower herbage		572.18	569.79	554.71	1.92	11.24	6.99	1.32
10% Safflower herbage		613.95	685.37	496.27	2.11	10.96	6.75	1.28
20% Safflower herbage		651.90	653.31	477.55	2.11	11.42	7.14	1.35
	SEM	34.55	36.58	33.91	0.11	0.07	0.06	0.01
p value	L	0.207	0.243	0.267	0.261	0.530	0.530	0.530
	Q	0.908	0.738	0.923	0.715	0.168	0.168	0.168
0% Safflower straw		516.78	549.27	588.06	1.73	11.19	6.95	1.32
5% Safflower straw		562.59	518.35	517.00	1.94	10.92	6.72	1.28
10% Safflower straw		561.83	596.87	546.13	1.83	11.40	7.13	1.35
20% Safflower straw		590.31	633.22	530.08	1.95	11.34	7.07	1.35
	SEM	21.21	31.64	21.72	0.07	0.08	0.07	0.01
p value	L	0.318	0.305	0.513	0.445	0.209	0.209	0.209
	Q	0.856	0.631	0.577	0.760	0.512	0.512	0.512

GY_{24}: gas yield is total gas volume (mL) produced for g T-DMd at 24 h; MCP: microbial crude protein is produced at 24 h (mg/g DM); ME: metabolic energy as MJ/kg DM; NEL: net energy lactation as MJ/kg DM; SFCA: molarities of short chain fatty acid in fermentation fluid at 24 h; T-DMd: *in vitro* true-dry matter disappearance (mg) for g DM at 24 h (mg/g DM); T-OMd: *in vitro* true-organic matter disappearance; SEM: standard error of means; L: linear; Q: quadratic

NE_L and SCFA values. This result shows that it could be advisable to use safflower herbage advisable instead of corn silage and wheat straw in the beef cattle TMR. The safflower herbage can be characterized as quality forage due to the values of CP, fibre and ME.

The 5%, 10% and 20% of safflower straw in beef cattle did not change the *in vitro* cumulative total gas and methane production and *in vitro* T-DMd, T-OMd, GY_{24}, PF_{24}, ME, NE_L and SCFA values, indicating it could be preferred instead of wheat straw. However, the safflower plant used in the study was the Dinçer type and did not have thorns. This plant may not cause adverse effects in the *in vivo* feeding experiments in ruminants. It may be advisable to use thorny forms of the plant in goats.

As a result;

- Before flowering, the safflower herbage has ME, CP, aNDFom, ADFom and ADL values, which may contain a moderate/good quality forage,
- Although safflower straw has the equivalent CP content to reference wheat straw, the values of aNDFom, ADFom and ADL are lower than those of wheat straw,
- The husked safflower grain has a high content oil, moderate CP content and high fibre contents,
- The use of safflower grain may be recommended up to 5% in horse TMR and up to 20% in ruminant TMR,
- Up to 20% safflower herbage can be used in high quality forage in horse and beef TMR,
- Moreover, it may be argued that further investigation into the *in vivo* digestibility of these feed sources and the effects on performance and product quality need to be investigated.

Ethics Committee Approval: Ethics committee approval was received for this study from the Local Ethics Committee for Animal Experiments of Erciyes University (ERU-HADYEK), Kayseri-Turkey (Date: January 14, 2015; Decision number: 15/10).

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References

Association of Official Analytical Chemists., 1995. Official methods of analysis of AOAC International. Washington: AOAC.

Altin, T.B., Barak, B., Altin, B.N., 2012. Change in precipitation and temperature amounts over three decades in central Anatolia, Turkey. Atmospheric and Climate Sciences 2, 107-125. [CrossRef]

Asgharzadeh, F., Nasri, M.H.F., Behdani, M.A., 2013. Effects of nitrogen and phosphorus fertilizers on nutritive value of safflower forage and silage. Journal of Animal and Poultry Sciences 3, 66-75.

Baumler, E., Cuniberti, A., Nolascoa, S.M., Riccobene, I.C., 2006. Moisture dependent physical and compression properties of safflower seed. Journal of Food Engineering 72, 134-140. [CrossRef]

Blümmel, M., Orskov, E.R., 1993. Comparison of in vitro gas production and nylon bag degradability of roughages in predicting of food intake in cattle. Animal and Feed Science Technology 40,109-119. [CrossRef]

Bozan, B., Temelli, F., 2008. Chemical composition and oxidative stability of flax, safflower and poppy seed and seed oils. Bioresource Technology 99, 6354-6359. [CrossRef]

Cook, B.I., Anchukaitis, K.J., Touchan, R., Meko, D.M., Cook, E.R., 2016. Spatiotemporal drought variability in the Mediterranean over the last 900 years. Journal of Geophysical Research: Atmospheres 121, 2060-2074. [CrossRef]

Dschaak, C.M., Noviandi. C.T., Eun, J.S., Fellner, V., Young, A.J., ZoBell, D.R., Israelsen, C.E., 2011. Ruminal fermentation, milk fatty acid profiles, and productive performance of Holstein dairy cows fed 2 different safflower seeds. Journal of Dairy Science 94, 5138–5150. [CrossRef]

Ellis, J.L., Kebreab, E., Odongo, N.E., McBride, B.W., Okine, E.K., France, J., 2007. Prediction of methane production from dairy and beef cattle. Journal of Dairy Science 90, 3456–3467. [CrossRef]

Getachew, G., Pittroff, W., Putnama, D.H., Dandekar, A., Goyal, S., DePeters, E.J., 2008. The influence of addition of gallic acid, tannic acid, or quebracho tannins to alfalfa hay on in vitro rumen fermentation and microbial protein synthesis. Animal Feed and Science Technology 140, 444–461. [CrossRef]

Gilbert, J., 2008. International safflower production- an overview. 7th International Safflower Conference, Australian Oilseeds Federation, Australia.

Gumus, E., Kucukersan, S., 2016. Ruminantların beslenmesinde aspir kullanımı. Lalahan Hayvancılık Araştırma Enstitüsü Dergisi 56, 25-31.

Ingale, S., Shrivastava, K.S., 2011. Chemical and bio-chemical studies of new varieties of safflower (*Carthamus tinctorius* L.) PBNS-12 and PBNS-40 seeds. Advances in Agriculture & Botanics International Journal of the Bioflux Society 3, 127-138.

I.P.C.C., 2014. Summary for Policymakers. In: Climate Change 2014: Mitigation of climate change. contribution of working group iii to the fifth assessment report of the intergovernmental panel on climate change [Edenhofer, O., R. Pichs-Madruga, Y. Sokona, E. Farahani, S. Kadner, K. Seyboth, A. Adler, I. Baum, S. Brunner, P. Eickemeier, B. Kriemann, J. Savolainen, S. Schlömer, C. von Stechow, T. Zwickel and J.C. Minx (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Kara, K., Aktug, E., Ozkaya, S., 2016. Ruminal digestibility, microbial count, volatile fatty acids and gas kinetics of alternative forage sources for arid and semi-arid areas as in vitro. Italian Journal of Animal Science 15, 673-680. [CrossRef]

Kara, K., Baytok, E., 2017. Effect of different levels of psyllium supplementation to horse diet on *in vitro* fermentation parameters and methane emission. Journal of the Faculty of Veterinary Medicine Istanbul University 43, 12-18.

Kara, K., Guclu, B.K., Baytok, E., 2015. Comparison of nutrient composition and anti-methanogenic properties of different Rosaceae species. Journal of Animal and Feed Sciences 24, 308-314. [CrossRef]

Kara, K., 2016. Effect of dietary fibre and condensed tannins concentration from various fibrous feedstuffs on *in vitro* gas production kinetics with rabbit faecal inoculum. Journal of Animal and Feed Sciences 25, 266–272. [CrossRef]

Landau, S., Friedman, S., Brenner, S., Bruckental, I., Weinberg, Z.G., Ashbell, G., Hen, Y., Dvash, L., Leshem, Y., 2005. Safflower (Carthamus tinctorius L.) as a novel pasture species for dairy sheep in the Mediterranean conditions of Sardinia and Israel. Small Ruminant Research 59, 239-249. [CrossRef]

Landau, S., Friedman, S., Brenner, S., Bruckental, I., GWeinberg, Z., Ashbell, G.,Hen, Y., Dvash, L., Leshem, Y., 2004. The value of safflower (Carthamus tinctorius) hay and silage grown under Mediterranean conditions as forage for dairy cattle. Livestock Production Science 88, 263-271. [CrossRef]

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 Development 28, 7-55.

Ministry of Agriculture, Fisheries and Food., 1984. Energy allowances and feeding systems for ruminants. Reference Book 433. HMSO, London: MAFF.

National Research Council., 2001. Nutrient Requirements of Dairy Cattle.7th Revised Edition. National Academy Press, Washington, DC (USA): NRC.

National Research Council., 1989. Nutrient Requirements of Horses. 5th Revised Edition. National Academy Press, Washington, DC (USA): NRC.

Oguz, M.N., Oguz, F.K., Buyukoglu, T.I., 2014. Effect of different concentrations of dietary safflower seed on milk yield and some rumen and blood parameters at the end stage of lactation in dairy cows. Revista Brasileira de Zootecnia 43, 207-211. [CrossRef]

Paya, H., Taghizadeh, A., Janmohammadi, H., Moghaddam, G.A., Khani, A.H., Alijani, S., 2014. Effects of microwave irradiation on in vitro ruminal fermentation and ruminal and post-ruminal disappearance of safflower seed. Journal of Biodiversity and Environmental Science 5(2), 349-356.

Sahebi, M., Kafilzadeh, F., Heidary, M., 2011. Composition and in vitro rate of gas production of canola and safflower seed affected by chemical treatment. Researches of the First International Conference, Babylon and Razi Universities, Iran.

Stanford, K., Wallins, G.L., Lees, B.M., Mündel, H.H., 2001. Feeding value of immature safflower forage for dry ewes. Canadian Journal of Animal Science 81, 289-292. [CrossRef]

Sunvold, G.D., Hussein, H.S., Fahey, G.C., Merchen, N.R., Reinhart, G.A., 1995. In vitro fermentation of cellulose, beet pulp, citrus pulp, and citrus pectin using fecal inoculum from cats, dogs, horses, humans, and pigs and ruminal fluid from cattle. Journal of Animal Science 73, 3639-3648. [CrossRef]

Sweeney, C.R., 2012. In vivo and in vitro digestibility of a complete pelleted feed in horses. Master's Theses and Project Reports, the Faculty of California Polytechnic State University. San Luis Obispo CA. [CrossRef]

Turkish Statistical Institute., 2016. Erişim adresi: [http://www.tuik.gov.tr /PreTablo.do?alt_id=1001], Erişim Tarihi: 18.09.2016.: TSI.

Van-Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber and non starch polysaccharides in relation to animal nutrition. Journal of Dairy Science 74, 3583-3597. [CrossRef]