Investigation of the effectiveness of tomato pulp on the in vitro fermentation of working horse diets

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ABSTRACT
The aim of this study is to determine the effectiveness of dried tomato pulp, as a soluble dietary fibre source, using [0 (control group), 2.5, 5.0, 10.0, and 20.0% (experimental groups)] as dry matter-DM in horse total mixed ration (TMR) on the in vitro fermentation parameters. The in vitro total gas production (TGP), methane emission, gas yield (GY), metabolic energy (ME), in vitro true dry matter disappearance (T-DMD), short-chain fatty acids (SCFA) molarities, partial factor (PF), microbial crude protein production (MCP), and pH value at 24 h of fermentation in horse TMRs were determined. In the study, in vitro TGP, GY, PF, ME, MCP, SCFA, and methane emission of horse TMRs were not changed by 2.5% and 5.0% tomato pulp supplementation (P > 0.05). The in vitro TGP (73.6 vs. 85.6 and 105.9 mL/g DM), GY (189.4 vs. 210.8 and 286.3 mL for g T-DMD), PF (5.9 vs. 5.2 and 4.2 mg/mL for GY), ME (5.3 vs. 5.6 and 6.1 MJ/kg DM), MCP (270.1 vs. 258.2 and 215.1 mg/g DM), SCFA (0.32 vs. 0.37 and 0.46 mmol/0.2 g), and methane emission (0.28 vs. 0.33 and 0.48 mL/g DM) of horse TMRs were positively affected by 10 and 20% tomato pulp supplementation (P < 0.05). The in vitro T-DMD values of TMRs and pH value of in vitro digestion fluid were similar for both control and experimental groups (P > 0.05). As a result, it was demonstrated that tomato pulp, which uses as dietary fibre and CP source, did not affect negatively the digestion values of the working horse diet. However, it has been considered that tomato pulp using at 10 and 20% ratios increased the in vitro digestion values of the working horse diet. Determining the in vitro digestion levels of tomato pulp, which is a source of soluble dietary fibre, for horses will guide future in vitro and in vivo studies.

Introduction
Horses are monogastric herbivores whose digestive system is anatomically developed for them to ingest small amounts of forage in a continuous manner. The digestive system of the horse is quite unique. The system has a relatively small but efficient stomach for grain utilization and a large caecum and colon for utilization and fermentation of forage. The purpose of the digestive system of the horse is to process feedstuffs into their component nutrients for absorption and utilization by the body. Structural carbohydrates, such as cellulose, along with other carbohydrates that escaped digestion in the small intestine are fermented in the large intestine. The microbial enzymes in the large intestine can digest cellulose, starch, and sugars into volatile fatty acids (VFA, in other words; short-chain fatty acids with C3–C3 carbons), which may supply up to 25% of the energy used by the horse. Except in extreme stress conditions, bacterial action in the large intestine of healthy adult horses produces their daily B vitamin requirements. The caecum is the primary site of water absorption. The rate of passage in the large intestine is considerably slower than in other portions of the digestive tract. The average rate of passage in the large intestine is approximately 36–48 h (NRC 1989, 2007). The soluble undigested fibres can be fermented in the colon, thereby producing short-chain fatty acids (acetate, propionate, and butyrate) and branched-chain fatty acids. The end products of the fermentation are used by different tissues in the body as a source of energy (NRC 1989, 2007).

The digestibility of horse diets has been revealed by in vivo and in vitro methods. While the in vivo method is the most accurate, it is costly, time-consuming, labor-intensive, and must be performed directly on a horse (Bush et al. 2001). Apart from studies using direct horse feces inoculum plus buffer mixture to detect in vitro digestion in horses, there are studies using horse feces inoculum and fermentation medium (vitamin, mineral, fatty acid, and yeast extract) (Sweeney 2012; Kholif et al. 2015; Ersahince and Kara 2017; Kara and Baytok 2017). Gas production profiles and parameters are indicators of the rate and extent of degradability of feedstuffs in the horse’s caecum and ventral colon, while total VFA and the type of VFA are fermentation end products from microorganisms of materials escaping enzymatic digestion and serve as energy sources for horses. An increase in propionate can improve the horse’s overall energy balance, given that propionate is glycogenic (Ersahince and Kara 2017). Thus, feces as a microbial inoculum for in vitro digestibility studies in horses has many practical and economic advantages over caecal fluid as it does not require the use of surgically prepared animals and can be collected from any individual or several animals (Abdouli and Attia 2007; Earing et al. 2010).
Cumulative gas production profiles of feeds have a wide variety, demonstrating the potential of the pressure transducer technique to evaluate the digestive kinetics of feeds used in horse diet (Murray et al. 2009). However, it is important to note that in vitro techniques are not designed to accurately measure absolute digestibility but to estimate it and compare the relative digestibility of different feeds (Tassone et al. 2019).

The fibrous feedstuffs in the horse diet can be forage (mainly meadow hay and herbage) and concentrate feeds with high fiber content (mainly grain bran, grain with husk, and agro-industrial by-products) (NRC 2007). Tomato-processing by-product, also known as tomato pulp, consists of peel and seeds and represents around 4% of the fruit weight (Del Valle et al. 2006). The tomato industry generates large amounts of by-products, and these by-products representing 10-30% of total processed tomatoes contain tomato seeds, peels, pulp, and cores (Rahmatnejad et al. 2009). Seeds and peels present industrial by-products (NRC 2007). Tomato-processing by-products, and these by-products representing 10-30% of total processed tomatoes are rich in nutritional value (Isik and Topkaya 2016). The in vitro gas production from insoluble fraction and potential gas production values of tomato pulp were higher than those of common fibrous feedstuffs (wheat bran and lucerne meal) and uncommon fibrous feedstuffs (maize bran, rice bran, lentil bran, and pomegranate peel) for herbivorous rabbit, which has a functional fibre fermentation in the large intestine (Kara 2016). The tomato pulp, which has 17% digestible dietary fibre, about 20% neutral detergent fibre (NDF), about 35–59% total dietary fibre (TDF), 26% total sugars, 13.7–19.0% crude protein (CP), 7.55% pectins, and 55% non-structural carbohydrate, can be a fibrous-energy-protein source for horse diet (Del Valle et al. 2006; Kara 2016).

Studies often evaluate pure ingredients without additives and are inoculated with faeces without prior enzymatic digestion (Ershahince and Kara 2017; Cagri and Kara 2018); however, there is limited information on the fermentation of horse diets (Kara and Baytö 2017). However, positive and negative associative effects were observed when the mixtures were incubated, compared to the values expected from incubation of the pure components (Niderkorn and Baumont 2009). Therefore, there is a need to evaluate the fermentation of mixed diets rather than pure ingredients. The tomato pulp can be an ideal dietary fibre source for the large intestine and an energy-protein source due to functional nutrient content its.

In this study, it was aimed to reveal the fermentative digestions of a mixed ration (containing different ratios of tomato pulp) instead of a pure feedstuff. The aim of the present study was to determine the effect of 2.5, 5.0, 10.0, and 20.0% in horse total mixed ration tomato pulp on digestive parameters using feces as inoculum.

Materials and methods

The samples of tomato pulp and feedstuffs

Tomato pulp was collected as wet from a local factory in Kayseri province of Turkey. The wet tomato pulp was oven-dried (Binder, Germany) at 65°C for 36 h. The feedstuffs of horse TMR were meadow hay, wheat straw, oat grain, wheat bran, flaxseed meal, di-calcium phosphate (DCP), and salt. The dried tomato pulp and other feedstuffs samples which were grounded in size to pass through a 1 mm sieve (IKA Werke, Staufen im Breisgau, Germany) were mixed for laboratory TMR’s (100 g, as DM). The control TMR included 20.9 Mcal digestible energy (DE)/kg DM and 104.5 g CP/day (Table 1).

Oat grain and wheat bran in the control TMR of horses were reduced and replaced with tomato pulp between 2.5, 5, 10, and 20% as a DM basis. Each TMR sample was prepared at approximately 500 g. The control and treatment TMR’s adjusted as iso-caloric and iso-nitrogenic (Table 1).

Chemical analyses

The total mixed ration of a horse was milled through a 1 mm sieve for use in chemical analysis and in vitro gas production. The ash, crude protein (CP) and ether extract (EE) contents were estimated 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 analyzed using a glass crucible on FIWE3 fibre analyser (Velp, Italy) (Van-Soest et al. 1991). The NDF was detected using sodium sulfite and thermo-stable α-amylase (Megazyme, Ireland) (aNDF). The NDF and ADF were given without ash content (aNDFom and ADFom). Total starch contents of horse total mixed ration with and without tomato pulp were analyzed using Megazyme assay (Megazyme, cat. no. K-TSTA-100A) procedures (Bray Business Park, Bray, Co. Wicklow, Ireland).

Total dietary fibre (TDF), soluble dietary fibre (SDF), and insoluble dietary fibre (IDF) contents are determined according to the AOAC enzymatic-gravimetric method (Prosky et al. 1988). The basis of this method was the isolation of dietary fibre by enzymatic digestion of the rest of the constituents of the feedstuffs. The residue was determined gravimetrically. The starch of the feedstuff (approximately 1.0 g) was digested by combining amylase (pH = 6.0; temperature, T = 100°C; time, t = 30 min) and amyloglucosidase (pH: 7.5; T = 60°C; t = 30 min). The protein of the feedstuff was digested with protease (pH: 4.5; T = 60°C; t = 30 min). Ethanol (95%) was added to precipitate the soluble fibre and incubated for 60 min. Filtration was carried out in crucibles (Velp, Italy) by using a filtration system (Velp Scientifica CSF 6, Italy). Protein and ash were measured in each residue in order to correct the values for

| Table 1. Ingredients and chemical compositions of horse TMR used in the present study. |
| Feedstuffs | Tomato pulp supplementation, % |
| Meadow hay | 48.0 | 48.0 | 48.0 | 48.0 |
| Wheat straw | 27.0 | 27.0 | 27.0 | 27.0 |
| Oat grain | 16.0 | 16.0 | 16.0 | 12.0 |
| Wheat bran | 8.0 | 5.5 | 3.0 | 3.0 |
| Flaxseed meal | 8.0 | 8.0 | 8.0 | 8.0 |
| Tomato pulp | 2.5 | 5.0 | 10.0 | 20.0 |
| DCP | 0.3 | 0.3 | 0.3 | 0.3 |
| Salt | 0.7 | 0.7 | 0.7 | 0.7 |
| DM (kg/day) | 10.0 | 10.0 | 10.0 | 10.0 |
| DE (Mcal/kg KM) | 20.9 | 20.9 | 20.9 | 21.0 |
| CP (g/day) | 1045 | 1036 | 1028 | 1039 |

DM: Dry matter; DE: Digestible energy; DCP: Di-calcium phosphate; CP: Crude protein.
dietary fibre. Dietary fibre analyses were performed using Megazyme chemicals (Ireland) and were in duplicate. Analyses were carried out as duplicates. Hemicellulose (HC), soluble fibre (SF), water-insoluble pectin (WIP), and digestible fibre fractions (DgF) levels of feedstuffs were calculated using the following formulas (Kara 2016):

\[
\text{HC}(%, \text{DM}) = a\text{NDF}o_m (%)-a\text{ADF}o_m (%)
\]
\[
\text{SF}(%\text{DM}) = \text{TDF} (%) - a\text{NDF}o_m (%)
\]
\[
\text{WIP} (%) = \text{SF} (%) - \text{SDF} (%) 
\]
\[
\text{DgF} (%) = \text{HC} (%) + \text{SF} (%)
\]

In vitro digestion technique for horses

The in vitro digestion technique in horses was carried out according to Sunvold et al. (1995) and Sweeney (2012), which incubated feed sample in faeces inoculum and fermentation medium, which included solution A, solution B, trace mineral solution, water-soluble vitamins, haemin solution, short-chain fatty acids, resazurin, yeast extract, trypticase, Na₂CO₃ and cystein-HCl*H₂O (Table 1). Feces samples used as an inoculum in the current study obtained from two thoroughbred working horses (6–7 years of age, 480–500 kg of body weight) fed a diet containing about 70% forage and about 30% concentrate feed, on DM basis.

Feces samples were diluted at a 1:10 ratio with 0.9% sterile serum physiologic solution (Poliﬂeks, Polifarma, Istanbul, Turkey) using a laboratory-type blender (Waring Products Division, Torrington, CT, USA). Diluted feces inoculum was filtered through four layers of cheesecloth under constant CO₂ gas (anaerobically) and used in the in vitro digestion technique. The diet for a horse, which had 550 kg body weight and moderate maintenance energy and nutrient requirement, was studied as substrate. The in vitro digestion technique was carried out in glass syringes with 100 ml volume (Fortuna®, Poulten & Graf Ltd., Wertheim, Germany) using the medium mixture, prepared according to Sunvold et al. (1995), and Sweeney (2012). The samples (500 ± 10 mg as DM) were incubated with a medium mixture (30 ml) (Table 1) and feces inoculum (5 ml) in glass syringes (n = 6). The syringes were closed using clips and then the initial volume was recorded and incubated in a water bath with a thermostat (Special Waterbath, Yapar Stainless Steel Ltd., Kahramanmaras, Turkey) at 39.0 ± 0.2°C up to 24 h. In addition, six blank syringes (no template; medium mixture plus feces inoculum) were used to calculate the total gas production.

Table 2. Composition of in vitro fermentation medium.

| Component                        | Amount     |
|----------------------------------|------------|
| Solution A                       | 330.0 ml/L |
| Solution B                       | 330.0 ml/L |
| Trace mineral solution           | 10.0 g/L   |
| Water-soluble vitamins           | 20.0 g/L   |
| Folate: biotin solution          | 5.0 g/L    |
| Riboflavin solution              | 5.0 g/L    |
| Haemin solution                  | 2.5 g/L    |
| Short chain fatty acids          | 0.4 g/L    |
| Resazurine                       | 1.0 g/L    |
| Distilled H₂O                    | 296.0 g/L  |
| Yeast extract                    | 0.5 g/L    |
| Trypticase                       | 0.5 g/L    |
| Na₂CO₃                           | 4.0 g/L    |
| Cystein HCl·H₂O                  | 0.5 g/L    |

\(\text{GP}_{24} = \text{MG}_{24} - \text{TDM}_d \times \text{GF}_{24}\)

\(\text{MCP} = \text{MG}_{24} - \text{TDM}_d \times \text{GF}_{24} \times 2.2 \text{mg/ml}\)

\(\text{DM}_{24} = \text{GP}_{24} \times \text{TDM}_d / \text{GF}_{24}\)

Determination of in vitro true dry matter disappearance values

Three of the fermentation syringes for horses were stopped after 24 h, and then the in vitro true dry matter disappearance (T-DMd) values. The in vitro dry 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) to obtain dry matter residue which was dried at 105°C and then burning the residue at 550°C. In vitro T-DMd was calculated as 1−[(DM residue−DM blank)/initial DM] × 100.

Statistical analysis

Statistical analysis of study data was performed using SPSS 17.0 software (IBM Corp., Armonk, NY, USA). One-way variance
analyses (ANOVA) were implemented for homogeneous variances. The linear, quadratic, cubic effects (polynomial contrast) for using different levels of tomato pulp in TMRs were also determined. Significance was defined at \( P \) values of <0.05.

### Results

The tomato pulp included 9.74% CP, 45.20% NSC, 43.78% TDF, 26.73% IDF, and 17% SDF in DM. Digestible fibre fractions (hemicellulose and soluble fibre) of tomato pulp was 29.63% of DM (Table 3).

The starch contents of control TMR and treatment TMRs with tomato pulp were ranged from 8.53 to 6.90% in DM. The NDF contents of control TMR and treatment TMRs with tomato pulp were ranged from 34.29 to 34.69% in DM. The CP contents of all TMR’s were in among 10.62 and 10.28% in DM (Table 4).

The horse TMRs in the present study included 20.8-21 Mcal/kg DM digestible energy (DE) and about 10% crude protein (CP). In the present study, GY24, ME, SCFA, GY26, PF24, MCP, and methane emission was not changed 2.5 and 5% tomato pulp (\( P > 0.05 \)); but increased 10 and 20% tomato pulp (\( P < 0.05 \)). The in vitro TGP for g DM of horse TMR’s at 24 h were ranged about 74 from 106 ml; and increased by 10 and 20% supplementation of tomato pulp (\( P = 0.035 \); cubic effect). The 0.28 ml methane production of the control group reached 0.48 ml for the treatment group with 20% tomato pulp (\( P = 0.093 \)). The ME values of horse TMR’s were ranged from 5.25 to 6.13 MJ/kg DM; and increased by 10 and 20% tomato pulp (\( P = 0.035 \)). The T-DMd values of TMRs and pH of in vitro digestion fluid were similar for treatment and experimental groups (Table 5).

### Discussion and conclusion

In vitro digestion techniques are widely used to understand ruminal feed digestion in ruminants using inoculum with rumen fluid content (Kara 2015, 2018, 2021). In recent years, digestion/fermentation values completed mixed feed or forage feedstuffs have been made as the in vitro for horses using horse feces inoculum (Sweeney 2012; Kholif et al. 2015; Kara and Baytok 2017; Cagri and Kara 2018). However, in the techniques made to understand the digestion of feeds in colonic fermentation provided by high colon bacterial enzymes in horses, digestion in the entire digestive tract should be provided. Dietary fibre mostly comprises carbohydrate polymers, which resist hydrolysis by digestive enzymes in the mammalian small intestine but can be fermented by large intestinal bacteria. One of the main benefits of dietary fibre relates to its fermentability, which affects microbial diversity and function within the gastro-intestinal tract, as well as the by-products of the fermentation process (Williams et al. 2017). Fibrous compounds in the diet of monogastric animals are important for intestinal fermentation and are divided into soluble and insoluble dietary fibre. It has obvious that dried tomato pulp, which is rich in SDF, will have a positive effect on large intestine fermentation in horses. In the in vitro digestion experiment, adding tomato pulp up to 10% as DM to diet did not change the total gas production and estimated digestion values, but the addition of 20% significantly increased the in vitro gas production and estimated digestion values.

Apart from the gases produced by colon fermentation, it is understood that the rate of short-chain fatty acids, which is the end product of carbohydrate fermentation, increases up to 20% with the use of tomato pulp, which is a soluble dietary fibre source, and colon health is positively affected. Horses can provide long-term glucose from the short-chain fatty acids produced in their colons (NRC 2007). It has also been understood that this carbohydrate source, which has a low glycaemic index, is necessary for the health of the horses’ metabolism.

Diet composition, particularly carbohydrate composition, may affect the microbial population of the large intestine as well as the proportions of VFA that are produced. Feeding a high-starch diet (30% in DM) has been reported to decrease the concentration of cellulolytic bacteria but increase the concentration of total anaerobic bacteria, lactic acid using bacteria, lactobacilli, and streptococci in the caecum (Medina et al. 2002). Soluble DF can increase the viscosity of cyme depending on its chemical structure. These dietary fibre compounds can lead to a reduced glycaemic response, delaying gastric emptying and nutrient release as well as by inhibiting the action of α-amylase, thus regulating blood glucose – a critical mechanism related to the development of insulin resistance and then Type 2 diabetes (Williams et al. 2017).

In the presented study, it is important to increase the levels of soluble fibre source instead of starch as an energy source, to provide long-term energy needed for horses, to increase the

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**Table 3.** Fibre substances and some nutrient compositions of tomato pulp.

| Nutrient matter | %, DM | Nutrient matter | %, DM |
|-----------------|-------|-----------------|-------|
| Ash             | 15.29 | HC              | 11.67 |
| CP              | 9.74  | SF              | 17.96 |
| EE              | 3.95  | WIP             | 0.91  |
| NSC             | 45.20 | DGf             | 29.63 |
| TDF             | 43.78 | αNDFom          | 25.82 |
| IDF             | 26.73 | ADFom           | 14.15 |
| SDF             | 17.05 | ADL             | 6.12  |

DM: Dry matter content as % in feed; Ash: Ash content as % in DM; CP: Crude protein as % in DM; EE: Diethyl ether extract as % in DM; NSC: non-structural carbohydrates (100−Ash−EE−CP−αNDFom) (NRC 2001); αNDFom: Thermolabile α-amylase treated neutral detergent fibre content was corrected for ash as % in DM; TDF: Total dietary fibre as % in DM; SDF: Soluble dietary fibre as % in DM; IDF: Indigestible dietary fibre as % in DM; Hemicellulose as % in DM (αNDFom − ADFom); SF: Soluble fibre as % DM (TDF−αNDFom); WP: Water-insoluble pectin as % DM (SF−SDF); DGf: Digestible fibre fractions (HC+SF) as % DM.

**Table 4.** Analysis values of horse TMR used in the present study.

| Tomato pulp supplementation, % | Control TMR | 2.5 | 5.0 | 10.0 | 20.0 |
|-------------------------------|-------------|-----|-----|------|------|
| Items (%, in DM)              |             |     |     |      |      |
| CP                            | 10.62       | 10.45 | 10.58 | 10.39 | 10.28 |
| EE                            | 3.35        | 3.30  | 3.41  | 3.25  | 3.30  |
| Ash                           | 10.70       | 10.34 | 10.87 | 10.59 | 10.95 |
| CF                            | 15.21       | 15.25 | 15.74 | 16.29 | 16.97 |
| NDF                           | 34.29       | 34.78 | 35.45 | 35.96 | 34.69 |
| NFC                           | 41.04       | 41.13 | 39.69 | 39.81 | 40.78 |
| Starch                        | 8.53        | 8.20  | 7.91  | 7.62  | 6.90  |

CP: Crude protein; NDF: assayed with a heat stable amylase and expressed exclusive of residual ash; EE: Diethyl ether extract; CF: Crude fibre; NFC: non-fibrous carbohydrate; NFC is = 100%−(CP% + NDF% + EE% + Ash%).
number of probiotic bacteria in the intestine, and to maintain blood glucose level. The starch level of forage materials used in horse diets varies between about 1–3% in DM. The starch level of Gramineae grain and by-products varies between 17 and 77% in DM (NRC 2007). Although the starch level was reduced with tomato pulp supplementation instead of oat grain and wheat bran in the treatment diets of the present study, it was observed that there was no decrease in the in vitro digestion level. There were even positive effects on some digestive parameters. In the presented study, it is understood that dried tomato pulp is a good source of soluble dietary fibre. Determining the in vitro digestion levels of tomato pulp, which is a source of soluble dietary fibre, for horses will guide future in vitro and in vivo studies. It will be a guide for the usability of sources containing soluble and insoluble dietary fibre in different ratios in the diet of horses and for testing this in vitro. Researchers will be able to base on these findings in order to increase the diversity of dietary fibre components, which are important for the health of horses.

Many studies about the reduction of methane emissions from animal production (especially ruminant rearing systems) have been carried out at the present time. Share in global warming is not as high as ruminants. But in the whole gastro-intestinal tract, for example, gastric enzymatic, small intestine; enzymatic and large intestine fermentative digestion of horse diet with tomato pulp should be performed with an in vitro model.

### Disclosure statement

No potential conflict of interest was reported by the author(s).

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