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# EFFECTS OF TANNINIFEROUS OAK (*QUERCUS HARTWISIANA*) LEAVES ON GAS PRODUCTION IN *IN VITRO* RUMEN FERMANTATION SYSTEM*

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**Summary:** The aim of the current study was to investigate the effects of tanniniferous oak leaves on the gas production in *in vitro* rumen conditions. For that purpose, amounts of different types of tannins in the leaves were measured spectrophotometrically. Gas production in *in vitro* rumen fermentation system was measured in absence and presence of a tannin binding substance, polyethilen glycol (PEG), after 24 h of incubation at 39 C in a water bath and thus tannin effect was calculated. A battery of tannin assays including total phenolics, total tannins, condensed tannins, free gallic acide, gallotannins, and protein precipitable phenolics were carried out in the leaves. At the end of incubation, the corrected mean gas production for oak leaves for 24 h was 13.5 and 22.0 ml, repsectively with and without PEG addition and hence tannin effect was found to be 163 %. It was also found out that total phenolics and total tannins, gallotannins, free gallic acid, condensed tannins, protein precipitable phenolics as percentage of total phenolics were 13.4, 11.7, 0.4, 0.1, 4.3 and 27.3 %, respectively. In conclusion, it appears that there are various types of tanniniferous substances in the oak leaves and they appear to reduce digestibility in the *in vitro* rumen conditions.

**Key Words:** Oak leaves, tannins, *in vitro* gas production, rumen.

## Tanen İçeren Meşe (Quercus Hartwisiana) Yapraklarının *İn Vitro* Rumen Fermantasyon Sisteminde Gaz Üretimine Etkileri

**Özet:** Bu çalışmanın amacı, tanen içeren meşe yapraklarının *in vitro* rumen şartlarında gaz üretimi üzerine etkilerini araştırmaktı. Bu amaçla, yapraklardaki tanenlerin tür ve miktarları spektrofotometrik yöntemlerle belirlendi. *İn vitro* rumen fermentasyon sisteminde gaz üretimi, tanen bağlayıcı madde olan polyethylen glycol (PEG)'ün varlığında ve yokluğunda 39 C'deki su banyosunda 24 saat inkübasyondan sonra belirlenmiş ve tanen etkisi aradaki farktan hesaplanmıştır. Total fenolik, total tanen, serbest gallik asit, gallotanen, kondanse tanen ve protein bağlayabilen fenolik tespitini içeren bir seri tanen analizi yapıldı. İnkübasyon sonunda, PEG ilave edilmediğinde 13.5 ml, PEG ilavesinde ise 22.0 ml gas üretildiği ve bu nedenle de tanen etkisinin % 163 olduğu belirlendi. Total fenolik, total tanen, gallotanen, serbest gallik asit, kondanse tanen ve protein bağlayabilen fenolik miktarlarının ise sırasıyla % 13.4, 11.7, 0.4, 0.1, 4.3 ve 27.3 olduğu belirlendi. Sonuç olarak, meşe yapraklarında değişik tipte tanen içeren maddeler bulunduğu ve bunların da *in vitro* rumen şartlarında sindirimi azalttığı belirlenmiştir.

**Anahtar Sözcükler:** Meşe yaprağı, tanenler, *in vitro* gaz üretimi, rumen.

## INTRODUCTION

*In vitro* rumen fermation systems offer a cheap, practical and reliable technique for the assessment of behaviour of feedstuffs with the microorganisms of the rumen[1,2]. Of those systems, gas production ($CO_2$ and $CH_4$) technique is widely used as a result of its precision in the prediction of *in vivo* organic matter digestibility, metabolisable energy and rumen protein degradability[1,3,4]. Tanniniferous compounds in feedstuffs can affect the rumen microbes[5]. As their digestion in rumen is not well understood, further research is needed to understand their interaction with rumen microbes in terms of gas production and digestibility.

Oak leaves are among the roughages fed to the ruminant animals during scarcity of food in some areas of Turkey and in the other parts of the world[6]. Although they are rich in protein, their nutritious affect is offset by the tannins they possess. Tannins either bind to proteins (condensed tannins) and prevent digestion or alternatively they are absorbed through the intestines and thus cause toxicity in the animal (hydrolisable tannins such as gallic acide)[5,7]. Therefore, it is important to find out the tannin type and content in the oak leaves.

However, it is not always easy to associate the tannin effect with one method because of the diversity of tannin types and their protein binding capacity in different types of tree leaves. Therefore, a battery of assays have been developed to analyse the plants for tanniniferous substances. In vitro gas production system is one of the most reliable method in terms of finding tannin effect[8]. For that purpose, polyethylen glycol (PEG) is added into the syringes containing sample and rumen fluid and thus PEG is bound to tannins as a result of their high affinity.

The aim of the current study was, therefore, to find out the tannin types and content in the oak leaves and

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to assess gas production capacity of that leaves in the presence and abscence of PEG in *in vitro* gas production system.

## MATERIALS AND METHODS

Processing the leaf material: The oak leaves were collected from an area covered by oak trees in the middle of the August and they were all mixed before the analyses. The sample was then weighed and shade dried. It was ground finely (60 mesh) by Miley mill.

*In vitro* rumen fermentation system: Hohenheim Gas Production System was used as *in vitro* rumen fermentation system[1,8]. This system consisted of 50 ml glass syringes and a water bath set to 39 C. Briefly, oak samples (0.375 g) in triplicates were placed carefully at the tip of the syringes and 30 ml rumen buffer solution was vacumed into the syringes by using repeat dispenser. After clipping the tip of syringes, they were suspensed in the water bath with the help of a carrier. Gas production was recorded at 0, 3, 6, 9, 12, and 24 h.

For the estimation of tannin effect on gas production, another triplicates of oak samples were prepared simultenously and PEG 6000 was added just after the addition of samples into the syringes. The amount of PEG added was twice the amount of the samples, i.e. around 0.750 g.

Tannin analyses: Of the dried and ground leaf samples, 0.200 g was taken into a beaker of approximately 25 ml capacity and placed into an ultrasonic water bath for 20 min at room temperature after adding 10 ml 70 % acetone or 50% methanol depending on the assay. The contents of beaker were transferred into centrifuge tubes and then were centrifuged at 3000*Xg* at 4 C.

Total phenolics and tannins were measured using Folin-Ciocalteau method according to Makkar et al.[9]. Condensed tannins were measured according to Porter et al.[10]. The amount of gallotannin and free gallic acids were determined by the method reported by Inoue and Hagerman[11]. The amount of protein-precipitable phenolics was measured according to Makkar et al.[12] by extracting the leaf samples by using 50 % methanol.

Chemical analyses of the leaf samples: The chemical analyses of oak leaves were carried out according to A.O.A.C.[13] (Table 1).

**Table 1.** Chemical composition of oak (Q. hartwisiana) samples used in the study. Crude fibre, crude protein, ash, ether extracts and nitrogen-free extractives are given as percentage of dry matter.
**Tablo 1.** Çalışmada kullanılan meşe (Q. hartwisiana) yapraklarının kimyasal kompozisyonu. Ham selüloz, ham protein, kül, ham yağ ve azotsuz öz maddeler kuru maddede % olarak verilmiştir.

| | % |
|---|---|
| Dry matter | 35.4 |
| Crude fibre | 21.7 |
| Crude Protein | 18.4 |
| Ash | 6.8 |
| Ether Extract | 1.6 |
| N-free extractives | 51.5 |

## RESULTS

Gas production of oak leaves in *in vitro* fermentation system is given Table 2.

The correction factor was calculated according to gas production of hay standard and it was found to be 1.06. Corrected mean gas production, calculated by adjusting the results to 0.200 g and deducting 13.2 ml gas produced for blanks, found to be 13.5 for oak and 22.0 for PEG added oak. Therefore, "PEG effect" or "tannin effect" was calculated to be 163 %.

Oak samples were also analysed for tannin content and type. The results are given in Table 3.

**Table 2.** Gas production of oak leaves at 0, 3, 6, 9, 12, and 24 h *in vitro* fermentation system.
**Tablo 2.** Meşe yapraklarının, *in vitro* fermentasyon sisteminde 0, 3, 6, 9, 12 ve 24. baatlerdeki gaz üretimleri.

| | | | Gas readings (ml) | | | | | |
|---|---|---|---|---|---|---|---|---|
| OAK+PEG | Replicate | Substrate (g) | 0h | 3h | 6h | 9h | 12h | 24h |
| Oak | A | 0.374 | 30 | 40 | 47 | 52 | 56 | 67 |
| Oak | B | 0.377 | 31 | 41 | 48 | 52 | 56 | 68 |
| Oak | C | 0.373 | 30 | 40 | 46 | 51 | 55 | 67 |
| Oak+PEG | A | 0.377+0.745 | 31 | 44 | 53 | 61 | 67 | 84 |
| Oak+PEG | B | 0.370+0.740 | 30 | 42 | 52 | 60 | 65 | 80 |
| Oak+PEG | C | 0.375+0.759 | 31 | 43 | 53 | 61 | 66 | 83 |

**Table 3.** Tanniniferous components of oak (Q. hartwisiana) leaves
**Tablo 3.** Meşe (Q. hartwisiana) yapraklarının tanen içerikleri.

| | % in DM |
|---|---|
| Total phenolics | 13.4 |
| Tannins | 11.7 |
| Gallotannins | 0.4 |
| Free gallic acid | 0.1 |
| Condesed tannins | 4.3 |
| Protein precipitable phenolics as % of total phenolics | 27.3 |

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## DISCUSSION

The study showed that tannins in oak leaves might have a great impact on the digestibility. Addition of PEG into the syringes increased gas production by 163 %. In other words, if there was no tannins in the leaves, the digestion would hypothetically increase by up to two times. This, however, does not show with certainity that the tannins in the oak leaves are all deleterious for the digestion. Because, the results in the current study refers to the rumen conditions but not to the lower digestive system. One of the well known effect of tanniniferous plants is the protection of digestion of proteins in the rumen and hence to produce rumen by-pass proteins. The degree of digestion in lower parts of the digestive tract can not be assessed by the current study. However, the data obtained clearly shows that the digestion of oak in the rumen is preturbed greatly as a result of tannins they possess. It is known that tannins not only prevents the digestion of proteins but also of carbohydrates and minerals, and they are all needed for optimal rumen microbial activity[5].

In the study carried out by Makkar et al.[5], various negative effects of tannins in oak has been reported on the *in vitro* digestion. They showed that tannins in oak reduces RNA synthesis by rumen microbes and inhibit the process of microbial attachment/adhesion to the feed particles[5] and hence they reduce the digestion of nutrients. In addition, they observed negative effects of oak leaves on almost all rumen microbial enzymes they studied. Taken with the results of the current study, it therefore appears that the tannins in oak leaves reduce digestion by affecting rumen microbial activity.

Tannin type and content known to be very important in the assessment of antiruminal effects of the tanniniferous plants. Makkar and Singh[14] reported a great variation in total phenolics content, condensed tannins, and protein precipitation capacity of various oak species. For example total phenolics content varied between 0.8 to 28.4 % depending on the age of the leaf and the oak species. The oak species studied in the study (Q. hartwisiana), yielded 13.4 % total phenolics. Condensed tannins, however, was between 0.4 and 5.5 % in the study of Makkar and Singh[14] and it was 4.3 % in the current study. Condensed tannins (proanthocyanidins) are not absorbed through the digestive tract and they bind proteins and thereby reduce their digestion[15,16]. They might also damage the mucosa of the gastrointestinal tract and therefore might reduce the absorption of nutrients and thereby reduce the absorption of essential aminoacids such as lysine and methionine. Although both condensed tannins and hydrolisable tannins (e.g. tannic and gallic acids) form tannin protein complexes in similar manner, hydrolisable tannins might be hydrolyzed in the acidic gastric environment and therefore bound proteins might be released[7,16,17]. Hydrolisable tannins can be absorbed through the gastrointestinal tract and they are toxic to ruminants[7]. Their toxication causes hemorrhagic gastroenteritis, necrosis of the liver and kidney damage with proximal tuberal necrosis[7]. Methaemoglobinaemia is observed in ruminants when tannins are received orally in high amounts[18-20]. Both condensed tannins and hydrolisable tannins exist in the oak leaves studied and therefore all effects associated with them is likely to be observed.

In conclusion, amounts of tannins is high and several types of tannins exist in oak leaves, which in turn appears to be reducing the digestion process by affecting the rumen microbial activity. Therefore, further studies are required to find out the digestion *in vivo* and strategies to inhalt these effects *in vivo*.

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