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Forage Yield, Nutritional Value and Phytotrepic Traits of Hops (*Humulus lupulus* L.)

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Abstract
Medicinal plants increases animal yield and quality; they also play an alternative role in the treatment of animal diseases thanks to their phytotrepic properties. One of these plants is hop (*Humulus lupulus* L.). A hop contains significant amounts of polyphenols, crude protein, and crude ash, and it's also easy digestibility. It contributes to animal health, yield, and quality due to these traits. This study, it was aimed to determine the hay yield and quality of hop. The five different age groups (3, 5, 10, 15, and 20) of two varieties of hop (Brewers Gold and Aroma) were used in the study. The samples were investigated for hay yield, protein yield, acid detergent fiber (ADF), neutral detergent fiber (NDF), potassium (K), phosphorus (K), calcium (Ca), magnesium (Mg), total phenolic (TPC), total flavonoid (TFC), condensed tannin (CT) and total alkaloid content (TA). The highest hay yield was determined in the 5 age group (568.57 and 663.38 kg da^{-1}, respectively) of both varietites and in the 10 age group (568.71 kg da^{-1}) of Aroma variety. The highest protein yield was determined at 5 and 10 years of age (107.67 and 89.06 kg da^{-1}, respectively) of Aroma variety. The K, P, Ca and Mg contents were ranged between 1.680-2.477%, 0.320-0.420%, 0.817-1.570% and 0.420-0.720%, respectively. The lowest TA of 2.95% was determined in the 15 age group of Aroma variety. The CT of hop varied between 1.38%-5.42%, the TPC ranged between 11.78-43.41 mg GA g^{-1}, and the TFC 11.87-13.32 mg QE g^{-1}. As a result, it has been determined that hop can be considered as an alternative roughage source in terms of the all properties examined. Besides, there were differences between the age groups and varieties of the plant in terms of forage yield, quality, and although it was determined that the hay of the Brewers Gold variety of the 5 age group performed better than the other treatments, it is possible to use the parts of both varieties in every age group as roughage easily..

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1. Introduction

Phytotherapy is defined as the use of medicinal plants to prevent and alleviate the effects of diseases in humans and animals. Medicinal plants can be used on their own as well as it’s being purified by methods such as extraction, squeezing, and distillation or some of them can be prepared. Besides, they can be applied as powders, extracts, pastes, and infusions (Kaya, 2009). Russo et al. (2009) reported that traditional herbal treatment methods are preferred in many countries due to the inaccessibility of modern treatment methods due to economic and geographical reasons and the expense of commercial veterinary drugs.

Recently, the interest in alternative treatment methods against chemical products and antibiotics used in animal husbandry has started to increase. Kowalczyk et al. (2013) reported that the use of externally supported antibiotic supplements in animal nutrition was banned in 2006; therefore, medicinal plants can be used as an alternative to antibiotics. Besides, the medicinal plants can be used in diseases, chronic diseases, and recurrent infections of livestock production (Davidović et al., 2012).

Plant secondary compounds are powerful and effective tools available to improve agroecosystems and animal health. A growing body of research has shown that phytochemicals such as tannins, flavonoids, phenolic compounds in an animal’s diet promote animal health, reduce greenhouse gas emissions and improve nutrient utilization (Ramírez-Restrepo and Barry, 2005; Kaymak Bayram et al., 2022). Previous studies indicated that the positive effect of secondary metabolites (flavonoids and phenolic) on the productivity, quality and health of animals (Seradj et al., 2014; Paula et al., 2016).

Increasing feed costs and the desire to increase the yield and quality of livestock has created the need for alternative forage plants. As an alternative forage plant, plants that grow spontaneously in nature come to the fore. However, some plants can be used as fodder plants for parts other than their intended use. One of these plants is hop. It is important in terms of animal yield and quality with its plant nutritional traits, and it also draws attention in terms of animal health with its secondary metabolites.

A hop (*Humulus lupulus* L.) is a perennial herb from the cannabis family. Although the plant is used in brewing, yeast, and bread making, its young shoots are also used as vegetables (İncekara, 1964). Hop were first used for their antimicrobial properties because of it is effective for injured animals to rub their wounds. In addition, it has been observed that the animals whose birth is approaching are calmer after eating the plant, their birth is easier, and the milk yield is increased. On the other hand, it has been determined that hop have anticonvulsant and hypnotic effects, and therefore animals that eat hop are less restless than other animals. This situation reveals that the plant has an estrogenic and calming effect.

This study was aimed to determine hay yield and quality of different age in hop varieties.

2. Material and Methods

In the study, Brewers Gold and Aroma varieties of hop (*Humulus lupulus* L.) 3, 5, 10, 15, and 20 years old were used as plant material. Hop samples were collected from farmers in August of the Pazaryeri district of Bilecik (Turkey) (Figure 1). After the hop was harvested from the field, the bolls of the plant were separated and the remaining parts were evaluated.

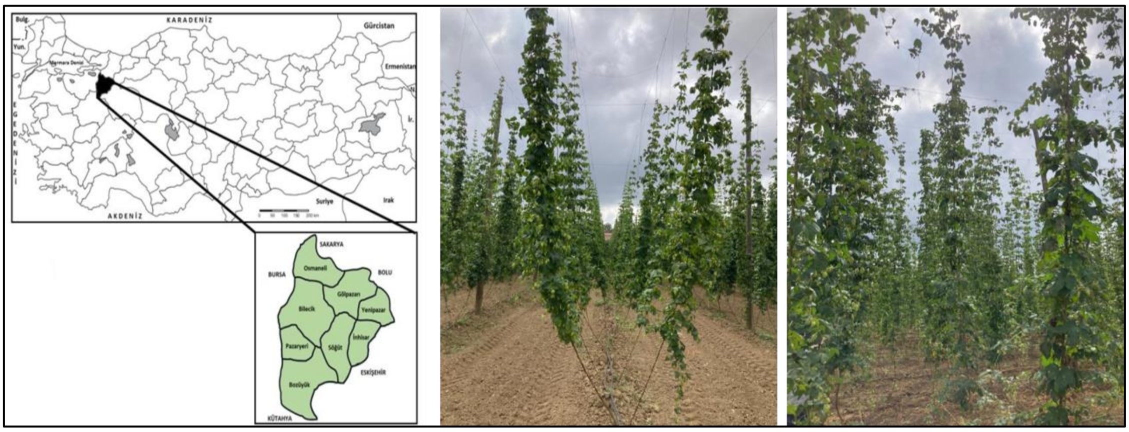

Figure 1. The image of the hop and the region where it is grown (İbrik, 2020)

Hop samples were dried 65 °C until constant weight and then, it is determining hay yield. Crude protein ratio, acid detergent fiber (ADF), neutral detergent fiber (NDF), and mineral contents (calcium (Ca), potassium (K), magnesium (Mg), and phosphorus (P)) were determined by using Near Reflectance Spectroscopy (NIRS, 'Foss 6500') with software package program 'IC-0904FE'.

For the secondary metabolites of hop samples (1 g) were total extracted with 10 ml of 1% HCl/methanol v/v for 1 h at room temperature. Then, total phenolic content (TPC) was determined by using Singleton et al. (1999) with some modifications, while the total flavonoid content (TFC) was determined by using Arvouet-Grand et al. (1994) with some modifications.

Condensed tannin was performed by using Bate-Smith (1975) with some modification. The hop samples 0.01 g weighed and added 6 ml tannin solution and boiled in a water bath for 1 h. Then reading was performed in a spectrophotometer at 550 nm wave length.

The total alkaloid contents of the samples were determined by modifying the INEN (2005) method. Accordingly, 1.2 g Al_2O_3 was be added to 0.2 g sample and mixed until a powder obtained. 1 ml KOH (150.4 g L^{-1}) was added to the powder mixture and mixed until a homogeneous consistency obtained. The mixture was taken into a centrifuge tube and 6 ml of chloroform was added to it and centrifuged at 3000 g for 5 minutes. The filtrate was collected in a glass bottle with the help of a filter. Chloroform, centrifugation and filtrate collection were repeated at least 10 times. It was evaporated at 30°C (until 1 ml remains) until there were no alkaloids left in the extract. To analyze the alkaloid amount, 5 ml of NaOH (0.40 g L^{-1}) and 2 drops of methyl red indicator was added and titrated with sulfuric acid (0.01 ml). The total amount of alkaloids was calculated as g 100 g^{-1} according to the formula below.

TA = 0.248* V/ sample weight (g)

Data were analyzed by using the MSTAT-C computer software program in split plot design in a randomized complete block. The main plots formed the varieties sub-plots and the age groups. Significant differences among the mean values were compared by Duncan multiple-range test.

3. Result and Discussion

Hay and protein yield of hop were given Table 1. The analysis of hop samples showed that age ad age × variety interaction were significant ($p<0.01$) while variety not significant. The highest hay yield of hop was determined from 5 age of both variety (568.57 and 663.38 kg da^{-1}, respectively) and 10 age of the Aroma variety (568.71 kg da^{-1}) according to the age × variety interaction. Hay yield of hop was the lowest in the samples from 15 and 20 age of Aroma variety (231.15 and 228.81 kg da^{-1}, respectively). The hay yield was higher in the 5 age compared to the other age of hop. The protein yield of both 5 and 10 years of Aroma variety (107.67, 89.06 kg da^{-1}, respectively) was higher than other treatments. When compared to the age, the highest protein yield obtained from 5 ages (86.41 kg da^{-1}). It was determined that both hay and protein yield decreased with advancing age.

The unused parts of hop (consisting of stems, leaves and unused flowers) are much larger than the amount used in beer production. The bolls of the plant correspond to approximately 20% of the total plant, and, this means that 80% of the plant is thrown away. In addition to its yield, the high protein content of the plant makes its evaluation in terms of animal nutrition high.

Table 1. Hay and protein yield of hop

Ages	Hay yield (kg da^{-1})			Protein yield (kg da^{-1})		
	Brewers Gold	Aroma	Mean	Brewers Gold	Aroma	Mean
3	464.76 bc**	372.05 cd	***418.41 b****	78.17 bc**	52.79 d	***65.48 b****
5	568.57 ab	663.38 a	***615.98 a***	65.16 cd	107.67 a	***86.41 a***
10	326.80 de	568.71 ab	***447.75 b***	52.12 d	89.06 ab	***70.59 b***
15	350.63 d	231.15 e	***290.89 c***	47.97 d	28.20 e	***38.08 c***
20	415.14 cd	228.81 e	***321.98 c***	60.44 cd	25.60 e	***43.02 c***
Mean	***425.18***	***412.82***		***60.77***	***60.66***	

**: p<0.01. There is not a difference between the same letters in each column (p<0.05)

The hop ADF and NDF ratios were significantly different (p<0.01) amongst age, varieties and age × variety interactions (Table 2). The ADF and NDF ratios were between 24.60-40.99% and 38.81-55.88%, respectively according to the binary interaction. The averages of ADF and NDF ratios of Brewers Gold (29.05% and 45.23%) were lower than the variety of Aroma (36.29% and 50.96%). The ADF and NDF states the plant's digestibility and maturity. In forage, ADF and NDF should be ranged between 20-30% and 30-40%, respectively (Cole, 2020). In this study, ADF and NDF ratios of Brewers Gold and age of 10 exhibited better than compared to the other treatments.

Table 2. ADF and NDF ratio of hop

Age	Acid detergent fiber (%)			Neutral detergent fiber (%)		
	Brewers Gold	Aroma	Mean	Brewers Gold	Aroma	Mean
3	29.05 d**	36.52 b	***32.79 b****	42.29 e**	46.51 d	***44.40 c****
5	30.00 d	30.62 d	***30.31 c***	46.06 d	45.32 de	***45.69 c***
10	24.60 e	40.99 a	***32.79 b***	38.81 f	54.00 a	***46.41 c***
15	31.38 cd	38.31 ab	***34.85 a***	50.62 bc	55.88 a	***53.25 a***
20	30.22 d	35.01 bc	***32.62 b***	48.37 cd	53.10 ab	***50.73 b***
Mean	***29.05 B****	***36.29 A***		***45.23 B****	***50.96 A***	

**: p<0.01. There is not a difference between the same letters in each column (p<0.05)

The effect of the age and interaction of age × variety on K and P contents of hop was significant (Table 3) (p<0.01, p<0.05). Besides, the effect of the variety on P contents was significant (p<0.01), while the effect on K contents was not significant (Table 3). The highest K content was determined in the 3 age of Brewers Gold variety (2.477%) and the 20 age of Aroma variety (2.383%). The lowest K content (1.680%) was obtained in the 10 age of Aroma variety. The P content of hop samples ranged between 0.320-0.420% in terms of interaction. The average P of Brewers Gold (0.373%) were higher than of Aroma (0.347%) (Table 3). The potassium plays an important role in osmotic pressure regulation and water balance in the animal's body while phosphorus is involved in every metabolic reaction and energy transfer within the animal body (Ahemad et al., 2009; Yogeshpriya and Selvara, 2018). Accordingly, the ratio of K and P in forages should be 0.80% and 0.21%, respectively. (Kidambi et al., 1993; Tekeli and Ateş, 2005). In this study, the K and P content of all hop was at the desired level (Table 3).

Table 3. The potassium and phosphorus content of hop

Ages	Potassium (%)			Phosphorus (%)		
	Brewers Gold	Aroma	Mean	Brewers Gold	Aroma	Mean
3	2.477 a**	2.310 b	***2.393 a****	0.407 a*	0.360 b	***0.383 a****
5	2.257 b	1.980 c	***2.118 b***	0.350 b	0.400 a	***0.375 a***
10	1.930 c	1.680 d	***1.805 c***	0.420 a	0.320 b	***0.370 a***
15	2.040 c	2.327 b	***2.183 b***	0.337 b	0.330 b	***0.333 b***
20	2.020 c	2.383 ab	***2.202 b***	0.353 b	0.327 b	***0.340 b***
Mean	***2.145***	***2.136***		***0.373 A****	***0.347 B***	

*: p<0.05; **: p<0.01. There is not a difference between the same letters in each column (p<0.05)

The effect of the age, variety and age × variety interaction on Ca and Mg contents of hop was significant (Table 4) (p<0.01, p<0.05). According to the interaction, Ca and Mg contents ranged between 0.817-1.570% and 0.420-0.720%, respectively. The variety of Brewers Gold was more than variety of Aroma in terms of both mineral nutrients. The Ca and Mg contents of roughage should be 0.3% and 0.1%, respectively (Kidambi et al., 1993; Tekeli and Ateş, 2005). In the study, it was above the desired values for both elements.

Table 4. The calcium and magnesium content of hop

Ages	Calcium (%)			Magnessium (%)		
	Brewers Gold	Aroma	Mean	Brewers Gold	Aroma	Mean
3	1.410 abc**	1.403 abc	***1.407 a****	0.627 bc**	0.647 bc	***0.637 a****
5	1.327 bc	1.500 a	***1.413 a***	0.597 cd	0.680 ab	***0.638 a***
10	1.570 a	1.250 c	***1.410 a***	0.720 a	0.533 d	***0.627 a***
15	1.250 c	0.957 d	***1.103 b***	0.537 d	0.457 e	***0.497 b***
20	1.470 ab	0.817 d	***1.143 b***	0.580 cd	0.420 e	***0.500 b***
Mean	***1.405 A****	***1.185 B***		***0.612 A****	***0.547 B***	

*: p<0.05; **: p<0.01. There is not a difference between the same letters in each column (p<0.05)

The effect of the age, variety and age × variety interaction on condensed tannin and total alkaloid of hop was significant (Table 5) (p<0.01, p<0.05). Kumar and Singh

(1984) indicated that high tannin in feed negatively affects protein digestion, and microbial and enzyme activities in ruminants, while Önal Aşcı and Acar (2018) indicated that the feeds with low condensed tannin led to an increase in protein content of milk. On the other hand, condensed tannins are inhibited some hydrogen-producing protozoans and methane-producing organisms that use hydrogen directly in the rumen, and reduce greenhouse gas emissions (Martin et al., 2016). Besides, the condensed tannins show an anthelmintic effect, reduce animal internal parasites and increase productivity in the animals (Luscher et al., 2016). Accordingly, the condensed tannin content in the feed should not exceed 2-3%. In the present study, the condensed tannin content was below 2-3% in variety of Brewers Gold 3, 5, 10, 15 ages and variety of Aroma in age of 3 (Table 5).

According to the interaction, the highest total alkaloid content of hop was determined from 3 age of a variety of Brewers Gold (3.72%), while the lowest was from 15 age of Aroma variety (2.95%) (Table 5). Knowing the amount of the alkaloid content of forages is very important because they are responsible for the bitter taste and lower palatability. High alkaloids in feeds cause toxicity to animals. Therefore, low alkaloid content is desirable in the forages. When compared to the ages, the highest total alkaloid content obtained from 3 ages of hop samples (3.40%). Besides, Brewers Gold average total alkaloid content (3.32%) was more than Aroma variety (3.08%) (Table 5). Okafor et al. (2020) reported that the average alkaloid content of hop was at 4.0%.

Table 5. Condensed tannin and total alkaloid of hop

Ages	Condensed tannin (%)			Total alkoloid (%)		
	Brewers Gold	**Aroma**	**Mean**	**Brewers Gold**	**Aroma**	**Mean**
3	1.38 e**	2.20 de	***1.79 d****	3.72 a*	3.09 bc	***3.40 a****
5	1.83 de	3.79 bc	***2.81 c***	3.12 bc	3.22 bc	***3.17 b***
10	2.05 de	3.86 bc	***2.96 c***	3.23 bc	3.13 bc	***3.18 b***
15	2.82 cd	4.79 ab	***3.81 b***	3.29 b	2.95 c	***3.11 b***
20	3.31 c	5.42 a	***4.37 a***	3.25 bc	3.02 bc	***3.13 b***
Mean	***2.28 B****	***4.01 A***		***3.32 A****	***3.08 B***	

*: p<0.05; **: p<0.01. There is not a difference between the same letters in each column (p<0.05)

The effect of the age, variety, and age × variety interaction on the total phenolic content of hop was significant (p<0.01), while they were not significant on the total flavonoid content (Table 6). Some researchers indicated that total phenolic and flavonoid contents have antioxidant and antimicrobial effects, and they have significant potential to improve animal yield and quality (O'Connell and Fox, 2001; Robbins, 2003; Frozza et al., 2013). Previous studies show that flavonoid and phenolic compounds were a positive effect on the productivity, health of animals, rumen fermentation, and control of nutritional stress such as bloat and acidosis (Seradj et al., 2014; Paula et al., 2016). According to the interaction of age × variety, total phenolic and flavonoid content of hop ranged between 11.38-43.41 mg GA g^{-1} and 11.87-13.32 mg QE g^{-1}, respectively. The variety of Brewers Gold was more than variety of Aroma in terms of total phenolic content. Da Rosa Almedia et al. (2020) reported that the average total phenolic and flavonoid content of hop was

33.93 mg GAE g^{-1} and 54.47 mg QE g^{-1}. Environmental conditions, cultural applications, soil types and the varieties could cause such differences.

T**able 6.** Total phenolic and total flavonoid content of hop

Ages	TPC (mg GA g^{-1})			TFC (mg QE g^{-1})		
	Brewers Gold	**Aroma**	**Mean**	**Brewers Gold**	**Aroma**	**Mean**
3	31.11 bc**	11.78 e	***21.45 b****	12.98	12.47	***12.73***
5	29.62 bc	19.76 d	***24.69 b***	13.26	13.11	***13.19***
10	27.86 c	11.38 e	***19.62 b***	11.87	12.53	***12.20***
15	43.41 a	29.49 bc	***36.45 a***	13.32	12.50	***12.91***
20	30.03 bc	37.05 ab	***33.54 a***	13.09	12.90	***13.00***
Mean	***32.41 A****	***21.89 B***		***12.91***	***12.70***	

**: p<0.01. There is not a difference between the same letters in each column (p<0.05). TPC: Total phenolic content; TFC: Total flavonoid content

4. Conclusion

As a result, hop has been determined that hop can be evaluated as roughage in terms of the examined traits. Besides, there were differences between the age groups and varieties of the plant in terms of forage yield, quality, and although it was determined that the hay of the Brewers Gold variety of the 5 age group performed better than the other treatments, it is possible to use the parts of both varieties in every age group as roughage easily.

Declaration of Author Contributions

The authors declare that they have contributed equally to the article. All authors declare that they have seen/read and approved the final version of the article ready for publication.

Declaration of Conflicts of Interest

All authors declare that there is no conflict of interest related to this article.

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