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Effect of Mixture Ratios on Silage Quality of Legume-Grass: Berseem (*Trifolium alexandrinum* L.)-Triticale (*xTriticosecale* Wittmack) Study

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ABSTRACT

Background: This research was carried out to determine the effects of mixtures of different ratios of berseem (*Trifolium alexandrinum* L. cv. Derya) (BS) and triticale (x*Triticosecale* Wittmack cv. Tatlıcak) (T) on silage quality.

Methods: The research was conducted at the Siirt University Faculty of Agriculture, Türkiye. Silage applications including BS and T sole (100% BS and 100% T) and binary mixture ratios (20% BS+80% T, 40% BS+60% T, 60% BS+40% T and 80% BS+20% T) were the subject of the research.

Result: As a result of the research, silage odour, structure, colour, total physical score, pH, dry matter, Fleig score, crude protein, acid detergent fiber, neutral detergent fiber and relative feed value varied between 11.33-14.00 points; 2.67-4.00 points; 1.00-2.00 points; 15.33-20.00 points; 4.62-5.98; 25.46-30.31%; 16.59-80.18; 10.30-19.12%; 30.86-36.89%; 33.27-55.93% and 100.09-180.71, respectively. Overall, the results revealed that the mixing ratio significantly affected the feed value and functional components of berseem-triticale silage. When all features were evaulated together, an 80% berseem+20% triticale silage mixture was recommended.

Key words: Berseem, Crude protein, Fleig score, Silage, Triticale.

INTRODUCTION

Due to the increasing demand for meat and dairy products in parallel with the growth of the world's population, livestock farming has become a vital element of food security (Singh *et al*., 2026). In Türkiye, where the sown rate of forage crops is low, livestock farming relies heavily on rangeland-based livestock farming. However, especially in geographical areas where a continental climate prevails, the period during which rangeland can be used is reduced due to the long and harsh winter months. This situation results in the animals being fed in barns for a large part of the year. In barn farming, ensuring a sufficient supply of high-quality roughage is essential for efficient livestock production. In this context, forage crops grown within field agriculture are of great importance. The cultivation of forage crops, which can be evaluated as green forage, hay and silage, is also the insurance of livestock enterprises. In Türkiye, which has approximately 38.6 million hectares of agricultural land, forage crops are grown on about 2 million hectares, yielding 32 million tons of green fodder (TSI, 2026). However, this forage production is insufficient to meet the roughage needs of the existing livestock. Therefore, the conservation of green fodder is considered important for the livestock sector (Kengoo *et al*., 2023), which is regarded as one of the main pillars of the rural economy.

The cheapest and easiest way to preserve green forage is to make silage. A quality silage feed also makes an important contribution to balanced and healthy animal nutrition. The silage of forage crops can not only improve the utilization value of forage plants and avoid the waste of forage; it can also effectively solve the problem of livestock's lack of food in winter (Pariz *et al*., 2017). In addition, silages are considered to be the most economical feed material for feeding ruminant animals (Bhatt *et al*., 2025). Türkiye produces approximately 27 million tons of silage (TSI, 2026). Increasing this production is important for the development of rural livestock farming.

The purpose of making silage is to preserve the nutritive value of the harvested crop (Wang *et al*., 2024; Yıldız, 2024). Factors affecting the nutritional value of silage include factors influencing the physical structure and chemical composition of the plant, such as harvest maturity, plant species and cultivars, sowing time, planting method, irrigation, fertilization, weed and pest control, plant microbiology and ecological conditions, as well as harvesting

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management (machinery, cutting height, wilting, bruising and chopping) and ensiling management (silo type, compression/density, sealing and type and dosage of silage additives) (Wang *et al*., 2024). Among plant species, annual legume forage crops are widely used for silage production. For this purpose, berseem (*Trifolium alexandrinum* L.) (BS), which has agricultural characteristics such as high biomass production, fast growth, high number of harvests, high protein content and long-term green fodder supply (Açıkbaş and Özyazıcı, 2022; Mandloi *et al*., 2026), has ensiling potential. However, as with many legume forage crops, it is quite difficult to ensile alone because of its low dry matter (DM) and water-soluble carbohydrate (WSC) contents and high buffering capacity (Sahoo, 2018). Therefore, it is a more recommended practice to ensile legumes with grasses and/or some cereals to facilitate the fermentation process and to obtain more balanced, nutritious and energy-rich feed for ruminants. Triticale (*xTriticosecale* Wittmack) (T) is a grass that can be used for silage production by cutting during the milk stage and whose silage yield can be higher than rye, oat and sorghum in some cases (Oliveira *et al*., 2021; Ma *et al*., 2022); if the silage rules are followed, high-quality silages can be obtained (Soundharrajan *et al*., 2023) and is used as a silage crop in many cases. In addition, characteristics such as low buffering capacity and high fermentable carbohydrates of grasses may also constitute an advantage for triticale in silage production. The use of legumes together with triticale provides good fermentation quality for silage (Bumbieris Junior *et al*., 2021). In this sense, silages made from mixtures of berseem and triticale can be an interesting roughage source in terms of both obtaining high-quality silage feed and providing nutritionally balanced feed for animal rations. In other words, the mixed silage of berseem and triticale can not only improve the feed utilization rate, but also solve the problem of feed nutritional imbalance.

Today, silage research focuses on the quality of high -quality raw materials or mixed silage. In this sense, it is of great importance to know and/or determine the mixing ratios when silaging legumes as a mixture with grasses. As a matter of fact, in many studies conducted with various legume and grass species, it has been reported that a higher quality silage feed is obtained when the species are ensiled as a mixture compared to ensiling them alone and that the mixture ratios significantly affect the silage quality (Yucel *et al*., 2018; Özyazıcı *et al*., 2023; Mosebi *et al*., 2025). This research was carried out to determine the effects of different ratios of mixtures of berseem and triticale on silage quality.

MATERIALS AND METHODS

Site description and plant material

The research was conducted at Siirt University Faculty of Agriculture (37°58′13.20″N- 41°50′43.80″ E; 887 m). Silage plants were grown in the vegetation period of December 2022 to May 2023. The soils where silage plants are grown have a clay-loam texture, are salt-free, are neutral in character and have little calcareous content. The organic matter content of the soil is “very low”, the available phosphorus content is “low” and the available potassium amount is “excessive”. While the average temperature value for the vegetation period was 10.6°C, the long-term (1939-2023) temperature average of the same period was 9.0°C. The total rainfall in the same period was 282.4 mm and the long-term average rainfall was recorded as 564.2 mm (TSMS, 2023). The ‘Derya’ variety of berseem and ‘Tatlıcak’ variety of triticale were used as silage material.

Agronomic practices for silage cultivation

Each silage plant was grown in separate plots in 10 rows with 5 replications. Berseem was planted with a sowing rate of 25 kg ha^{-1} at 25 cm row spacing and triticale was planted with a sowing rate of 450-550 pieces m^{-2} at 20 cm row spacing. The sowing was carried out on December 8, 2022. Agricultural practices such as fertilization, weed, pest and disease control were applied homogeneously for each plot throughout the growing season.

Silage preparation

In the study, the pure silage of the species (100% BS and 100% T) and binary mixture silages (20% BS + 80% T, 40% BS + 60% T, 60% BS + 40% T and 80% BS + 20% T) were considered as research subjects. The harvest time of triticale was taken into account in silage making. Triticale for silage was harvested during the milk maturity period. In the same period, berseem was harvested in its flowering stage. The harvested silage materials were wilted for a while to reduce moisture content; next, the materials were chopped to lengths of approximately 0.5-1 cm using a forage cutter. The forage materials were weighed and mixed according to the mixing ratios; then, they were filled into 2-liter glass jars using a sequential filling technique. Prepared silage jars were left to ferment in the dark at room temperature (25±2°C) for 60 days. The silage experiment was conducted according to the randomized plots design with 3 replications.

Silage physical and chemical quality analysis

The silages were opened after 60 days. Physical properties such as odour, structure and colour of silages were examined. Physical examinations were performed subjectively by three subject experts and scores were given according to the scoring method developed by DLG (1987). In addition, the physical quality class of the silages was determined according to the DLG score, which consists of the sum of odour, structure and colour scores (DLG, 1987) (Table 1).

Dry matter ratio (Bulgurlu and Ergül, 1978) and pH (Heiss *et al*., 1985) were determined in silage samples and Fleig score (FS) was calculated with the help of Equation 1 (DLG, 1987).

$$FS = [220 + (2 \times \text{silage DM ratio} - 15)] - 40 \times \text{silage pH value} \quad ...(1)$$

In addition, silage quality class with FS [FS= 100-81 (I-Very good), 80-61 (II-Good), 60-41 (III-Satisfactory-

Medium), 40-21 (IV-Low-less value), 20-0 (V-Bad)] was also evaluated (DLG, 1987).

Dried samples were ground using a laboratory mill with a 1 mm screen and subsequently stored for chemical analysis. Crude protein (CP), acid detergent fiber (ADF) and neutral detergent fiber (NDF) ratios of the silages were determined using the #IC-0904FE calibration set (WinISI, 2023) with a Near Infrared Reflectance Spectroscopy device (Brogna *et al*., 2009). Additionally, silage relative feed value (RFV) was determined according to the principles reported by Van Dyke and Anderson (2000). In evaluating the quality grade of silage roughage according to the CP, ADF, NDF and RFV data, the classification in Table 2 was used (Rohweder *et al*., 1978).

Statistical analysis

The data obtained from the study were subjected to variance analysis according to the randomized parcels trial design; according to the F test results, the differences between the groups were determined with the TUKEY multiple comparison test (Açıkgöz and Açıkgöz, 2001).

RESULTS AND DISCUSSION

Effect of mixing ratios on silage physical properties

The scores of berseem and triticale ensiled at different mixing ratios in terms of physical parameters are given in Table 3. Significant differences were found between the mixture ratios in terms of silage odour ($p<0.01$), structure ($p<0.05$) and DLG score ($p<0.01$). These differences occurred between the pure silage of berseem and/or mixtures containing high amounts of berseem and other subjects (Table 3). When opened the silages should have a distinctive “slightly sour, fruity and aromatic” odour and should not have a buttery acidic odour. In addition, the leaves and stems of the silage materials maintain their structure and colour at the time of ensiling, which are important physical indicators

Table 1: Physical examination key developed by DLG and silage quality class.

Physical examination key	
1. Odour	Score
No butter acid odour, slightly sour, fruity and aromatic odour	14
A small amount of butter acid, strong sour odour and slight escalation	8
Moderate butter acid odour, strong escalation-musty odour	4
Strong butter acid or ammonia odour, very slight sour odour	2
Strong decomposition, ammonia or musty odour	0
2. Structure	
Intact leaves and stems	4
A slightly deteriorated structure of leaves	2
A deteriorated structure of leaves and stems, musty and dirty	1
Rotten leaf and stalk	0
3. Colour	
Preserved its colour at the moment it was silaged (brown in withered silage)	2
Slightly changed colour (yellow to brown)	1
Completely changed colour (reseda green)	0
Quality class according to the physical properties of silage	
Quality class	DLG score
I- Very good	20-18
II- Good	17-14
III- Medium	13-10
IV- Low (low value)	9-5
V- Corrupted (useless)	4-0

Table 2: Quality standards of legume, grass and legume-grass mixture.

Quality standard	CP (%DM)	ADF (%DM)	NDF (%DM)	RFV
Prime	>19	<31	<40	>151
Premium (1) (very good)	17-19	31-35	40-46	151-125
Good (2)	14-16	36-40	47-53	124-103
Fair (3)	11-13	41-42	54-60	102-87
Poor (4)	8-10	43-45	61-65	86-75
Reject (5)	<8	>45	>65	<75

of quality silage. Silages containing high amounts of berseem generally received low scores in physical indicators. In contrast, all treatments with a high proportion of triticale in the mixture had higher scores (Table 3). Compared to grasses/cereals, legume forage crops can be more easily spoiled during silage fermentation, especially in terms of colour and structure, due to their more delicate leaf and stem structures. This is the most important reason why total physical score values decrease with the increase in legume ratio in silage mixtures. According to DLG scores, silage of "good-very good" quality was obtained (Table 3). Silage studies conducted with legume forage crops and grasses mixed at different ratios support the current research results (Özyazıcı *et al.*, 2022; Öten and Köse, 2024).

Effect of mixing ratios on silage pH, DM and FS

pH, DM and FS data of silages are presented in Table 4. In silage mixtures, silage pH and DM ratio showed significant variations according to the proportions of legume and grass plants in the mixture ($p<0.01$). In parallel with the increase in the berseem ratio in the mixture, the silage pH value increased while the DM ratio decreased (Table 4). This can be explained by the fact that the easily fermentable carbohydrate content of berseem, a legume plant, is low, whereas triticale contains a high amount of WSC in its structure. Additionally, the DM content of plants belonging to the grass family is generally higher than that of plants belonging to the legume family. Similar results to the current research findings were also obtained from silage studies with different legume-grass mixture ratios such as lucerne-sweet maize stalk (Wang *et al.*, 2021), forage pea/grass pea/vetch-wheat (Ertekin, 2023) and vetch-triticale (Öten and Köse, 2024).

pH is one of the main factors affecting the degree and profile of fermentation (Kung *et al.*, 2018), ensuring that the silage feed is kept in a stable form (Bernardes *et al.*, 2015). Filya (2001) reported that the pH range for quality silage should be between 4.6 and 4.8. In the present study, except for the subjects with high levels of triticale, silage pH values of other applications were found to be above this optimal value in the literature. The higher pH in these silages is probably attributable to the lower WSC concentration and higher buffering capacity value, which together are thought to inhibit silage pH decrease during fermentation. Dry matter ratio is one of the important parameters that determine silage quality. Klamem *et al.* (2005) reported that approximately 25-40% DM content in silage is a good roughage for animal feeding. It can be said that the DM ratio of the silages obtained in the current study is at an ideal level for feed rations.

Table 3: Average scores of physical properties of sole and mixed silages of BS and triticale.

Mixture ratios	Odour	Structure	Colour	DLG score	Silage quality class
100% BS	11.33b	2.67b	1.33	15.33c	Good
100% T	14.00a	4.00a	2.00	20.00a	Very good
20% BS + 80% T	13.67a	4.00a	2.00	19.67a	Very good
40% BS + 60% T	14.00a	4.00a	1.67	19.67a	Very good
60% BS + 40% T	14.00a	3.00b	1.33	18.33ab	Very good
80% BS + 20% T	14.00a	2.67b	1.00	17.67b	Good
Mean	13.50	3.39	1.56	18.44	Very good
F value	30.929**	4.995*	2.922^{ns}	21.221**	
Coefficient of variation (%)	2.47	18.83	26.24	3.61	

Differences between means indicated with the same letter in the same column are statistically insignificant, *: $p<0.05$ and **: $p<0.01$ are significant within the error limits, ns: Not statistically significant.

Table 4: Effect of mixing ratio on silage pH, DM and FS.*

Mixture ratios	pH	DM	FS	Silage quality class
100% BS	5.98a	25.46d	16.59b	Bad
100% T	4.92b	30.31a	68.95a	Good
20% BS + 80% T	4.62b	29.99a	80.18a	Good
40% BS + 60% T	4.73b	28.81b	73.29a	Good
60% BS + 40% T	5.51ab	27.99b	40.72ab	Low-less value
80% BS + 20% T	5.91a	26.42c	21.44b	Low-less value
Mean	5.28	28.16	50.19	Satisfactory-medium
F value	8.758**	94.319**	11.037**	
Coefficient of variation (%)	6.65	1.23	28.75	

*: Differences between means indicated with the same letter in the same column are statistically insignificant, **: $p<0.01$ are significant within the error limits.

In the study, the FS, determined by using the relationship between the DM content and pH value of the silages, was also significantly affected by the mixing ratios ($p<0.01$). FS value decreased with the increasing proportion of berseem in the mixture. According to the silage FS results, it was determined that the silage obtained from 100% berseem was in the "bad" quality class, the silage mixtures containing 100% triticale and high proportions of triticale were in the "good" quality class and the silage mixtures containing high proportions of berseem were in the "low" quality class. According to the average FS value, the silages obtained from the study were found to be satisfactory (Table 4).

Nutritional value of legume-grass mixtures of silage

CP, ADF, NDF and RFV data of silages are presented in Table 5. The silage forage quality traits were significantly ($p<0.01$) influenced by the berseem-triticale mixture ratios. The highest CP and RFV were determined in 100% BS (19.12% and 180.71, respectively) and 80% BS + 20% T (18.90% and 172.41, respectively) applications. The lowest CP and RFV were determined in 100% triticale (10.30% and 100.09, respectively) silage. While CP and RFV increased depending on the increase in legume ratio in the mixture, ADF and NDF ratios decreased (Table 5). This can be explained by the fact that the CP content of graminaceous plants is lower than that of legumes, whereas their cell wall components are higher. These results are consistent with the findings of Soufan and Al-Suhaibani (2021); Liu *et al.* (2023) and Eren *et al.* (2025).

Crude protein is an important quality parameter of forages and is one of the factors affecting animal productivity. Therefore, it is important to include CP in a feed ration at a rate that meets the needs of ruminants. Meen (2001) emphasized that the CP content in feed rations should be at least 7%. In the current study, the CP content of the berseem-triticale silages was at a level that could meet the needs of ruminants.

ADF is a forage quality indicator that estimates the digestibility of forage, while NDF estimates how much forage will be consumed. Low concentrations of NDF and ADF are associated with high DM intake and high digestibility (Ghebregziabiher *et al.*, 2025). Therefore, a high ADF and NDF content in feed is not desirable. RFV provides a comprehensive estimate of forage quality by considering digestibility, fiber content and other nutritional components (Tlahig *et al.*, 2024). According to the classification in Table 2, it was seen that the silages were between fair and prime feed quality standards in terms of ADF, NDF and RFV.

Table 5: Effect of mixture ratio on the nutritional value of silage forage.*

Mixture ratios	CP	ADF	NDF	RFV
100% BS	19.12a	31.37c	33.27d	180.71a
100% T	10.30d	36.89a	55.93a	100.09c
20% BS + 80% T	11.25cd	35.92a	52.20a	108.72c
40% BS + 60% T	13.10c	34.75ab	47.29b	121.62bc
60% BS + 40% T	15.83b	32.55bc	41.47c	142.69b
80% BS + 20% T	18.90a	30.86c	35.11d	172.41a
Mean	14.75	33.72	44.21	137.71
F value	56.764**	12.990**	80.899**	40.103**
Coefficient of variation (%)	5.92	3.55	4.00	1.11

*: Differences between means indicated with the same letter in the same column are statistically insignificant, **: $p<0.01$ are significant within the error limits.

CONCLUSION

According to the research results, it was concluded that the best option for mixed silages of berseem and triticale is the 80:20 combination to obtain maximum quality silage. The current research findings might help bridge the knowledge gap of selecting the best mixture ratio of berseem and triticale to obtain silage with good qualitative traits. However, future studies with different plant species in multiple seasons will strengthen the findings of the current study and shed light on the applicability of mixing ratios.

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Conflict of interest

All authors declare that there is no conflict of interest related to this article. This research received no external funding.

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