Scientific Papers. Series D. Animal Science. Vol. LXVIII, No. 2, 2025
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

ACCLIMATIZATION OF THE JAPANESE WAGYU BEEF BREED IN THE MIDDLE BALKAN MOUNTAIN IN BULGARIA

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Abstract

The study was conducted in 2023 in the village of Palitsi, at the Elena Vaga cow farm. Two groups of animals of the Wagyu breed were formed. The first group was 19 heifers calved in Bulgaria in 2022. The other was 18 female calves born in 2022. The yield of fresh and dry grass mass, botanical composition and chemical analysis of the herbage have been studied. Female calves at three months weighted 95 kg, at 6 months - 178 kg, at 9 months - 225 kg, and at 12 months - 312 kg. In September, February and April, there was a negative increase in cows. The highest positive increase was in May, June and October. The yield of fresh and dry mass with hay maturity was higher than that of pasture maturity. A high percentage of participation in the grain group in the spring was established. Grass plantings in spring were characterized by a lower CB content and a higher NFE, and in hay maturity were higher values of SP, minerals, Ca and better in vitro digestibility of dry matter.

Key words*: acclimatization, botanical and chemical composition, grass stand, live weight, Wagyu.*

INTRODUCTION

There is an increase in the number of beef cattle worldwide, and this is related to the desire to use a cheaper way of farming for fattening. As such, the so-called combined cultivation - pasture with different duration of the barn period was developed. In recent years, there has also been an increase in the number of specialized beef cattle in our country. One of the breeds that has gained particular popularity is the Japanese Wagyu breed. The meat of animals of this breed is distinguished from other breeds by its intense marbling (Gotoh et al., 2011; Horii et al., 2009; Albrecht et al., 2011). In addition, they adapt very well to pasture conditions.

The name of the Wagyu breed comes from the Japanese language and has two roots - "Wa" - Japanese and "gyu" - beef. For centuries, animals of this breed have been used for harness. The Japanese Wagyu cattle breed is primarily used for cultivating, processing and harvesting rice.

In 1937, the Japanese Black breed was officially recognized (Morita et al., 2000). It is interesting to note that only 3,000 cattle could qualify as purebred Wagyu lines. According to Wagyuinternational, today Wagyu are reared and bred in varying degrees of purity around the world. Ninety-six percent of all animals are reared in Japan. Japanese Wagyu consists of 4 breeds of cattle - Japanese Black, Japanese Brown, Japanese Shorthorn and Japanese Large. Japanese Black cattle are the predominant breed. It produces meat with the highest degree of marbling compared to the other three breeds of cattle and makes up over 90% of all cattle in Japan. Australia is the country that ranks second in population of Wagyu after Japan. Wagyu cattle reared in Australia are of the Japanese Black breed. Almost 85% of the production is exported worldwide. For Australians, Wagyu meat is a preferred product for consumption.

According to Wagyuinternational.com, Wagyu cattle are reared in many parts of the world. Such are South Africa, America, Brazil, Canada, Costa Rica, Mexico, Asia, China, Indonesia, New Zealand, Philippines, Vietnam, Europe, etc. Beef from every continent, country and farm has its own flavour profile and qualities. The Wagyu industry in Europe is still quite small. After the year 2000, it began to develop in member states of the European Union - Denmark, Germany, the Netherlands,

Hungary, Ireland, Spain and Sweden. This breed of cattle is also bred in Great Britain, but it consists of crosses with conventional European cattle. The largest is the Wagyu association in Germany. The breed is also widespread in the Balkans - Greece, Turkey and Romania. In 2022, 19 purebred female animals were imported from Germany to Bulgaria, fertilized by embryo transfer. Currently, the herd has grown to 38 female animals and one male calf.

The main function of pastures is the production of feed for ruminants. In addition, they are a source of biomass for obtaining energy, protect the soil from water and wind erosion, utilize not a small part of CO_2, protect groundwater from pollution, part of the landscape around us, a place for recreation and walks. The production of meat at the expense of the grass on the hills and mountains seems very attractive. Nutrient satisfaction from a pasture depends on its nutrient resource, the composition and digestibility of the forage, and its daily consumption (Edwards, 2003). Studies by Todorova (2000), Todorova & Kirilov (2002) reported significant variation in the Weende composition, energy nutritive value and mineral composition of pasture grass from the Central Balkan region. According to Todorova et al. (2003), fresh mass yield from pastures decrease with increasing altitude. The participation of leguminous grasses in the grassland decreases with increasing altitude from 295 to 1540 m. For the maximum utilization of grass, the duration of animal grazing is very important. Russian scientists are of the opinion that when there is enough grass, it is economically most profitable to fatten cattle mainly with grass (Cherekaev, 1975, Vostrikov & Dorotyuk, 1982).

Pasture-based production systems represent a significant sustainable supplier of animal-based foods worldwide. For such systems, increasing evidence highlights the importance of plant diversity for the proper functioning of soils, plants and animals (Distel et al., 2020). The main problem in high-yielding animals on a grazing diet is obtaining an adequate net energy intake to meet the animal's requirements, apart from methane emissions (Mahanta et al., 2020). The pasture system for fattening is promising and suitable for ecological production. Ensuring sufficient grass mass, timely start and end of grazing on individual plots, regular control of height and yield, ensure the balance in consumption of fresh mass (Kennedy et al., 2005). Ingestive behaviour determines nutrient intake and thus feed efficiency and sustainability of ruminants through their ability to adapt to diverse available resources (Boval & Sauvant, 2021).

Biodiversity in pasture grasses is an important factor in obtaining a forage mass of high quality and nutritional value to meet the food needs of the livestock sector (Provenza et al., 2007). The ratio of plant groups in the composition of grass biomass affects the intake, assimilation and digestibility of forage by ruminants (Franzke et al., 2010). Animal nutrition is a central problem in animal husbandry, related to all important parameters of its development – productivity, product quality, reproduction, health, animal welfare, the economy of the industry and environmental protection (Todorov, 2014).

The purpose of the study was to follow the acclimatization of the Japanese Wagyu beef breed in our climate.

MATERIALS AND METHODS

The study was conducted in 2023 in the lands of Palitsi village, Elena (Veliko Tarnovo region) in the "Elena Vagu" Ltd. cow farm. Two groups of animals were formed. The first group consists of 19 heifers born in Germany, fertilized by embryo transfer with sexed sperm, imported and calved in Bulgaria in 2022. The other group consists of 18 female calves, offspring of these cows, born in 2022. Both groups are of the Wagyu meat breed. They are reared on pasture. Calves were reared with their mothers until weaning on pasture and then fed supplemental calf feed after 6 months in the growing-to-fertilization period. Cows were also fed a supplementary cow feed with 16% CP. They had unlimited access to water. The weight development of cows and calves was monitored for a period of one year. Average live weight of calves and average daily gain of cows by month were recorded.

The study included transition - type *Festuca rubra* L., *Agrostis capillaris* L. in pasture and

hay maturity. The following quantitative and qualitative indicators were monitored:

Fresh mass yield (kg/da) - determined after several samplings by measuring, along the diagonal of the grass stand (4 repetitions) and mowing the area of each plot (1 m^2). The samples were taken in two stages. Stage 1 - pasture maturity of the grass stand at the end of the third ten days of the month of April to the initial spindle phase for the wheat grasses. Stage 2 - hay maturity of wheat grasses (sweeping phase, grading) in the first ten days of July.

Dry mass yield (kg/da) - by drying plant samples (0.5 kg) under laboratory conditions (105°C) and recalculating for an area of 1 da based on the dry matter content.

Botanical composition of the grass (%) - determined by weight by analyzing grass samples with a size of 0.250 m^2 (taken immediately before mowing). By species and groups, the percentage participation of cereal and leguminous meadow grasses, and of various grasses (total) was established. The botanical analysis of the natural vegetation was carried out at two stages during the growing year: spring and summer. Stage 1 until the initial spindle phase for the wheat grasses and immediately before the onset of the budding phase for the leguminous plants; Stage 2 sweeping phase, grading of wheat grasses (hay maturity) of the grass stand.

A chemical analysis of representative pooled samples from herbivory studies was performed. The dry grass samples were dried, ground and analyzed in the chemical laboratory of the Institute of Mountain Animal Breeding and Agriculture, Troyan according to the classical chemical Weende and Van Soest methods. The main chemical composition of the dry biomass includes the following indicators: Crude protein (CP, %) - determined by the Kjeldahl method; Crude fats (CF, %) – by extraction in a Soxhlet type extractor; Crude fiber (CFr, %) according to the Weende analysis; Ash (%) – in a muffle furnace at 550°C; Calcium (Ca, %) - according to Stotz (complexometric); Phosphorus (P, %) - with vanadate-molybdate reagent according to the method of Gerike and Kurmis (Sandev, 1979), spectrophotometer (Agilent 8453 UV - visible Spectroscopy System), measuring in the 425 ηm region and NFE (%) = 100-(SP , % + SSl, % + CM, % + Ash, % + Moisture, %).

The fibrous components of the cell walls were determined as a percentage of dry matter and included: Neutral-detergent fibers (NDF, %), Acid-detergent fibers (ACF, %) and Acid-detergent lignin (ADL, %) according to the detergent analysis of Goering and Robertson (1970). The polyosides Hemicellulose and Cellulose were calculated empirically. The degree of lignification is expressed as a percentage ratio of ADL/NDF. The potential energy nutritional value of the fodder was evaluated according to the Bulgarian system such as Milk feed units and Feed units for growth (Todorov et al., 2010). *In vitro* enzymatic digestibility of dry matter (DDM, %), according to Aufrere (1982) by two-phase pepsin-cellulase method.

RESULTS AND DISCUSSIONS

The milk yield of the mother is judged by the growth of the calves. Although milk yield is not a priority breeding trait for beef cows, higher milk yield cows raise better calves.

Table 1. Average live weight of calves in kg from Elena and Palitsi village

Factor /months/	**n/number/**	**X+ Sx/** live weight, kg/	**S**	**C**
3 months	18	95.06+5.45	23.10	24.30
6 months	18	178.72+4.96	21.03	11.77
9 months	18	225.00+6.69	28.39	12.62
12 months	18	312.50+3.16	41.84	13.39
Total	**72**	**202.82+9.94**	**84.33**	**41.58**

The data from Table 1 show that female calves of the Wagyu breed at three months have a live weight of 95.06±5.45 kg, at 6 months they weigh 178.72±4.96 kg, at 9 months they have a live weight of 225± 6.69 kg, and at 12 months - 312.50±9.86 kg. Forster (2010) indicated that during the lactation period growth of Aberdeen Angus cattle is strongly influenced by maternal milk yield, which depends on calving season

and cow live weight. The results obtained in our research in the first three months confirm it Gociman et al. (2019, 2020). The obtained live weight in the next three months (Table 1) was realized from milk and pasture, since the calves are reared together with their mothers and thus, they get used to eating grass. After weaning, female calves are fed supplementary feed during the growing period until conception. Law et al. (2013) found that Aberdeen Angus cows with higher live weight at calving and higher and sustained milk yield produced calves with higher live weight at weaning. After their birth and reaching 3 months of age, the 2020 female calves studied by Nikolov & Karamfilov (2020) increased their live weight almost twice. During the first three months, the average daily gain is within 0.700-0.750 kg, during 4-7 months it is 0.830-0.950 kg. The growth of beef cattle calves during the lactation period depends on breed, sex, sire (Hoppe et al., 2010; Jakubec et al., 2003; Toušová et al., 2015). A similar gain during lactation was reported by Kolisnyk et al. (2018) after reviewing the Aberdeen Angus cattle breed in Ukraine. In general, the gain increases gradually during the lactation period, with the absolute monthly gain being within 20-26 kg. In the month after weaning, the average daily gain of the calves studied by Nikolov & Karamfilov (2020) decreased by approximately 25%. At 7 months of age, female calves reach approximately 32% of the live weight of adult cows of the breed. Female Wagyu calves in our study weighed 180 kg after weaning. While studying the growth of 205-day-old Aberdeen Angus calves, Hassen et al. (2004) found that mean daily gain increased continuously from birth to 250 days of age and then began to decline. The same authors found that female calves at the age of 261 days reached an average live weight of 271 kg. After a relative stabilization around 14 months, at 15- and 16-months growth again sharply declines to approximately 0.420-0.570 kilograms. This period coincides with the reaching of economic maturity and service of the heifers. The growth of animals both as a whole and within individual age groups is affected by the year, season and month of birth. Regardless of growth fluctuations during different periods, female calves reach optimal live weight for breeding (330-360 kg) at optimal age (15-16 months) (Nikolov & Karamfilov, 2020). The data obtained in our study are lower by about 20 kg/225 kg/ at the age of 9 months, during which the calves of this beef breed reach sexual maturity.

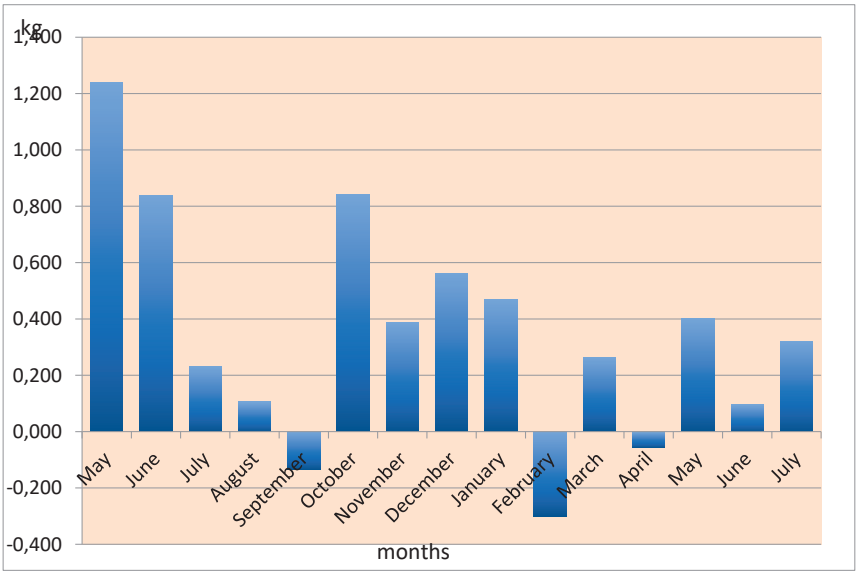

Figure 1. Average daily gain of cows in kg from Elena and Palitsi village

The graph in Figure 1 shows the average daily gain of cows, reared on the farm. The data indicates that during the months of September, February and April, the average daily gain was negative. Low gain was also observed during the months of July, August and March. During those months the cows received feed with 16% crude protein (CP) because of poor pastures. Highest gain was observed in May, June and October. During this period, the grass is the lushest and therefore the growth is the highest. The productivity of the pasture during the different months is shown in the climatogram (Figure 2).

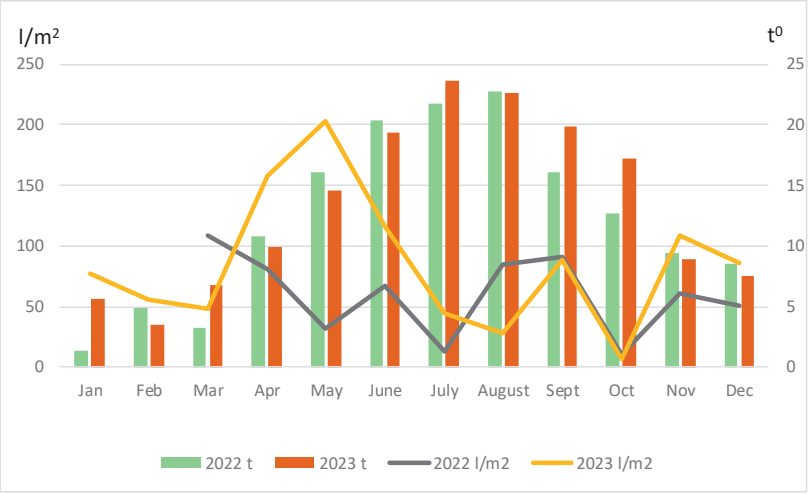

Figure 2. Climatogram

The gain in large ruminants depends on the quality of the used bulk forages - when their nutritional value is higher than 0.50 KE/kg of BW, such feeds should no longer be called roughages. Our data correspond to these norms (Table 5). First lactation cows are from 330 kg to 550 kg. The obtained results correspond to the data cited in the catalogue "Breeds of farm animals in the Republic of Bulgaria 2023".

Productivity of natural grass stand during spring and summer growth.

The annual biomass productivity of a natural grassland dominated by the cereal species *Festuca rubra* L. and *Agrostis capillaris* L. was monitored. Utilized for the needs of cattle, both for grazing and hay production. The average height during the spring growth of the meadow grass was determined (21.0 cm), and the obtained value determines the optimal growth of the species in the grass cover under the foothill conditions of the Middle Stara Planina. In grass studies, the yield of fresh and dry mass during spring growth (m. May) amounted to 584.00 and 151.02 kg/da (Table 2), and when the dominant grass species entered hay maturity, according to sampling in the first ten days in the month of July, the established productivity of fresh and dry mass amounts to 857.00 and 248.90 kg/day, which leads to a relatively good supply of bulky grass fodder suitable for feeding the cattle.

Table 2. Productivity of fresh and dry mass (kg/da) of natural grass stand - Elena and Palitsi village

Grass stand type – *Festuca rubra* L.-*Agrostis capillaris* L	Fresh mass, kg/da	Dry mass, kg/da
Grass stand in pasture maturity	584.00	151.02
Grass stand in hay maturity	857.00	248.90

The botanical composition was monitored by groups and species, and a predominance in the grass during the sampling period (during the spring growth of the grass) was found in the group of wheat grasses (63.50%). Legume meadow grasses and forbs occupy relatively equal shares in the grass cover of (18.20-18.30%), considering the early age of the undergrowth. We believe that the share of the three main groups during the spring growth of the grass species is in a favorable ratio, contributing to increasing the biological completeness of the grass chim used for food by the studied beef cattle breed Wagyu (Figures 3 and 4).

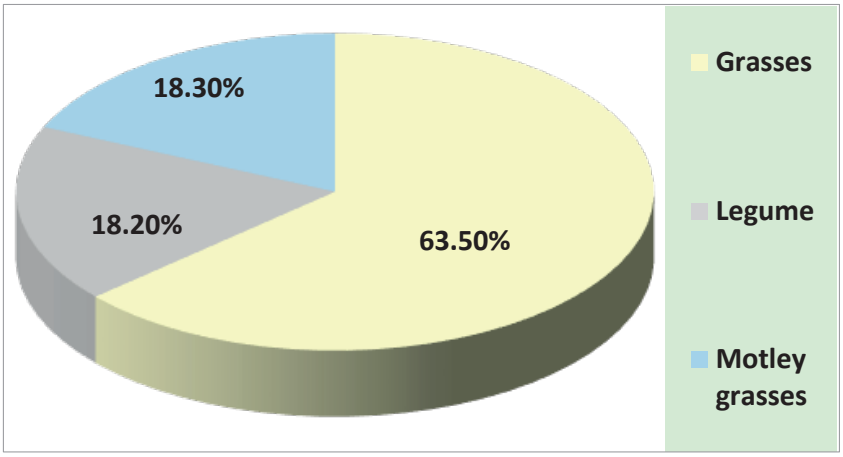

Figure 3. Botanical composition (%) of grass stand in spring growth divided by groups - Elena and Palitsi village

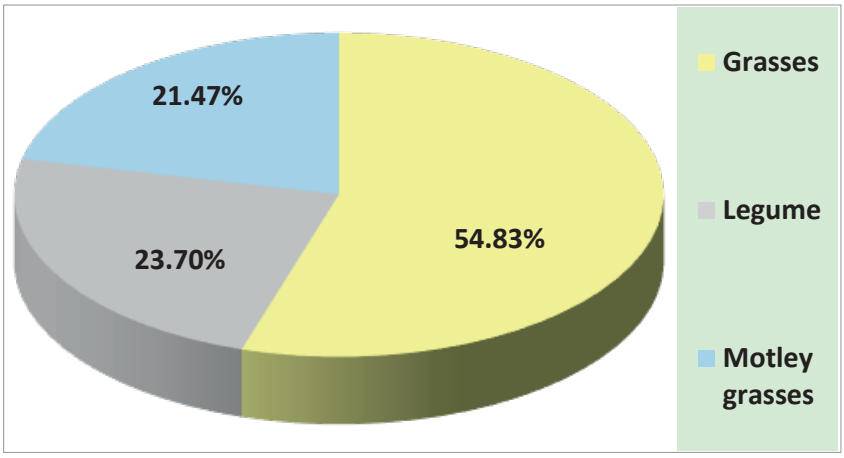

Figure 4. Botanical composition (%) of grass stand in hay maturity divided by groups - Elena and Palitsi village

The spectrum of species diversity during the spring growth in the natural meadow is represented by 7 species of wheat and legume meadow grasses of economic importance (Figure 5). The largest share is held by *Festuca rubra* L. (43.80%), followed by *Agrostis capillaris* L. (14.60%). Of the leguminous meadow grasses, three important representatives were registered, with *Trifolium pratense* L. and *Lotus corniculatus* L. registering an equal participation share of 7.30%.

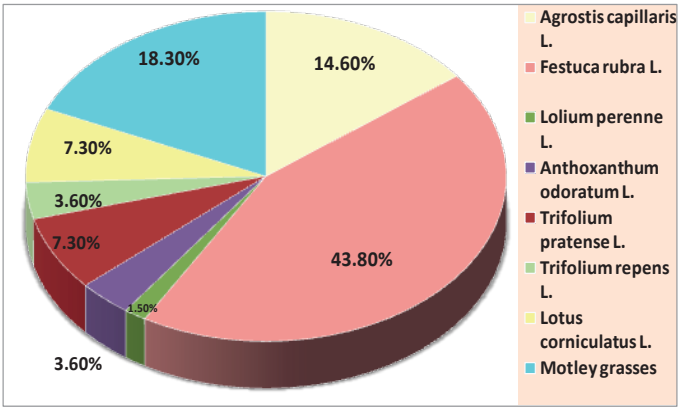

Figure 5. Botanical composition (%) in spring growth of turn divided by types - Elena and Palitsi village

The results of sampling when the grass stand reached hay maturity showed that the proportion of cereal grasses reached 54.83%, with leguminous meadow grasses registering an increase to 23.70%. A slight increase in the variety of herbaceous vegetation was also found considering the advancing age of the grass stand and the different cycle of growth and development characteristic of the grasses from the different botanical families - up to 21.47% (Figures 4 and 6). Given the spectrum of species diversity of cereal and legume meadow grasses, the examined herbage at hay maturity registered 10 main species of meadow grasses of economic importance (Figure 5). Under the influence of climatic factors and the development cycle of individual species, changes have been found in the defining species of the grassland. In this case, the species *Festuca rubra* L. gives up its dominant position to *Agrostis capillaris* L., which occupies the highest share (23.30%) of the wheat meadow grasses. The share of leguminous meadow grasses is increasing, reaching 23.70% of the total composition of the grassland, which reflects positively on the quality of the biomass.

When the grass stand reaches hay maturity, the general assessment is that the plant mass is well balanced in terms of the share of the three groups of meadow grasses. What was established is also confirmed by the favorable results in the chemical composition of the grassland.

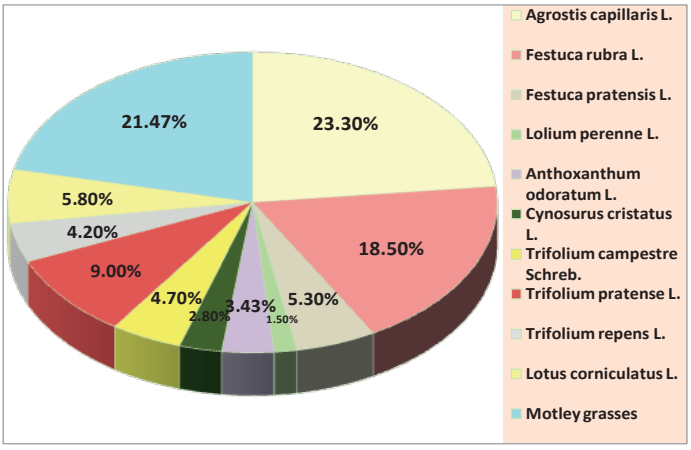

Figure 6. Botanical composition (%) of grass stand in hay maturity divided ono types - Elena and Palitsi village

Table 3 traces the main parameters influencing the composition of dry matter in the grass biomass of natural grass during spring growth (suitable for grazing) and when entering hay maturity. The crude protein content of the dry matter of the hay maturity biomass (12.29%) was 1.25% higher than that of the spring-grown grass mass, probably due to the higher involvement of legumes in the studied grass vegetation.

Table 3. Chemical composition of biomass of natural grass stand (in %) - Elena and Palitsi

Variants	Crude Protein (CP), %	Crude Fats (CF), %	Crude Fiber (CFr), %	Nitrogen-Free Extracts (NFE), %	Ash, %	Calcium, %	Phosphorus, %
Pasture maturity	11.04	1.35	38.34	33.28	6.28	2.13	0.521
Hay maturity	12.29	1.07	40.41	29.55	7.12	3.01	0.513

Grass stand at hay maturity is also characterized by a higher content of crude fiber (40.41%), mineral substances (7.12%) and calcium (3.01%) compared to pasture. The excess in the values of the indicators is by 2.07%, 0.84% and 0.88%, respectively. The concentration of the macroelement phosphorus has almost identical values in the researched grassland - 0.513% (hay maturity) and 0.521% (pasture maturity). The content of crude fat affects the palatability of the fodder mass. With higher values of the indicator is the herbage in pasture maturity (1.35%).

Table 4 shows the fibrous structural components of the cell walls and the in vitro dry matter digestibility. The data analysis indicates that the grass stand in pasture maturity has significantly higher values of the studied indicators, which affects and determines the lower digestibility of the dry mass.

Table 4. Fiber components in cell walls and *in vitro* digestibility of dry matter (%) of natural grass stand - Elena and Palitsi village

Variants	NDF, %	ADF, %	RFV, %	Hemicellulose, %	Cellulose, %	CFdig – Aufrere, %
Pasture maturity	65.73	36.57	6.41	29.16	30.16	61.73
Hay maturity	56.08	27.01	2.37	29.07	24.64	68.16

NDF - Neutral-detergent fiber; ADF - Acid-detergent lignin; RFV - Relative Feeding Value

The increase in the content of neutral-detergent fiber, acid-detergent fiber and acid-detergent lignin in the grass stand at pasture maturity was respectively 9.65% (for NDV), 9.56% (KDV) and 4.04% (for KDV) compared to that of hay maturity. Hemicellulose is a polyoside digestible by ruminants. The values of the trait in the grassland with different maturity are almost identical (29.07%-29.16%). Cellulose is a difficult or almost indigestible component of the fibrous composition of the plant cell, the content of which is 5.52% higher in the grass with pasture maturity of use.

With a higher in vitro digestibility of dry matter is the biomass of the grass stand at hay maturity (68.16%). The values of the indicator exceed by 6.43% that of the grassland with pasture maturity.

Lignin is a complex organic compound that binds cellulose fibers and hardens by strengthening plant cell walls (Nikolov & Karamfilov, 2020).

According to the degree of lignification (Figure 7), as a percentage of neutral and acid-detergent fibers in the composition of the grass mass, the grass stand with pasture maturity (9.75%) has a higher coefficient. The values of the indicator in the grassland with hay maturity are 5.52% lower.

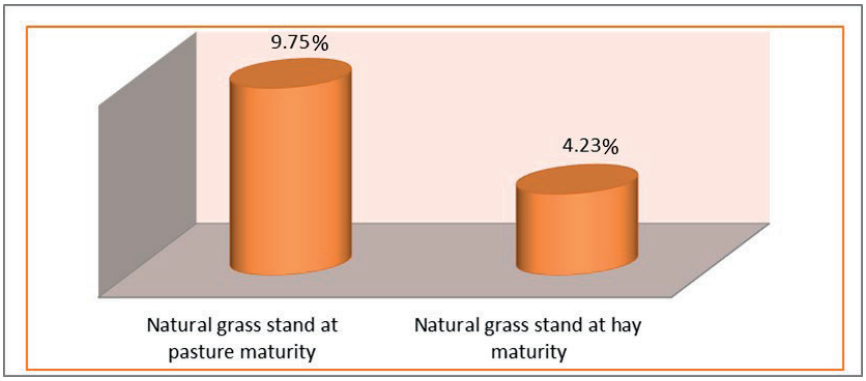

Figure 7. Coefficient of lignification level of natural grass stand, %

The indicators determining the energy value of the natural grass stand are an important factor for the quality of the obtained fodder and its efficient use (Table 5). The values of gross and exchange energy are close and correspond to those indicated by Todorov (2010). According to the obtained results, the quality of the grassland in pasture and hay maturity does not change significantly during the grazing period. The observed changes are related to the quantitative parameters in the composition of the grass community.

Table 5. Nutritional value of natural grass stand - Elena and Palitsi

Variants	GE, MJ/kg	EE, MJ/kg	FUM, amount in kg DM	FUG, amount in kg DM
Pasture maturity	16.83	6.87	0.62	0.56
Hay maturity	16.84	7.05	0.64	0.58

Gross energy (GE, MJ/kg DM), Exchangeable energy (EE, MJ/kg DM), Feed Unit for Milk (FUM, in kg DM), Feed units for growth (FUG, in kg DM)

CONCLUSIONS

Female calves of the Wagyu breed at three months have a live weight of 95.06±5.45 kg, at 6 months they weigh 178.72±4.96 kg, at 9 months they have a live weight of 225±6.69 kg, and at 12 months - 312.50±9.86 kg.

During September, February and April, a negative average daily gain was observed for cows. Low growth was also observed in July, August and March. The highest one was observed in May, June and October.

The yield of fresh and dry mass in the grassland with hay maturity was higher than that in pasture maturity by 46.7 and 64.8%, respectively.

The phytocoenological review of the plant composition in grass studies determined a high percentage of participation of the more economically significant plant species in the group of cereals during spring growth (63.50%) and 23.70% for legumes when entering hay maturity. A prerequisite for obtaining feed mass with higher quality and nutritional value has been established. The dominant species of the wheat and legume grasses are respectively: *Festuca rubra* L., *Agrostis capillaris* L., *Trifolium pratense* L., *Lotus corniculatus* L.

Grass stands in the spring growth mode are distinguished by a lower content of crude fibers (by 2.07%) and a higher content of nitrogen free extractive substances (by 3.73%). The distinctive feature of the grasses at hay maturity is the higher values regarding the content of crude protein (by 1.25%), minerals (by 0.84%), calcium (by 0.885%) and better in vitro digestibility of dry matter substance (with 6.43%).

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****Farm animal breeds in the Republic of Bulgaria* (2023). Catalogue, VI revised edition, Sofia, Bulgaria, 288p. ISBN 978-619-205-194-5