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BIOTECHNOLOGY IN ANIMAL HUSBANDRY

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ORGANIC BEEF PRODUCTION AS A SUSTAINABLE SOLUTION FOR THE EU MARKET - A CASE STUDY OF THE REPUBLIC OF SERBIA

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Review paper

Abstract: The European Green Plan and its implementation strategies defined very demanding and ambitious goals for establishing the first "climate neutral continent" in the world. The implementation of the "Farm to Fork Strategy" (F2F) strategy will have an extremely immense effect on quantitative changes in the area of animal production (reduction in the number of animals and volume of production), increase in prices of beef and products of animal origin, the decrease in citizens' standards and increase in inflation. The appearance of the so-called "leakage effects" can be expected in NON-EU regions with the lowest population density (LU/ha), such as Serbia and the countries of the Western Balkans. At the same time, this effect represents a development opportunity for organizing extensive to semi-intensive production of organic beef based on the Cow-calf system - grass-fed beef production. In addition to the production of organic meat, these systems will contribute to improving biodiversity, maintaining the microbiological and pedological structure of the soil, regulating the carbon cycle, preventing erosion and forest fires, stopping population migration from villages to cities, reducing the use of artificial fertilizers and biocides, etc.

Key words: organic production, beef, sustainable, EU market

Introduction

The European Green Plan (December 2019, European Green Deal) covers the most important sectors of economic and agricultural activities within the framework of the EU, which aims to mitigate climate change and further deterioration of the natural environment. The main goal is aimed at reducing the emission of gases with greenhouse effects (GHG) by 55% (compared to 1990) by the year 2030, that is the creation of the first "climate neutral continent" in the world by the year 2050. The realization of the set goals includes reforms in the most important sectors related to renewable energy sources, new climate laws, the establishment of a circular economy, reduction of air, water and land pollution, creation of sustainable agricultural production and food industry through higher quality and safety of food, less food waste, protection and preservation of the entire biodiversity. The countries of the Western Balkans have accepted the green agenda that refers to the region *(Guidelines - Green Agenda for the Western Balkans, 2020*), which is fully complementary to the European green plan. Within the framework of the general Green Plan, the "Farm to Fork Strategy" (F2F) strategy will have the most significant impact on agricultural and livestock production. Within this strategy, a key role is played by the action plan (period 2021-2027) for the development of organic production and preservation of biodiversity (*EC, 2020b, Regulation (EU) 2018/848*). This strategy represents a sustainable model of organic production and consumption, especially for small and medium-sized farms. In the forthcoming years, the mentioned strategies will have a significant impact on the restructuring of the volume and type of animal production, the movement of prices of products of animal origin, as well as on the overall market of agricultural and food products. The changes will not be visible only in the EU countries, but the influence of the strategies will reflect in the countries of the Western Balkans and Southeast Europe.

The production and market of beef in the EU are facing increasing pressures concerning campaigns to reduce red meat consumption, linking red/beef meat with environmental protection, negative impact on climate change, lack of welfare on farms, poor treatment of animals, lower and lower incomes generated on farms, etc. Furthermore, over the last 20 years, there has been a noticeable trend of a continuous decrease in the number of cattle, which in the year 2021 was at the lowest level with around 75.6 million animals (*Eurostat*, *2021*). This size of the cattle population enables the production of about 6.8 million tons of bovine meat (beef and veal carcasses). Furthermore, the population density of farmed animals per ha within the EU is remarkably uneven. In the year 2016, there was an average of 0.8 livestock units/ha in the range of 0.2 to 3.8, while the percentage of cattle was almost half of the total population of domestic animals. Furthermore, from the total number of cattle, 20.5 million are milk cows and 10.8 are fattening cows (*Eurostat, 2021).* However, the share of organic in the total livestock production is constantly increasing. The total organic agricultural sector is growing rapidly and currently represents 8.1% / 14.6 million ha in relation to the total arable area of the EU (*Trávníček et al., 2021*). The trend of increasing the number of farmed animals in organic production is also increasing and is at about 3% in relation to all farmed animal species, or 6% in relation to the percentage of cattle. In addition, the share of pasture used for the production of organic milk and meat is 5.6 million/ha (44%) (*EU Agricultural Markets Briefs, 2019*).

Commission's for markets, income and environment for the period 2021-2031 predicts a continuous decline in beef meat production of 8% but also a decline in demand (consumption of 0.3-0.9%) within the framework of the European Union (*EC - agricultural outlook, 2021*). In addition, in the period 2009-2020, beef meat production decreased by 1.7% *(Bundesanstzalt Bericht, 2021*). On the other hand, in accordance with the predicted population growth until 2031, migration movements of the population, and religious and traditional habits of consumers, it is predicted that the consumption of meat globally in this period will grow annually by 1.4% until 2030 (1.3% until 2050), and it is expected increase in demand for meat of all types on the world market by 0.3% (*OECD - FAO, 2021*; *Alexandratos et al., 2012*). This trend will certainly be reflected in the EU market in the form of changes in trade chains (meat deficit), export and import quotas in the EU, meat prices, regular supply of the market, etc. According to the estimates of the American Chamber of Commerce, since 2010, the European market has constant deficit of around 250,000 to 300,000 tons of beef, which is compensated mainly by imports from the United States and Latin American countries.

The aim of the present research was to investigate the sustainable model of organic beef production for the EU market through the case study of the Republic of Serbia, after implementation of the "Farm to Fork Strategy" (F2F).

The effects of the implementation of the Farm to Fork Strategy - F2F

The most important quantitative goals of the F2F strategy until 2030 refer to reducing the use of chemical pesticides by 50%, mineral fertilizers by at least 20%, reducing the sale of antibiotics in farm animal production and aquaculture by 50%, reducing food waste by 50% in the retail and consumer sectors, reducing the loss of nutrients in the soil by 50%, reducing the nitrogen (N-nitrate) balance by 50%, increasing organic production by at least 25%, etc. Groups of researchers (*Barreiro-Hurle et al., 2021; Beckman et al., 2020; Henning et al., 2021*) used different models to investigate the economic impacts on the implementation of F2F in the EU framework. The applied models mainly included a large number of different quantitative objectives of the strategy at the current level of technical and technological development of agriculture. The results of these studies unequivocally indicate that the F2F strategy will lead to a significant reduction in the volume of agricultural production, a reduction in the number of domestic animals and their products, with a simultaneous increase in food prices on the market of EU countries. Furthermore, *Henning et al. (2021),* state that the most pronounced decrease in the number of animals will be in fattening cattle (- 45%)

and dairy cows (- 13.3%), and estimates a decrease in the production of beef by 20% and milk by 6.3%, with an increase in the price of beef by 58%. Similar results were obtained in their research by *Barreiro-Hurle et al. (2021)*, and *Beckman et al. (2020)* who state that the decrease in beef production will be around 13.5% and milk from 10.0% to 11.6%. Given that the implementation of F2F requires additional, enormously high costs of adapting to new conditions on the market (rising prices, falling production, reduced net exports from the EU, etc.), the consequences of this will be felt to the greatest extent by consumers. Therefore, the implementation of the F2F strategy will directly affect the appearance of pronounced inflation and the loss of well-being among citizens. *Wesseler (2022)*, states that from an economic perspective, the implementation of F2F will obviously not be a free experiment within the EU. Furthermore, *Henning et al. (2021)* especially point out that the strategy will not justify its most important purpose and goal at all, because it will be "climate ineffective" due to the appearance of obvious leakage effects towards NON-EU countries. An additional 54.3 million is expected. t CO2 eq. in areas outside the EU borders. Of this, the most pronounced effect will be in animal production, namely in the production of beef (36 Mil. t CO2eq).

The strategy generally suggests the emergence of risks in households with low incomes and indicates the necessity for radical changes and transformations in the way of food production, processing, food supply chains, etc. In any case, the implementation of the F2F strategy will require significant technical-technological and institutional changes, such as innovative biotechnological solutions (*Wesseler, 2022)*.

The role of Ruminants

A large number of calls/tenders within the framework of EU funds are focused on finding the most optimal solutions in the beef production sector. Comprehensive and detailed studies like *Eip-Agri (2021)* based on respect for agroecological principles, offer long-term sustainable solutions through grass-based beef production systems. These systems often appear in different variants such as Cow-calf systems (suckler cows/beef) and contribute to the improvement of biodiversity, maintenance of microbiological activity of the soil and its pedological structure, regulation of the carbon cycle, prevention of erosion and forest fires, stopping population migration from villages to cities etc. In addition, their most important role is the transformation of inedible resources into food suitable for human consumption with high nutritional value, as well as contributing to the economic development of rural areas (*Gantner et al., 2022; Oltjen and Beckett, 1996; Eip-Agri, 2021; Domingos, 2022*). In rural areas where it is not possible to grow arable crops, and where pastures are used for grazing ruminants, this type of animal enables the long-term sustainability of the agro-ecological functions of the land, reducing the C footprint by its better binding in the soil (indirectly reducing enteric GHG emissions), improving water infiltration and its retention in the soil improves soil fertility, reduces the use of artificial fertilizers and biocides, etc. (*Teague, 2018; Grandin, 2022*). In this sense, adequate management of pastures and grazing through integrated crop-livestock systems provides numerous positive effects (*Planisich et al., 2021*).

Considering the above, the long-term ecological sustainability of the agroecosystem should be regulated by agropolitical measures, which provide a protocol for the regenerative management of crops and pastures.

Cattle and beef production in Serbia

From the aspect of farm size, degree of automation, present equipment and applied technology, cattle production in the Republic of Serbia (RS) is characterized by keeping cattle on small family farms of an extensive type, through medium semi-intensive farms, all the way to extremely specialized large farms with a pronounced degree of intensive and exclusively commodity production (*Kučević, 2022*). In 2021, 859,514 cattle were bred on 116,292 farms in Serbia (*RSZ 2021; SEEDEVa, 2020*). In an extremely fragmented structure of farm sizes (70% own 2-5 cows on average), about 2/3 of the production is distributed in the central part of Serbia, while is the remainder in the plains in the north of the country. Farms located in the north of the country are typically diversified crop/livestock agricultural holdings (*Kučević et al., 2019b*). In terms of breed composition, the Simmental breed and cattle of the Simmental type dominate with about 75%-80%, the second most important are black and white cattle and the Holstein-Friesian breed with about 15%, and the rest belong to autochthonous and specialized fattening breeds, crossbreeds with the aforementioned breeds, etc. (*Perišić, 2022; Bogdanović, 2016*). A number of fattening breeds are underrepresented, even in the controlled cattle population, it is about 1% (*Expert reports GOO, 2021*). The most represented fattening breeds are Aberdinangus, Limousin, Charolais and Hereford.

Beef production in the Republic of Serbia is below 1% compared to production in the EU (*Vlahović, 2015*). In 1990, the production of beef amounted to 140,000 tons, but in 2021 it would drop to only 77,000 tons (*RSZ, 2021*). The total gross domestic production was currently based on around 320,000 total slaughtered animals during the year (*Laćarac, 2020*). During that period, meat exports were around 7,000 tons with an average of 115% self-sufficiency. About 50% of beef is produced for an unknown customer, 20% for own needs and only about 30% for the organized market chain (*SEEDEVb, 2020*). The Republic of Serbia has had a quota of 8,700 tons per year for the export of "baby-beef" beef to the EU for decades, and this possibility of export is used by an average of about

10%.

Cattle and beef production in Serbia; Cow–calf system - grass-fed beef production

The Republic of Serbia has more than sufficient natural and social resources that enable the successful organization of both intensive commodity and traditional (and organic) cattle production (*Kučević, 2022*). A particular advantage relates to the possibility of establishing production in the Cow-calf system, which would be organized on meadows and pastures in hilly and mountainous areas (*Perišić, 2022*). The percentage of areas under meadows and pastures amounts to over 30% of the total agricultural land (about 1.5 million hectares). It is an ideal resource from the point of view of organizing cheap and profitable beef production. Unfortunately, a significant part of the area under meadows and pastures is not used due to poor yields of grass mass, poor quality, inaccessibility of the terrain, weediness, disordered property relations, etc. (*Kučević, 2022*). In addition to the mentioned resources, there are enough processing facilities (slaughterhouses, facilities for the production of animal feed) available, a sufficient number of which also have export permits. In the year 2019, the Veterinary Administration registered 439 approved facilities (361 red meat facilities and 78 white meat facilities) engaged in slaughtering, cutting and processing meat. In the RS, in addition to small craft facilities, there are around 120 processors of medium and larger capacities (*Mitrović, 2016*). Advisory and professional support to farmers is organized through an advisory system in agriculture that includes 35 agricultural and advisory services. With regard to facilities for housing cattle, it was determined that there are housing capacities for 2,557,926 animals and that 64% is of the maximum capacity (*Radivojević et al., 2014*).

According to available statistical data, there is still a sufficient number of available labour on agricultural farms (*Bogdanov and Rodić, 2014*). However, the age structure of the owners of the farm is unfavourable, considering that over 67% are older than 55 years of age and over 70% do not have the necessary IT education.

There are no precise data on the number of established cow-calf systems in the RS. Judging by the share of specialized fattening breeds in this type of production, the number of farms producing beef from specialized fattening breeds is insufficient. Given the above, it is necessary to strengthen the further import of fattening breeds and improve the establishment of national mother farms (nucleus of the herd). On the other hand, it is necessary to use the genetic potential of the most numerous Simmental breed, which can be directed to the production of quality beef through a successful selection program (defined breeding goal). A large number of farmers in EU countries have just decided to use the Simmental breed for meat production in the cow-calf system both in purebred and through crossbreeding programs (crossbreeding with fattening and dairy breeds) to achieve the heterosis effect in the F1 generation (*Kräußlich, 2005; Grogan et al., 2005*). Improvement of production traits of domestic cattle breeds cud be realized through the import of positively tested animals or semen in accordance with the breeding goal *(Kučević et al., 2005).* Respecting breed characteristics, vertical rezoning could be successfully organized in the cow-calf system (*Perišić, 2022*). Considering that Simmental is the most widespread breed in Serbia and because of the agro-climatic conditions, are the most common type of beef production systems *(Kučević et al., 2019a).*

The development and organization of extensive to semi-intensive beef production should be based on the maximum utilization of plant mass from meadows and pastures. The breeding technology in this system should be adapted to the selection of breed, the quantity and quality of grass mass, feeding possibilities, final body mass before slaughter, market requirements, etc. Available natural and social resources and adequate climatic conditions provide a comparative advantage for this type of production. However, currently, it is minimally and extremely ineffectively used. For the successful organization of the cow-calf system, considering the current state and characteristics of the beef production sector (socio-economic factors, demographic structure and migration - leaving the village, level of education of farmers, etc.), it is necessary to define a long-term action plan that should the following areas are improved:

- Strengthening cooperatives and clusters that are market- and export-oriented (improving the competitiveness of farmers, defining long-term contracts for production planning and secure purchase at market prices, cooperation with export agencies and chambers of commerce);
- Alignment of legislation with EU standards in the area of classification and evaluation of the quality of beef carcasses on the slaughter line (equalization and improvement of the quality of meat and halves, formation of adequate prices of meat concerning quality, etc.);
- New investment funds for starting and improving production (working capital of farmers through rural development programs);
- Education of farmers according to the specific requirements of the farm (needs in cultivation technology, knowledge from the IT sector and modern equipment, the introduction of standards necessary for exporting meat to foreign markets);
- Implementation of defined breeding goals and selection program (correct selection of breed in accordance with producing conditions and the preferred type of production);
- Prevention of illegal trade in livestock;
- More efficient management of meadows and pastures in order to increase the

yield and quality of plant mass (reseeding, selection of specific grass mixtures in accordance with the requirements of the soil and climate, enrichment of the soil with N-nitrogen through agroecological cycles, regulation of property-ownership relations, new legislation in this area etc.);
- More efficient organization of grazing (application of new technologies and IoT, sensors - precision livestock grass management, construction of solar farms on pastures, etc.);
- Improvement of the process of certification, marking and guarantee of product origin (organic production standards, names of origin and geographical indications, labels of traditional products, etc.);
- More efficient access to existing national subsidies, and their timely and purposeful realization (more efficient systems of information transfer to farmers);
- Improvement of rural-ethno tourism based on gastronomy with the consumption of locally produced beef and products of animal origin.

Based on a survey conducted with farmers engaged in the production of beef (different systems and technology) in several regions of Serbia, over 90% of the surveyed farmers expressed their willingness to double their production in 3-5 years (unpublished data of the author, research conducted in the year 2015). A prerequisite for such a thing is to improve a certain number of the above-mentioned areas. This would mean that in the short term, based on existing resources and capacities as well as the current number of livestock, the volume of meat production could be increased to the level of 10-15 years ago, i.e. 120,000 tons (*RSZ, period 2010-2020*). Increasing the volume of meat production could be further accelerated by the organized import of specialized fattening breeds of cattle and the formation of national nucleus farms. Using the example of an investment by purchasing 10,000 pregnant heifers of fattening breeds, in 10 years we could increase the initial number of breeding stock to over 30,000 female breeding animals (author's calculations based on recursive formulas, *Matić-Kekić et al., 2011)*. In this way, the potential of around 90,000 additional fatteners could be ensured.

Cattle and beef production in Serbia; Organic beef production

Serbia has fully developed legislative and institutional frameworks for organizing organic agricultural (livestock) production. The complete national legislation (Laws and Regulations) is harmonized with EU regulations (Council Regulation (EC) No. 834/2007 Commission (EC) No. 889/2008). At the Ministry of Agriculture, Forestry and Water Management, a special sector was established as a competent authority that performs tasks related to organic production. This body accredits control (certification) organizations under the SRPS EN ISO/IEC 17065 standard. A certain number of control organizations deal with the control and certification of organic products intended for export to the EU market.

Organic agricultural production is in an upward trend in the RS. Currently, about 21,000 ha of organic arable agricultural land is cultivated, of which about 6,000 ha are meadows. However, despite the regulated system that fully enables the organization of organic production, organic livestock production is extremely poorly developed, especially organic cattle production, which is at the level of 0.1% (*Organska proizvodnja u Srbiji, 2020*). Considering the growth trends of cattle in organic farming in the EU for the last decade *(EU Agricultural Markets Briefs, 2019; Kučević et al., 2016)*, the current state of organic livestock production should be improved.

Conclusions

The European Green Deal and the strategies within this concept will certainly lead to substantial changes in the meat market in the EU (especially beef) in the forthcoming decades. The most significant changes will be related to the decrease in the volume of agricultural production, the number of domestic animals and their products (fattening and dairy cows), changes in the volume and type of imports and exports, with an accompanying increase in food prices (meat and milk). Furthermore, the expected increase in inflation will have a negative impact on the level of well-being of EU citizens. On the other hand, the expected continuous increase in both consumption and demand for meat on the market may cause interruptions in the regular supply of the market and even supply deficits. Additionally to the mentioned, as a consequence of implementation strategies, the emergence of the so-called "leakage effects" can mostly be expected in NON-EU regions with the lowest population density (LU/ha), such as Serbia and the countries of the Western Balkans.

The production of beef in the Cow-calf system (suckler cows/beef) plays an extremely important role in improving biodiversity, maintaining the microbiological activity of the soil and its pedological structure, regulating the carbon cycle, preventing erosion and forest fires, holding population migration from villages to cities, etc. Therefore, this type of production can be reasonably expected in the future as well. A further step in the form of "upgrading" and advancement of this system in the form of technology and breeding methods would refer to the conversion of existing conventional farms to the production of organic beef. Such business decisions would be fully aligned with EU strategies for increasing the share of organic production, but also with current trends in production, consumption and demand for beef until the year 2030 and 2050, respectively.

The Republic of Serbia has exceptionally good comparative advantages in relation to the available natural and social resources and capacity. They enable the unobstructed development of extensive to semi-intensive beef production in Cow-calf systems (suckler cows/beef). This type of production should be maximally based on the utilization of plant mass from meadows and pastures. Given that the legislative and institutional frameworks in the RS for organizing organic livestock production are fully existing, it would be economically justified for this type of production to be certified according to the methods for organic beef production at one point. In support of this are the existing trade agreements, which enable the unobstructed export of beef (conventional and organic) to the EU market. If current long-term trends in meat considered, this production system will have a long-term sustainable perspective.

However, a prerequisite for the successful organization of this type of both conventional and organic production at the national level is the definition of a long-term action plan that should improve existing weaknesses and deficiencies in a number of the mentioned sectors. Finally, the implementation of such an action plan should be established in a timely manner in relation to the beginning of the implementation of EU concepts and strategies.

Organska proizvodnja goveđeg mesa kao održivo rešenje za potrebe EU tržišta – studija slučaja Srbija

Denis Kučević, Muhamed Brka, Miroslav Plavšić, Ksenija Čobanović, Tamara Papović, Vesna Gantner

Rezime

Evropski zeleni plan i strategije za njegovu realizaciju definisale su vrlo zahtevne i ambiciozne ciljeve za uspostavljanje prvog „klimatski neutralnog kontinenta“ na svetu. Implementacija strategije od “njive do trpeze” (Farm to Fork Strategie, F2F) će imati izuzetno veliki uticaj na kvantitativne promene u oblasti stočarske (animalne) proizvodnje kroz smanjenje broja životinja i obima proizvodnje, povećanje cena goveđeg mesa i proizvoda animalnog porekla, pada standarda građana i rasta inflacije. Nakon implementacije F2F strategije, može se očekivati i pojava tzv. “efekta curenja” (leakage effects) u regionima i zemljama izvan Evropske unije sa najmanjom gustinom naseljenosti uslovnih grla po hektaru. To se najpre odnosi na Republiku Srbiju i zemlje Zapadnog Balkana. Međutim, novi regionalni izazovi upravo mogu biti i razvojna šansa za pomenute države, kroz organizovanje ekstenzivne do poluintezivne proizvodnje organskog goveđeg mesa zasnovane po sistemu “krava tele – krava dojilja”, bazično organizovane u regijama bogatim livadama i pašnjacima. Osim proizvodnje organskog mesa, ovi sistemi će doprineti unapređenju biodiverziteta, održavanju mikrobiološke i pedološke strukture zemljišta, regulisanju karbonbskog ciklusa, sprečavanju erozije i šumskih požara, zaustavljanju migracija stanovništva od sela ka gradovima, smanjenu upotrebu veštačkih đubriva i biocida i slično.

Ključne reči: organska proizvodnja, goveđe meso, održivost, tržište EU

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GENETIC PROGRESS EVALUATION OF GROWTH TRAITS IN MOGHANI SHEEP

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Abstract: For evaluation of growth traits' genetic progress, pedigree information of 39408 Moghani sheep, between 1986 to 2016, at the breeding station of Moghani sheep was used. Studied traits included birth weight, weaning weight, six months weight, nine months weight, and one yearling weight. Variance components and genetic parameters of traits were estimated by restricted likelihood and six animal models using Wombat software. Direct heritability of birth weight, weaning weight, six months weight, nine months weight, and one yearling weight was (0.07, 0.09, 0.15, 0.13, and 0.20 respectively). Breeding values for the calculation of genetic trends for each trait were estimated by using the univariate best animal model. Phenotypic, genetic, and environmental trends of traits were estimated via regression of phenotypic average, breeding value average, and difference of breeding value from phenotypic value divided by birth year. The calculated genetic trends of birth weight, weaning weight, six months weight, nine months weight, and yearling weight were 0.008, 0.012, 0.054, 0.74, and 0.145 kg/year respectively, except for birth weight, the others were significant ($p<0.01$). The genetic gain of traits including birth weight, weaning weight, six months weight, nine months weight, and one yearling weight was estimated (0.213, 1.071, 1.171, 1.164, and 1.324 kg respectively). At evaluated years, many fluctuations were observed in the genetic trend of traits. In other words, it seems at the studied years, there wasn't a comprehensive plan for genetic breeding and improvement of body weight traits in Moghani sheep.

Key words: breeding value, genetic progress, genetic trend, phenotypic value, phenotypic trend

Introduction

Moghani sheep is one of the most valuable breeds of Iranian sheep and is the most populous breed of Iranian sheep after the Baluchi breed (about 2.5 million) *(Jafaroghli et al., 2010).* Moghani sheep is one of the most important meat breeds among tail sheep, of which in terms of large body size, resistance to climate change, the production capacity of heavy lambs, high growth rate, ability to triple lambing in two years, higher lamination rate, lower lamb mortality, and high genetic potential, it is known to be suitable for meat production (*Savar Sofla et al., 2011*).

The main center of maintenance and breeding of this breed is Moghan plain, but it exists in other areas such as Meshginshahr, Sarab, Ardabil, and even in some other provinces. The breeding system of these sheep is mainly decentralized and is done through migration between summer and winter pastures in mountainous and plain areas (*Ghavi Hossein Zadeh, 2015*).

Meat production is one of the most important criteria for determining the economic profit of sheep breeding in Iran. To examine ways to increase the income of sheep herds and select animals for proper production, we must first define economic traits as selection goals, then suggest appropriate selection methods to improve those traits according to the prediction of response to selection (*Matika et al., 2003*).

Selection of animals based on breeding values and estimation of genetic parameters and important economic traits are essential for designing optimal breeding strategies for farm animals (*Kosgey et al., 2006*). Therefore, estimating genetic parameters and the relative importance of the effect of various genetic factors is necessary not only for the preservation of indigenous breeds but also for setting goals and designing breeding programs. It is also necessary to better understand the genetic mechanism of different traits, determine the criteria and goals of selection, breeding value and use it in selection programs and predict the expected response of selection programs based on one or more traits (*Mohammadi et al., 2013*).

One of the main goals of breeding programs is to change the average value of breeding important economic traits in the shortest possible time and the right direction. Genetic improvement is possible by selecting parents with higher breeding values (*Kosgey et al., 2006*). In a society where selection is made and mating between animals is planned, it is necessary to examine the resulting changes in the average breeding value of the population as a result of selection to determine the effectiveness or inefficiency of that breeding program (*Rashidi and Akhshi, 2007*).

Therefore, the genetic trend is usually estimated for the stage at which the selection was made. Estimation of genetic and environmental trends in a population makes it possible to evaluate selection methods and reveals the role of environmental factors such as nutrition, health, and reproduction (*Hanford et al., 2005*). Because animal breeding values are cumulative over time, therefore, the average breeding value of animals each year shows the genetic level in that year (*Sargolzaei and Edriss, 2005*).

Since the evaluation of breeding programs is determined by estimating the genetic and phenotypic trends of the studied traits in a specific time frame and the genetic trend indicates an improvement in the average breeding value of the animals, this study aimed to estimate the parameters, genetic trends, and the rate of genetic progress related to growth traits in birth weight, weaning weight, six months weight, nine months weight, and one yearling weight Moghani sheep.

Materials and Methods

To estimate the genetic parameters of the growth traits of Moghani sheep, pedigree information and growth traits (birth weight, weaning weight, six months weight, nine months weight, and yearling weight) available in the Moghani sheep breeding station were used. This station is located 30 km southeast of Pars Abad city, Ardabil province. At this station, male and female lambs at the age of 18 months are ready to mate. Rams are used for one year, but ewes can be used for up to 6 years depending on their physical condition and reproductive function. Male and female lambs are kept in separate herds from six months of age.

In this study, 4859 birth weight records, 3450 weaning weight records, 2028 six months weight records, 1955 nine months weight records, and 1865 one yearling weight records collected during 30 years (1986 to 2016) were used. Pedigree information was used after initial editing (including correction and removal of duplicate records related to different periods of animal production and error). The pedigree characteristics of the present study data obtained using CFC 1.0 (*Sargolzaei et al., 2006*) software are given in Table 1.

Table 1. Pedigree information of Moghani sheep breed

Description	Number
No. of animals in the whole population	39408
No. of animals with both known parents	19034
Dams	13399
Sires	1481
No. of animals with no progeny	24528
No. of animals with progeny	14880
No. of base population	3492

Data was edited using Excel and Microsoft Visual FoxPro 9.0 software. During the data editing phase, due to the small number of triple lambs, the records of these lambs and other records whose information was not accurate or complete were deleted from the data file.

Significance testing of fixed effects, included in the operational model for each trait, was carried out using the general linear model (GLM) procedure (SAS 9.2), and the least-squares means of the traits were determined (*SAS, 2008*). Considered fixed effects in the model were the gender of lamb (male and female), birth year in 20 classes (1993-2013), damage at lambing in 6 classes (2-7 years old), and birth type in 2 classes (singletons and twins), respectively. The interactions between fixed effects were not significant and therefore excluded. The statistical model used was as follows [1]:

$$Y_{ijklm} = \mu + Y_i + A_j + S_k + LS_l + e_{ijklm} \quad [1]$$

in which Y_{ijklm} is the record of the desired traits, μ is the population mean, Y_i is the effect of the year of birth, A_j is the effect of damage at lambing, LS_l is the birth type, and e_{ijklm} is the residual effect.

(Co) variance components and corresponding genetic parameters for the various traits were estimated by restricted maximum likelihood (REML) method fitting an animal model. Wombat 1.0 software was used for this purpose (*Meyer, 2006*). Initially, six univariate models (shown below) were used to estimate genetic parameters.

$$\mathbf{y} = \mathbf{Xb} + Z_1 a + e$$

Model 1

$$\mathbf{y} = Xb + \mathrm{Z}_1 a + Z_2 \mathrm{c} + \mathrm{e}$$

Model 2

$$\mathbf{y} = Xb + \mathrm{Z}_1 a + Z_3 \mathrm{m} + \mathrm{e} \qquad Cov\,(a, m) = 0$$

Model 3

$$\mathbf{y} = Xb + \mathrm{Z}_1 a + Z_3 \mathrm{m} + \mathrm{e} \qquad Cov(a, m) = A\sigma_{am}$$

Model 4

$$\mathbf{y} = Xb + \mathrm{Z}_1 a + Z_2 \mathrm{c} + Z_3 \mathrm{m} + \mathrm{e} \qquad Cov\,(a, m) = 0$$

Model 5

$$\mathbf{y} = Xb + \mathrm{Z}_1 a + Z_2 \mathrm{c} + Z_3 \mathrm{m} + \mathrm{e} \qquad Cov(a, m) = A\sigma_{am}$$

Model 6

Where is a vector of records on the different traits; , , , , and are vectors of fixed effects, direct additive genetic effects, maternal additive genetic effects, maternal permanent environmental effects, and the residual effects, respectively. , , , and are corresponding design matrices associating the fixed effects, direct additive genetic effects, maternal additive genetic effects, and maternal permanent environmental effects to the vector of . Also, refers to the covariance between maternal and indirect genetic effects. An Akaike's information criterion (AIC) test was used to determine the most appropriate model according to the following formula [2]:

$$AIC_i = -2\ Log\ L_i + 2P_i \quad [2]$$

in which, log $\boldsymbol{L_i}$ is the maximized log-likelihood of the respective model i at convergence and $\boldsymbol{P_i}$ is the number of parameters obtained from each model; the model with the smallest AIC was chosen as the most appropriate model.

The total heritability ($h^2{}_t$) of the studied traits was calculated from the following formula [3]:

$$h^2{}_t = \frac{\sigma_a{}^2 + 0.5\,\sigma_m{}^2 + 1.5\,\sigma_{am}}{\sigma_p{}^2} \qquad [3]$$

in which $\sigma_a{}^2$, $\sigma_m{}^2$, σ_{am}, and $\sigma_p{}^2$ are direct additive genetic variance, maternal additive genetic variance phenotypic variance, the covariance between direct, and maternal additive genetic variance and phenotypic variance, respectively.

Breeding values for the calculation of genetic trends for each trait were estimated by using the univariate best animal model. Phenotypic, genetic, and environmental trends of traits were estimated via regression of phenotypic average, breeding value average, and difference of breeding value from phenotypic value divided by birth year. Genetic progression for each trait was estimated from the difference between the mean population breeding values at the end of the period and its value at the beginning of the period.

Results and Discussion

Table 2 shows the descriptive statistics of the examined traits. The number of records of growth traits studied decreased with the increasing age of lambs (4859 records for birth weight compared to 1145 records for yearling weight), which could be due to mortality, data editing, deletion of some lambs, or failure to record records, and the sale of lambs at older ages.

Table 2. Descriptive statistics of growth traits in the Moghani sheep breed

Traits	Birth weight	Weaning weight	6 months weight	9 months weight	Yearling weight
No. of records	4859	2145	2028	1319	1145
Mean (kg)	4.47	21.26	33.12	39.82	40.32
S.D (kg)	1.79	5.11	5.61	4.30	14.75
C.V (%)	40	24	17	11	36
Min (kg)	1.85	10	24	28	31.50
Max (kg)	5.94	39	52	53.50	65.01

The effect of birth year on all studied traits was significant ($p < 0.01$). Climatic factors, management, nutrition, and health vary over the years. Variable climatic conditions such as rainfall and ambient temperature affect the quality and quantity of forage, which leads to significant changes in the amount of food available to the animal and the provision of necessary needs, and ultimately the weight of lambs at different ages (*Rashidi et al., 2008; Mohammadi and Sadeghi,* *2010*). Therefore, differences in the management systems and fluctuations of weather conditions in different years can be the main reason for the significant effect of the year of birth on the studied traits. Which was consistent with the results reported in the Sakiz, Moghani, Lori, and Kordi breeds (*Jafaroghli et al., 2010; Ceyhan et al., 2011; Yeganehpor et al., 2015; Shahdadi and Saghi, 2017*).

The effect of maternal age on all studied traits was significant ($p < 0.01$). Differences between maternal behavior and how lambs are cared for at different ages of ewes can be a reason for this effect. The rate of development of physical growth, especially the environment of the uterus, body weight, reproductive system, and more milk production by the mother at an older age can also be related to this effect. In addition, increasing the age of the ewes affects the amount of milk produced, and despite providing enough milk to feed the lambs, the birth weight increases (*Rashidi et al., 2008; Jiang et al., 2011*), which was consistent with the results reported in the Iran-Black, Lori, Kordi, and Kermani breeds (*Kamjoo et al., 2014; Kargar et al., 2014; Yeganehpor et al., 2015; Shahdadi and Saghi, 2017*).

The effect of birth type on all growth traits was significant ($p < 0.01$), which was consistent with the results reported in Sakiz, Moghani, Lori, and Kordi breeds (*Kamjoo et al., 2014; Kargar et al., 2014; Yeganehpor et al., 2015; Shahdadi and Saghi, 2017*). Due to the use of all uterine and maternal conditions, single lambs have more birth weight, while in twin lambs the energy and nutrients required by the fetus are divided between the twins. In this case, fewer facilities for the mother environment will be available to each of the twin lambs (*Rashidi et al., 2008*).

The effect of lamb sex on all traits was significant ($p < 0.01$), which was consistent with the results reported in Kordi and Moghani breeds (*Jafaroghli et al., 2010; Shahdadi and Saghi, 2017*). In all traits, male lambs had higher averages than female lambs. This significant effect can be due to differences in the type and amount of sex hormones secreted that cause animal to grow. For example, estrogen has a limiting effect on long bones in females, which can be one of the reasons why female lambs are smaller and weigh less than male lambs (*Jafaroghli et al., 2010*). Due to the insignificance of the interactions on the fixed factors, none of them was used in the final model.

Table 3 shows the average performance of traits by different levels of fixed effects.

Table 3. Average growth traits based on different fixed effects (kg)

Fixed effects	Traits				
	Birth weight	Weaning weight	6 months weight	9 months weight	yearling weight
Birth year	**	**	**	**	**
Dams age	**	**	**	**	**
2	4.00 ± 0.001^{i}	20.35 ± 0.041^{f}	32.33 ± 0.037^{f}	39.01 ± 0.009^{f}	39.47 ± 0.007^{e}
3	4.14 ± 0.032^{e}	20.47 ± 0.029^{e}	32.75 ± 0.018^{e}	39.27 ± 0.055^{e}	40.07 ± 0.082^{d}
4	4.32 ± 0.027^{c}	20.79 ± 0.071^{d}	32.89 ± 0.004^{d}	39.49 ± 0.019^{d}	40.32 ± 0.034^{c}
5	4.26 ± 0.013^{d}	21.52 ± 0.054^{c}	33.37 ± 0.041^{c}	39.76 ± 0.061^{c}	40.36 ± 0.063^{c}
6	5.00 ± 0.037^{b}	21.79 ± 0.014^{b}	33.57 ± 0.075^{b}	40.29 ± 0.005^{b}	41.00 ± 0.005^{b}
7	5.12 ± 0.069^{a}	22.12 ± 0.017^{a}	33.76 ± 0.038^{a}	41.13 ± 0.007^{a}	41.12 ± 0.074^{a}
Birth type	**	**	**	**	**
Single	5.00 ± 0.033^{a}	22.03 ± 0.045^{a}	33.68 ± 0.053^{a}	40.10 ± 0.029^{a}	40.96 ± 0.068^{a}
Twine	3.94 ± 0.072^{b}	20.49 ± 0.071^{b}	32.56 ± 0.017^{b}	39.54 ± 0.008^{b}	39.68 ± 0.073^{b}
Sex	**	**	**	**	**
Male	4.56 ± 0.037^{a}	21.56 ± 0.049^{a}	33.42 ± 0.083^{a}	40.00 ± 0.061^{a}	40.99 ± 0.067^{a}
Female	4.29 ± 0.007^{b}	20.96 ± 0.026^{b}	32.82 ± 0.054^{b}	39.64 ± 0.030^{b}	39.65 ± 0.017^{b}
** p<0.01;similar letters for levels of each fix effect indicate a non-significant difference at p<0.01					

The components of (co) variance and genetic parameters of the studied traits are shown in Table 4. The results showed that with increasing age, the amount of direct heritability of traits increased, which may indicate a decrease in maternal effect with age on the studied traits. Reasons for increasing the heritability of traits with age include factors such as increased expression of genes that have an increasing effect on body weight and also a decrease in variance due to maternal effects relative to the direct genetic variance of the animal (*Ahteghadi et al., 2015*).

Table 4. (Co) variance components and genetic parameters estimates for the studied traits

Parameter	Traits				
	Birth weight	Weaning weight	6 months weight	9 months weight	Yearling weight
Model fitted	3	3	2	1	1
σ_a^2	0.25	1.23	2.44	2.54	3.83
σ_m^2	0.54	0.96	-	-	-
σ_{pe}^2	-	-	0.75	-	-
σ_e^2	2.78	10.59	12.32	17.00	17.45
σ_p^2	3.57	12.78	15.51	19.54	21.28
σ_{am}	-	-	-	-	-
$h_d^2 \pm SE$	0.07 ± 0.01	0.09 ± 0.04	0.15 ± 0.03	0.13 ± 0.01	0.20 ± 0.02
$h_m^2 \pm SE$	0.15 ± 0.03	0.07 ±0.01	-	-	-
$c^2 \pm SE$	-	-	0.05 ± 0.05	-	-
h_t^2	0.15	0.13	0.15	0.13	0.20

σ_a^2: direct genetic variance, σ_m^2: maternal additive genetic variance, σ_{pe}^2: maternal permanent environmental variance, σ_e^2: residual variance, σ_p^2: phenotypic variance, σ_{am}: covariance between direct genetic and maternal additive genetic, h_d^2: direct heritability, h_m^2: maternal heritability, c^2: ratio of maternal permanent environmental effect to phenotypic variance, h_t^2: total heritability.

Direct heritability of birth weight was estimated to be 0.07, which was consistent with the results reported in the Moghani breed by *Jafaroghli et al. (2010).* Also, compared to the reported values of 0.1 and 0.11 in the Kordi breed (*Naghavian et al., 2015; Shahdadi and Saghi, 2017*), 0.36 and 0.34 in the Lori breed (*Beyranvand et al., 2013; Shahdadi and Saghi, 2017*), 0.14 and 0.23 in the Kermani breed (*Kargar et al., 2014; Khosravi et al.,2018*), 0.36, 0.24 and 0.31 to 0.54 in Makooei, Zandi and Moghani breeds, respectively (*Hoque et al., 2009; Jiang et al., 2011; Mohammadi et al., 2012*) were less than and higher than the reported value of 0.007 in Makooei breed (*Azizi et al., 2015*). The high heritability of this trait compared to a similar study might indicate an increase in the genetic efficiency of the selection because the true breeding value of the animal has been more accurately estimated through its phenotype.

Direct heritability of weaning weight was 0.09, which was consistent with the results reported in the Moghani breed (*Jafaroghli et al., 2010*). Also, it was lower than the reported values of 0.33 and 0.57 in the Kermani breed (*Kargar et al., 2014; Khosravi et al., 2018*), 0.19, 0. 23, and 0.28 in the Kordi breed *(Mohammadi et al., 2009; Naghavian et al., 2015; Shahdadi and Saghi, 2017*), 0.20, 0.21, 0.27, and 0.47 in Makooi, Moghani, Baluchi and Shal breeds (*Hasani et al., 2009; Hossein-Zadeh and Ardalan, 2010; Jiang et al., 2011; Amou Posht-e Masari et al., 2015*), and it was higher than the reported value 0.024 in the Moghani breed (*Azizi et al., 2015*). The reason for these differences can be due to differences in records, breed, management, and nutritional conditions of the studied herds.

Direct heritability was obtained at six months weight of 0.15, which was lower than the reported values of 0.21, 0.26, and 0.32 in the Kordi breed (*Shokrollahi and Zandieh, 2012: Naghavian et al.,2015; Shahdadi and Saghi, 2017*), 0.16, 0.28, 0.30, 0.32, and 0.48 in Iran-Black, Baluchi, Iran-Black, Zandi, Shal and Makooei breeds (*Beygi Nasiry et al., 2005; Hossein-Zadeh and Ardalan, 2010; Jiang et al., 2011; Mohammadi et al., 2011; Amou Posht-e Masari et al., 2015*) and higher than the reported value of 0.05 in the Lori breed *(Beyranvand et al., 2013*), 0.009 and 0.13 in the Moghani breed (*Hossein-Zadeh and Ardalan, 2010; Azizi et al., 2015*). The reason for these differences can be related to the components of different genetic and phenotypic (co) variances, especially their ratio in different breeds, which change under the influence of selection, recording conditions, environmental fluctuations, and models used to analyze the studied traits.

The direct heritability of the nine months weight was estimated to be 0.13, which was higher than the values of 0.11 and 0.12 reported in the Moghani and Kermani breeds (*Hossein-Zadeh and Ardalan, 2010; Khosravi et al., 2018*), and lower than the reported values 0.181, 0.19, 0.22, 0.29, 0.31, 0.33, 0.37, and 0.41 in the Moghani, Iran-Black, Kordi, Baluchi, Zandi, Makooei and Shal breeds (*Beygi Nasiry et al., 2005; Jafari et al., 2012; Mohammadi et al., 2012; Kamjoo et al., 2014; Kargar et al., 2014; Azizi et al., 2015; Naghavian et al., 2015; Amou Posht-e Masari et al., 2015*). The lower estimate of the heritability of this trait in the current study may indicate that direct selection based on this trait does not significantly improve the production efficiency of this population.

Direct heritability for one yearling weight was 0.20, which was consistent with the values reported in the Kordi breed (*Shahdadi and Saghi, 2017*) which was lower than the reported values of 0.24 in the Kordi breed (*Naghavian et al., 2015*), 0.41, 0.36, 0.31, 0.28, and 0.28 in Makooei, Kermani, Baluchi, Shal and Zandi breeds (*Beygi Nasiry et al., 2015; Jafari et al., 2012; Mohammadi et al., 2012; Kargar et al., 2014; Amou Posht-e Masari et al., 2015*), and higher than the reported values 0.076, 0.1 and 0.18 in the Moghani and Iran-Black breeds (*Hossein-Zadeh and Ardalan, 2010; Jafari et al., 2012; Kamjoo et al., 2014; Azizi et al., 2015*), 0.16 in the Kermani breed (*Khosravi et al., 2015*). The heritability of this trait may be higher than in similar studies due to increased expression of genes that have an increasing effect on body weight and can also be due to reduced variance due to maternal effects compared to direct genetic variance of the animal.

Maternal heritability of birth weight and weaning weight traits were 0.15 and 0.07, respectively, which were consistent with the results reported in the Lori breed (*Beyranvand et al., 2013*). Maternal heritability decreases with age, which may be due to reduced lamb dependence on breast milk. The results of this study showed that maternal genetics, like animal genetics, have an effect on birth weight and weaning weight traits. Therefore, more attention should be paid to maternal effects to improve growth traits in the pre-weaning ages.

In general, it can be said that the estimated genetic parameters in each community for each economic trait are specific to that community and several factors may affect it. The structure and volume of available information, the degree of specificity of animal relationships in the pedigree, selection in the herd, method of estimating variance components and genetic parameters, livestock breed, and environmental conditions in the herd are different. Also, using different breeding programs makes a difference between different estimates (*Elfadilli et al., 2000; Hoque et al., 2009*).

Table 5 shows the results of the Akaike criterion for determining the most appropriate model. The studied models for the studied traits were compared using the Akaike criterion (AIC), and the model with the lowest Akaike criterion was selected as the most appropriate model. This criterion shows the extent to which the use of a statistical model causes data loss. In other words, this criterion establishes a balance between model accuracy and complexity (*Farhangfar et al., 2007*).

Table 5. AIC values for traits (AIC values for traits in bold numbers (as the best) had the highest values in other models)

Model	Traits				
	Birth weight	Weaning weight	6 months weight	9 months weight	Yearling weight
1	72763.602	-68936.241	-24443.830	**-1476.432**	**-921.341**
2	72765.602	-68938.412	**-24450.661**	-1474.813	-914.730
3	**72772.331**	**-68943.111**	-2445.325	-1470.326	-916.339
4	72742.836	-68938.274	-2447.871	-1472.713	-919.335
5	72764.137	-68940.337	-2445.885	-1476.012	-916.768
6	72764.033	-68931.023	-2446.323	-1473.756	-920.367

To genetically evaluate the growth traits (including birth weight, weaning weight, six months weight, nine months weight, and one yearling weight) of Moghani sheep, genetic, environmental, and phenotypic trends of these traits were investigated. Table 6 shows the genetic, environmental, and phenotypic trends of the traits.

Table 6. Estimates of genetic, phenotypic, and environmental trends (kg/year) for body weight traits at different ages

Traits	Phenotypic trend	Environmental trend	Direct genetic trend
Birth weight	$0.018 \pm 0.032^{*}$	0.010 ± 0.021^{ns}	0.008 ± 0.011^{ns}
Weaning weight	$0.067 \pm 0.014^{**}$	$0.055 \pm 0.017^{**}$	$0.012 \pm 0.012^{**}$
6 months weight	$0.139 \pm 0.006^{*}$	$0.085 \pm 0.003^{**}$	$0.054 \pm 0.003^{**}$
9 months weight	-0.111 ± 0.002^{ns}	$-0.185 \pm 0.002^{*}$	$0.074 \pm 0.081^{**}$
Yearling weight	$-0.274 \pm 0.014^{**}$	$-0.419 \pm 0.007^{*}$	$0.145 \pm 0.063^{**}$
ns: Non significant, * Significant at P<0.05, ** Significant at P<0.01			

The phenotypic trend of birth weight, weaning weight, six months weight, nine months weight, and one yearling weight was estimated to be 0.018, 0.067, 0.139, -0.111, and -274 kg/year, respectively. *Dorostkar et al. (2010)* in the Moghani breed reported the phenotypic trend of these traits of 0.0371, 0.0531, -0.0206, -0.3839, and -0.143 kg/year, respectively. A phenotypic trend of these traits was reported in Kordi breed 0.0371, 0.0531, -0.0206, -0.3839, and -0.143 kg/year, by *Shahdadi and Saghi (2017)* and 16, 328, 227, 298, and 405 g/year by *Naghavian et al. (2015)*. The observed differences between different estimates can be due to factors such as animal breed, genetic diversity within the population, management, and environmental conditions, and the method of estimating genetic parameters.

The negative phenotypic trend of the nine months weight trait was consistent with the results reported in the Lori, Zandi, and Kermani breeds (*Mohammadi et al., 2012; Naghavian et al., 2015; Yeganehpor et al., 2015*). This negative trend may be due to reasons such as poor management, fluctuations in environmental conditions, changes in weather conditions, and the level of health in the years under study. In unfavorable environmental conditions, the animal phenotype is affected by the environment, which prevents the emergence of animal genetic potential. It becomes difficult to predict breeding values in these conditions, which ultimately leads to an underestimation of the genetic progression per generation (*Hanford et al., 2005*). Therefore, it is necessary to provide suitable and optimal environmental conditions to maximize the genetic potential of the herd so that the phenotypic trend is aligned with the genetic trend.

The negative phenotypic trend of one yearling weight trait was consistent with the results reported in Moghani and Kordi breeds by *Dorostkar et al. (2010)* and *Naghavian et al. (2015).* The negative phenotypic trend indicates the negative trend of environmental factors. The average phenotype of animals is more influenced by environmental factors. The negative trend of environmental factors in the ages of nine months and one yearling can be due to inappropriate environmental factors at these ages.

The genetic trend of birth weight was estimated to be 0.008 kg/year which was not significant ($p>0.01$). The insignificance of the genetic process of birth weight indicates that no genetic change in the birth weight of this breed was observed during the studied years, which corresponds to the reported value of 117.01 g in the Lori breed (*Yeganehpor et al., 2015*). Also, it was higher than the reported values of 0.00014 and 0.0055 kg/year in the Moghani breed (*Dorostkar et al., 2010; Azizi et al., 2015*), -3, 2, and 53 g/year in Shal, Kermani, and Zandi breeds (*Sataei Mokhtari et al., 2009; Mohammadi et al., 2011; Amou Posht-e Masari et al., 2015*) and less than the reported values 20 g/year and 0.038 kg/year in Kermani and Menz breeds (*Grizw et al., 2007; Rashidi et al., 2008*). The reason for these differences may be due to different selection criteria in different breeds as well as the weight of lambs and different methods of estimating genetic trends in different breeds.

The genetic trend of weaning weight was 0.012 kg/year ($p <0.01$), which was higher than the values of 0.00213 and 0.0053 kg/year reported in the Moghani breed (*Dorostkar et al., 2010; Azizi et al., 2015*) and less than the reported values 125, 106, and 96.41 g/year in the Kermani breed (*Rashidi and Akhshi, 2007; Sataei Mokhtari et al., 2009; Mokhtari and Rashidi, 2010*), 64, 72.9, and 117.01 g/year in the Kordi breed (*Mohammadi et al., 2009; Naghavian et al., 2015; Shahdadi and Saghi, 2017*). The difference in the values obtained in the genetic trends of this trait in different studies depends on different structures such as the different genetic structures of the study population and different periods for evaluating herds.

The genetic trend of six months' weight was estimated to be 0.054 kg/year ($p < 0.01$), which was lower than the reported values of 0.388 kg/year in the Menz breed (*Grizw et al., 2007*), 88.24, 91, and 142 g/year in the Kermani breed (*Rashidi and Akhshi, 2007; Sataei Mokhtari et al., 2009; Mokhtari and Rashidi, 2010*), 59.63, 73, and 148.27 g/year in the Kordi breed (*Mohammadi et al., 2009; Naghavian et al., 2015; Shahdadi and Saghi, 2017*), 72 g/year in the Baluchi breed (*Mohammadi et al., 2011*) and higher than the reported values -18 g/year in the Shal breed (*Amou Posht-e Masari et al., 2015*), 0.00054 and 0.0052 kg/year in the Moghani breed (*Dorostkar et al., 2010; Azizi et al., 2015*) and 21 g/year in the Zandi breed (*Mohammadi et al., 2011*). The reason for estimating this trend more than some similar studies could be due to the use of rams with higher breeding values and the setting of controlled mating programs.

The genetic trend of nine months' weight was 0.074 kg/year ($p < 0.01$), which was higher than the reported values of 26.4 and 72 g/year in the Zandi breed (*Mohammadi et al., 2011; Mohammadi et al., 2012*), 0.0345 and 0.0061 kg/year in the Moghani breed (*Dorostkar et al., 2010; Azizi et al., 2015*), 24.31, 27, and 71.6 gr/year in Kermani, Shal, and Kordi breeds (*Sataei Mokhtari et al., 2009; Naghavian et al., 2015; Amou Posht-e Masari et al., 2015*) and was lower than the reported values 0.076 g/year in the Lori breed (*Yeganehpor et al., 2015*), 77, 81, 110.01 and 136.52 g/year in Baluchi, Kermani and Kordi breeds (*Hasani et al., 2009; Mohammadi et al., 2009; Mokhtari and Rashidi, 2010; Shahdadi and Saghi, 2017*). The reason for the low estimate of this trend compared to some similar studies may be due to the lack of attention to this trait in the selection and removal of unwanted animals with high breeding value at the age of nine months.

The genetic trend of one yearling weight was estimated to be 0.145 kg/year ($p<0.01$), which was lower than the reported values of 0.849 kg/year in the Moghani breed (*Dorostkar et al., 2010*), 156 and 495 g/year in Kermani and Menz breeds (*Grizw et al., 2007; Mokhtari and Rashidi, 2010*), and was higher than the reported values 0.0058 kg/year in the Moghani breed (*Azizi et al., 2015*), -3, 41.5, 65, 88, 98.4, and 122.2 g/year in Shal, Zandi, Baluchi and Kordi breeds (*Hasani et al., 2009; Mokhtari and Rashidi, 2010; Mohammadi et al., 2012; Amou Posht-e Masari et al., 2015; Naghavian et al., 2015; Shahdadi and Saghi, 2017*). The highest genetic trend was related to one yearling weight trait, which also had the highest heritability. This was predictable given that more genetic trends could be due to more incremental genetic changes (*Shaat et al., 2004*).

Vatankhah et al. (2005), reported that the genetic trend of traits in the country's breeding herds decreased and, in some cases, negatively. The main reasons for the lack of expected genetic improvement can be considered as factors such as unclear breeding goals for the studied breeds, and failure to use appropriate animal models to predict the breeding values of animals and evaluate them. Also, the inaccuracy of recording production traits and pedigree registration and the incompleteness of the predicted programs in breeding herds can be a reason for this issue. In addition, managerial and environmental fluctuations can be another factor in preventing genetic progression to the expected level.

The genetic progress after 30 years for birth weight, weaning weight, six months weight, nine months weight and one yearling weight was estimated to be 0.213, 1.071, 1.171, 1.164, and 1.324 kg, respectively. *Amou Posht-e Masari et al. (2015)* in the Shal breed reported the genetic progress of these traits at 1, 316, 404, 522, and 60 g, respectively. *Hasani et al. (2009)* in the Baluchi breed reported the genetic progress of these traits as 0.011, 1.488, 2.066, 2.062, and 2.043 kg, respectively. The rate of genetic progress of these traits in the Kordi breed 36.09, 1040.15, 1357.27, 1067.27, and 1153.26 g by *Shahdadi and Saghi (2017)*, and 0.017, 0.089, 1.319, 0.727, and 1.199 kg by *Naghavian et al. (2015)* has been reported.

Development and completion of a selection index for important economic traits along with appropriate economic coefficients can be an important step in genetic development and increase profitability in this breed. On the other hand, since genetic growth in different flocks of sheep depends on predetermined selection goals, the selection criteria depend on those goals and the appropriate environmental conditions. Also, key factors affecting the genetic growth of herds in different environments are genetic diversity, selection accuracy, generation interval, and selection intensity, therefore, we cannot expect that genetic progress estimates for traits in different herds are consistent (*Piper and Ruviskey, 1997*).

Conclusion

In all traits, the genetic trend of the traits was positive, but the breeding values of the animals fluctuated in different years, which could indicate a weakness in determining the goals and criteria for the correct selection of the studied traits. To improve the breeding status of the study population, due to significant genetic progress for the studied traits, productive animals can be selected based on breeding values and wait for the effects of this selection in the coming years.

Procena genetskog napretka osobina porasta kod ovaca rase moghani

Maryam Esrafili Taze Kand Mohammaddiyeh, Reza Behmaram

Rezime

Za procenu genetskog napretka osobina porasta korišćeni su podaci iz pedigrea za 39.408 ovaca rase moghani u periodu od 1986. do 2016. godine u stanici za uzgoj ovaca Moghani. Proučavane osobine su uključivale masu na rođenju, masu nakon odbijanja, masu u uzrastu od šest meseci, masu u uzrastu od devet meseci i masu u uzrastu od jedne godine. Komponente varijanse i genetski parametri osobina procenjeni su korišćenjem ograničene verovatnoće i šest animal modela, korišćenjem softvera Wombat. Direktna naslednost mase na rođenju, mase pri odbijanju, mase u uzrastu od šest meseci, mase u uzrastu od devet meseci i mase u uzrastu od jedne godine bila je (0,07, 0,09, 0,15, 0,13 i 0,20 respektivno). Vrednosti uzgoja za izračunavanje genetskih trendova za svaku osobinu su procenjene korišćenjem univarijantnog najboljeg animal modela. Fenotipski, genetski i ekološki trendovi osobina procenjeni su regresijom fenotipskog proseka, proseka priplodne vrednosti i razlike priplodne vrednosti od fenotipske vrednosti podeljene sa godinom rođenja. Izračunati genetski trendovi mase na rođenju, mase pri odbijanju, šestomesečne mase, devetomesečne mase i mase u uzrastu od jedne godine bili su 0,008, 0,012, 0,054, 0,74 i 0,145 kg/god, respektivno, osim mase pri rođenju, ostali su bili značajni ($p < 0,01$). Procenjen je genetski napredak osobina, uključujući masu pri rođenju, masu pri odbijanju, masu u uzrastu od šest meseci, masu u uzrastu od devet meseci i masu u uzrastu od jedne godine (0,213, 1,071, 1,171, 1,164 i 1,324 kg, respektivno). U procenjenim godinama primećene su mnoge fluktuacije u genetskom trendu osobina. Drugim rečima, čini se da u proučavanim godinama nije postojao sveobuhvatan plan za genetski uzgoj i poboljšanje osobina telesne mase kod moghani ovaca.

Ključne reči: oplemenjivačka vrednost, genetski napredak, genetski trend, fenotipska vrednost, fenotipski trend

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PREDICTING THE BODY WEIGHT OF INDIGENOUS GOAT BREEDS FROM MORPHOLOGICAL MEASUREMENTS USING THE CLASSIFICATION AND REGRESSION TREE (CART) DATA MINING ALGORITHM

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Abstract: Classification and regression tree (CART) is a tree-based data mining algorithm that develops a model to predict an outcome. This study purposed to create a model to predict the body weight (BWT) of Red Sokoto (RS), Sahel (SH), and West African Dwarf (WAD) goats using morphological measurements (such as body length, BL; head girth, HG; head width, HDW; face length, FAL; height at wither, HTW; rump length, RL; shoulder width, SW; rump width, RW; and rump height, RH). In total, 600 goats were used for this study (200 each of RS, SH, and WAD goats). Pearson's Moment Correlation was used to evaluate the degree of association between BWT and each morphological measurement. Concomitantly, CART analysis was performed to estimate which independent variable (morphological measurements) played a considerable role in the BWT (dependent variable) prediction. In RS and WAD goats, a positive and statistically significant ($p < 0.0001$) correlation was observed between BWT and each morphological measurement. However, in SH goats, both positive and negative statistically significant correlations were observed between BWT and morphological measurements. The CART analysis indicated that in RS and WAD goats, HG played a considerable role in BWT prediction, while, in SH goats, BL was considered the most critical independent variable in BWT prediction. Therefore, this study suggests that HG can be used as a prognostic index for BWT estimation in Red Sokoto and West African Dwarf, while BL can be used for Sahel goats. The SAS codes used are available via a GitHub repository (https://github.com/Soullevram/CART).

Key words: algorithm, body weight, data mining, goats

Introduction

Goat rearing dates back several years. Goats were among the first sets of animals to be domesticated (*Amills et al., 2017*) and were primarily meant to serve as a source of meat and milk. By-products from goats, such as skin and hair fibre, are processed to manufacture belts. In Nigeria and other parts of Africa, subsistent goat farming is the source of livelihood and food security for many households (*Meissner et al., 2013*). There are three common breeds of goats found in Nigeria: The West African Dwarf goat which is predominantly found in the southwestern part of the country, Sokoto Red, and the Borno Sahel both of which are predominantly northern breeds of goats. However, research institutions and farmers have started to import exotic and foreign breeds because of their perceived better performance. One of the most important traits in livestock production is body weight; this is because of the market value attached to it. The body weight of a goat is emblematic of its health and weaning period *(Yakubu and Mohammed, 2012)*. *Norris et al. (2015)* classified body weight and body conformation as the two most important traits determining whether a goat farm is making profits.

Morphological measurements, such as body weight (BWT), rump height (RH), and body length (BL), are very important in selection and breeding programs *(Sam et al., 2016)*. Most commercial livestock enterprises utilize weighing scales to estimate the body weights of animals. However, rural or subsistent goat farmers usually cannot afford a weighing scale. Consequently, in undeveloped or less developed farming communities, animal body weight is determined using a highly subjective approach, visual judgment *(Chinchilla-Vargas et al., 2018)*. Morphological measurements have been used to get accurate estimates and predictions of animal body weight in livestock *(Younas et al., 2013; Eyduran et al., 2017; Sandeep et al., 2017)*.

A litany of statistical methods such as regression models, principal component analysis, factor score analysis, and canonical correlation *(Cankaya and Kayaalp, 2007; Yakubu et al., 2009; Yakubu and Musa-Azara, 2013; Oguntunji and Ayorinde, 2014)* has been used to predict body weight from morphological body measurements. Although a wide array of prediction models has been employed in predicting the body weight of animals from body measurements, their viable interpretations do not necessarily mean that they are applicable. An actionable solution to the limitations of previous prediction models is the data mining algorithm known as the Classification and Regression Tree (CART) introduced by *Breiman et al. (1984)*. CART can be used to develop an easy-to-use- and -understand statistical model to make predictions using a nominal or an ordinal scale *(Olfaz et al., 2019)*. CART analysis helps to determine which morphological measurements play a considerable role in predicting the dependent variable, body weight *(Mathapo and Tyasi, 2021)*. CART can be used even when the data is unbalanced, intricated, and missing values *(Speybroeck, 2012)*. When CART statistical technique is used to analyse categorical or continuous data, a classification or regression tree is produced. CART analysis can be applied to a wide range of data types, such as categorical, numerical, surviving, and ratings data *(De'ath and Fabricus, 2000)*. Since CART is a non-parametric statistical technique, the validity of its results is not dependent on the fulfilment of statistical assumptions about the distribution of independent variables *(Zaborski et al., 2019)*. The output of this tree-based statistical algorithm is pictorial.

The visual appraisal method of body weight assessment used by rural farmers is inaccurate, and subjective and could lead to the undervaluation of an animal's market value. Consequently, an objective and simple delineation method, such as CART, is needed to assist goat farmers in the appraisal of their livestock. CART has been used to predict the body weight of Ouda sheep *(Yakubu, 2012)* and locally adapted Muscovy ducks *(Oguntunji, 2017)* in Nigeria. However, to the best of the authors' knowledge, scientific literature on the use of CART to forecast the body weight of indigenous goat breeds in Nigeria is scanty. The study, therefore, aimed to predict the body weight of three goat breeds indigenous to Nigeria from their morphological measurements using the CART data mining algorithm. The resulting regression tree will enable farmers, especially rural farmers, to make informed decisions regarding the market value of their livestock, breeding, and selection.

Materials and Methods

Study area and period

The data for this study was obtained at goat markets within Ibadan situated at 7.3775° N, 3.9470° E, in Nigeria. Data was collected from Akinyele, Oranyan, and Bodija goat markets in Ibadan, Oyo State, Nigeria. All data were collected between March and September 2021.

Experimental animals

Three goat breeds, namely Red Sokoto (RS), Sahel (SH), and West African Dwarf (WAD), were randomly selected for this study. A total of 600 goats consisting of 200 RS, 200 SH, and 200 WAD were assessed for their morphological measurements. Reported morphological features by *Adu and Ngere (1979)* were used as the baseline for assigning sampled individual animals to a breed. Animals that did not conform to strict breed descriptions and or were visibly pregnant were excluded from this study. Animals were aged between 1-3 years. The goats examined were owned by small-scale farmers who practiced an extensive management system.

Data collection

The body weight (BWT) and morphological measurements of 600 RS, SH, and WAD goats were examined. The morphological body measurements estimated were: body length (BL), head girth (HG), head width (HDW), face length (FAL), height at wither (HTW), rump length (RL), shoulder width (SW), rump width (RW), and rump height (RH). The morphological measurements were conducted according to the specifications of *Yakubu et al. (2010)* and *Okpeku et al. (2011)*. Morphological body measurements were documented in centimeters (cm) using a measuring tape, while the BWT measurements were recorded in kilograms using a 50kg spring balance.

Statistical analysis

All statistical analyses were performed using SAS 9.4 *(SAS Institute Inc., 2016)*. The PROC MEANS procedure of SAS was used to obtain the descriptive statistics for the body weight and morphological body measurements. PROC CORR was used to compute the Pearson Moment Correlation coefficients, to determine the association between the dependent variable and independent variable(s).

The regression tree (RT) was constructed using the HPSPLIT procedure in SAS. The HPSPLIT procedure produced a decision tree, which models a continuous or categorical response, presented as if-then statements. For this study, the predictor variable(s) were continuous. Testing or training data with known response values were used in building a tree model for this study. The splitting criteria used in this study were based on impurity. The residual sum of squares (RSS) splitting criterion was chosen. The cost-complexity (CC) pruning option *(Breiman et al., 1984; Quinlan, 1987; Zhang and Singer, 2010)* was used. 10-fold cross-validation (CV) was done to both the CC pruning option and model. The CV option of PROC HPSPLIT in SAS produces tables and plots that estimate the error metric of the parameters and the future prediction accuracy for each subtree *(Camdeviren et al., 2005)*. The error metric for RT is average square error (ASE). RSS and ASE were used to assess the model fit for the regression tree. Ultimately, the subtree with the minimal RSS and ASE values is selected as the final tree.

The training data contains lots of predictors, with some being more important than others. The most important predictors are identified based on their variable importance. The position of a variable, especially its closeness to the top of the tree, is not associated with the importance of that variable. Rather variable importance is estimated using the following: surrogate count, count, relative importance, and RSS. The final RT produced using the HPSPLIT procedure in SAS is labeled using base 62; that is, the encoding used are 0-9, A-Z, and a-z.

Results

Descriptive statistics for morphological body measurements

The descriptive statistics for the morphological body measurements obtained from the three goat breeds are presented in Table 1. The highest BWT mean value was observed in the Sahel (SH) breed, while the lowest mean BWT value was observed in the West African Dwarf (WAD) goat. The highest mean values for all morphological variables were observed in SH except for HTW. WAD had the lowest mean values for all morphological variables. Across all three breeds, the highest mean value for a morphological variable was recorded in HG, followed by RH in the Red Sokoto (RS) breed and BL in the West African Dwarf breed. The coefficient of variation for the RS breed ranged between 11.72% - 33.94%, 6.63% - 23.29% for the SH breed, and 12.47% - 50.54% for the WAD.

Table 1. Descriptive statistics for morphological measurements and body weight of Red Sokoto, Sahel, and West African Dwarf goats

	Red Sokoto Goat			Sahel Goat			West African Dwarf Goat		
Measurements	Mean	SD	CV (%)	Mean	SD	CV (%)	Mean	SD	CV (%)
BL (cm)	44.51	5.49	12.34	50.55	6.90	13.64	42.11	8.93	21.22
HG (cm)	63.06	7.39	11.72	68.39	4.53	6.63	54.65	12.52	22.91
HDW (cm)	11.74	1.96	16.70	12.89	1.57	12.19	11.69	1.87	16.04
FAL (cm)	15.40	2.03	13.20	17.00	1.58	9.30	14.05	2.50	17.81
HTW (cm)	12.53	2.23	17.77	11.96	2.79	23.29	12.47	3.06	24.54
RL (cm)	16.89	2.73	16.20	17.64	3.07	17.43	15.17	1.89	12.47
SW (cm)	31.65	4.73	14.95	37.12	4.30	11.60	27.01	5.48	20.29
RW (cm)	13.48	2.93	21.75	14.33	3.08	21.50	12.99	1.82	13.99
RH (cm)	58.44	7.89	13.50	64.67	4.77	7.38	40.95	5.64	13.77
BWT (kg)	20.43	6.93	33.94	22.79	4.56	19.99	12.41	6.27	50.54

BL: body length; HG: head girth; HDW: head width; FAL: face length; HTW: height at wither; RL: rump length; SW: shoulder width; RW: rump width; RH: rump height; BWT: body weight; SD: Standard deviation; CV: Coefficient variation.

Variable importance

The importance of each independent value in predicting the BWT of RS, SH, and WAD breeds is presented in descending order in Table 2. The highest relative importance was observed in HG for both RS and WAD breeds, while for the SH breed, the highest relatively important variable was BL. However, for the SH breed, no importance was attributed to the variables HDW and SW.

Correlation between BW and morphological measurements

Pearson moment correlation statistical test was used to assess the association between BWT and morphological measurements in the three breeds. In the Red Sokoto and West African Dwarf breeds, BWT was positively correlated, with a statistical significance at $p < 0.001$, with all the morphological measurements. In the Sahel breed, however, BWT was positively correlated with most of the morphological measurements, except HTW and RL, at different levels of significance (Tables 3-5).

Table 2. Variable importance in predicting the body weight of Red Sokoto goats, Sahel goats and West African Dwarf goats

Red Sokoto goats			Sahel goats			West African Dwarf goats		
Variable	Relative	Importance	Variable	Relative	Importance	Variable	Relative	Importance
HG	100%	69.32	BL	100%	41.11	HG	100%	76.10
RL	57%	39.43	HG	75%	30.70	RH	36%	27.04
RH	34%	23.38	RL	36%	14.73	BL	27%	20.44
RW	33%	22.65	RH	33%	13.51	FAL	24%	18.38
SW	32%	21.92	RW	26%	10.76	RL	14%	10.77
BL	31%	21.55	FAL	25%	10.31	HDW	13%	9.66
FAL	25%	17.24	HTW	15%	6.32	SW	12%	9.16
HTW	22%	15.45				HTW	10%	7.94
HDW	21%	14.48				RW	7%	5.11

BL: body length; HG: head girth; HDW: head width; FAL: face length; HTW: height at wither; RL: rump length; SW: shoulder width; RW: rump width; RH: rump height.

Table 3. Correlation coefficients between body weight and morphological measurements in Red Sokoto Goat

Measurements	BL	HG	HDW	FAL	HTW	RL	SW	RW	RH	BWT
BL		0.56***	0.29***	0.43***	0.39***	0.39***	0.52***	0.51***	0.67***	0.45***
HG			0.36***	0.57***	0.55***	0.60***	0.50***	0.49***	0.75***	0.78***
HDW				0.35***	0.03	0.50***	0.29***	0.02	0.34***	0.42***
FAL					0.29***	0.56***	0.45***	0.34***	0.58***	0.53***
HTW						0.51***	0.34***	0.33***	0.61***	0.48***
RL							0.48***	0.21*	0.69***	0.69***
SW								0.41***	0.59***	0.49***
RW									0.42***	0.38***
RH										0.67***
BWT										

BL: body length; HG: head girth; HDW: head width; FAL: face length; HTW: height at wither; RL: rump length; SW: shoulder width; RW: rump width; RH: rump height. *** p < 0.0001, ** p <0.001, * p < 0.01.

Table 4. Correlation coefficients between body weight and morphological measurements in Sahel Goat

Measurements	BL	HG	HDW	FAL	HTW	RL	SW	RW	RH	BWT
BL		0.32***	0.23**	-0.16	-0.71***	-0.58***	0.09	0.80***	-0.14	0.61***
HG			0.22*	0.38***	0.04	0.11	0.19*	0.18*	0.35***	0.56***
HDW				0.31***	0.06	0.13	-0.05	0.06	0.30***	0.19*
FAL					0.35***	0.45***	-0.04	-0.22**	0.50***	0.17*
HTW						0.76***	0.14	-0.79	0.38***	-0.35***
RL							0	-0.57***	0.40***	-0.27***
SW								0.1	-0.02	0.18*
RW									-0.32***	0.51***
RH										0.18*
BWT										

BL: body length; HG: head girth; HDW: head width; FAL: face length; HTW: height at wither; RL: rump length; SW: shoulder width; RW: rump width; RH: rump height. *** p < 0.0001, ** p <0.001, * p < 0.01.

Table 5. Correlation coefficients between body weight and morphological measurements in West African Dwarf Goat

Measurements	BL	HG	HDW	FAL	HTW	RL	SW	RW	RH	BWT
BL		0.78***	0.43***	0.79***	0.75***	0.48***	0.66***	0.64***	0.65***	0.82***
HG			0.27***	0.70***	0.66***	0.49***	0.61***	0.61***	0.63***	0.84***
HDW				0.33***	0.30***	0.19**	0.32***	0.35***	0.37***	0.33***
FAL					0.66***	0.43***	0.59***	0.59***	0.57***	0.76***
HTW						0.34***	0.52***	0.47***	0.46***	0.63***
RL							0.39***	0.51***	0.58***	0.50***
SW								0.64***	0.71***	0.73***
RW									0.70***	0.66***
RH										0.73***
BWT										

BL: body length; HG: head girth; HDW: head width; FAL: face length; HTW: height at wither; RL: rump length; SW: shoulder width; RW: rump width; RH: rump height. *** p < 0.0001, ** p <0.001,* p < 0.01.

CART models for the Red Sokoto, Sahel, and West African Dwarf goats

The optimal regression trees (RT) for all three breeds are presented in Figures 1-3. The CART model used for constructing the regression tree for all three breeds had BWT as the dependent, explained or predicted variable and morphological measurements as the independent, explanatory or predictor variable.

The optimum RT for Red Sokoto (RS) breed consists of 15 nodes; RT for Sahel (SH) breed has 13 nodes; and the RT for West African Dwarf (WAD) breed has 15 nodes.

For the RS breed, the root node (labelled node 0) has 200 number of observations (n) and an average (or mean) of 20 kg – which corresponds to the mean of BWT to the nearest whole number. Animals in node 0 were divided into two different nodes on the basis of HG, namely node 1 (< 59.18 cm) and node 2 (≥ 59.18 cm). The predicted average (13 kg) for node 1 was lower than that of node 2 (23 kg). Node 1 was further split into node 3 (< 10.08 cm) and node 4 ((≥ 10.08 cm) based on RW. Node 3 was subdivided into node 7 (< 10.06 cm) and node 8 ((≥ 10.06 cm) based on HDW. Both node 7 and 8 were terminal nodes, however, their predicted means were low. Node 4 was split on the basis of BL into node 9 (< 41.02 cm) and node A (≥ 41.02 cm). Node 2, on the other hand, had a number of observations and was split based on RL into node 5 (< 19.05 cm) and node 6 (≥ 19.05 cm). The relative importance of HG resurfaced in the splitting of node 5 into node B (< 68.17 cm) and node C (≥ 68.17 cm), as well as in the subdivision of node 6 into node D (< 69.04 cm) and node E (≥ 69.04 cm). Out of all the terminal nodes, node 10 – a child node of node k, whose splitting decision was based on HG ≥ 71.07cm – had the highest mean prediction (34.22 kg). Further demonstrating the relative importance of HG in BWT prediction of RS goats.

The RT for the SH breed has 200 n and an average of 22.79 kg at its root node, corresponding to the BWT mean of the breed. The root node was divided based on BL into node 1 (< 48.05 cm) and node 2 (≥ 48.05 cm). Node 1 was subdivided on the basis of HG into node 3 (< 67.23 cm) and node 4 (≥ 67.23 cm). Node 3 was further divided based on FAL into node 7 (< 17.08 cm) and node 8 (≥ 17.08 cm). Node 4, on the basis of RW, was subdivided into node 9 (< 13.15 cm) and node A (≥ 13.15 cm). On the other hand, node 2, based on HG, was subdivided into node 5 (< 75.06 cm) and node 6 (≥ 75.06 cm). Out of the two child nodes of node 2, only node 5 was subdivided into node B (< 20.16 cm) and node C (≥ 20.16 cm) based on RL. Among all the terminal nodes, node 6 was observed to be the best node because its mean prediction (32 kg) was the highest.

Contrastingly, the root node of the RT for WAD breed had an average (12.41 kg), which was lower than the averages of RS and SH. Node 0 was divided into node 1(< 60.15cm) and node 2 (≥ 60.15 cm) based on HG. On the basis of HG again, node 1 was subdivided into node 3 (< 48.09 cm) and node 4 (≥ 48.09 cm). Based on BL node 3 was subdivided into node 7 (< 53.40 cm) and node 8 (≥ 53.40 cm). Node 4, on the basis of RH, was subdivided into node 9 (< 39.18 cm) and node A (≥ 39.18 cm). However, node 2, whose n was less than node 1, was subdivided into node 5 (< 47.01 cm) and node 6 (≥ 47.01 cm). Based on FAL, node 5 was further divided into node B (< 16.03 cm) and node C (≥ 16.03 cm). Node 6 was subdivided into node D (< 41.25 cm) and node E (≥ 41.25 cm). Node E was the best node because it had the highest mean prediction (27.5 kg) than all other terminal nodes.

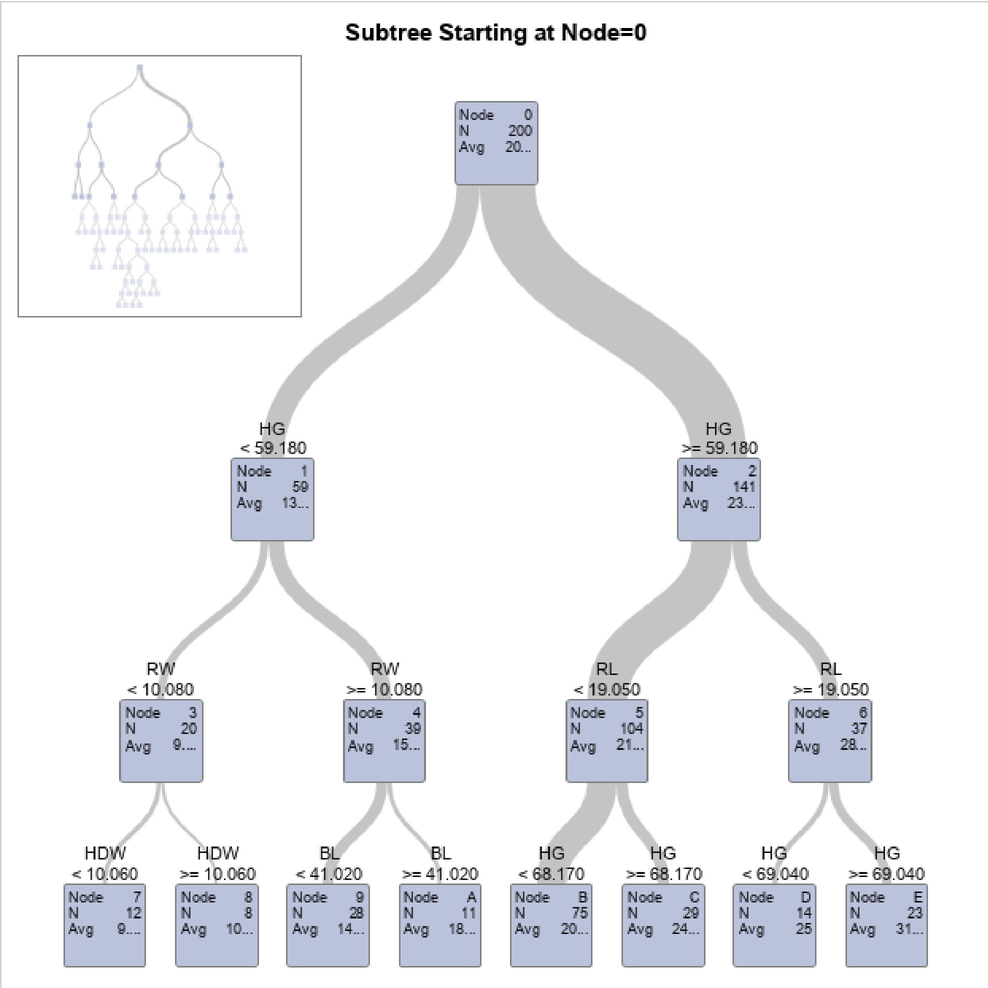

Figure 1. Classification and regression tree (CART) algorithmic model for Red Sokoto goats
HG: heart girth; RW: rump width; RL: rump length; HDW: head width; BL: body length

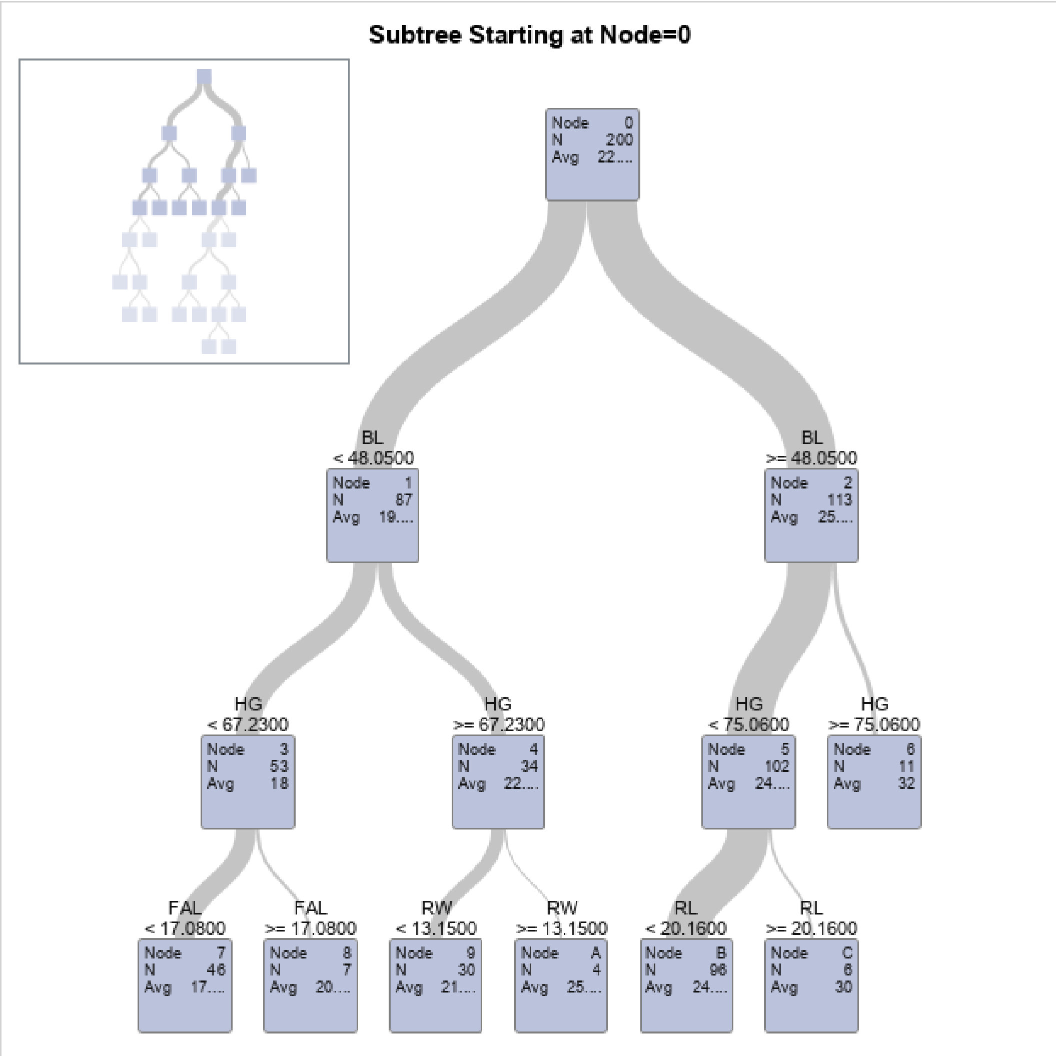

Figure 2. Classification and regression tree (CART) algorithmic model for Sahel goats
BL: body length; HG: heart girth; FAL: face length; RW: rump width; RL: rump length

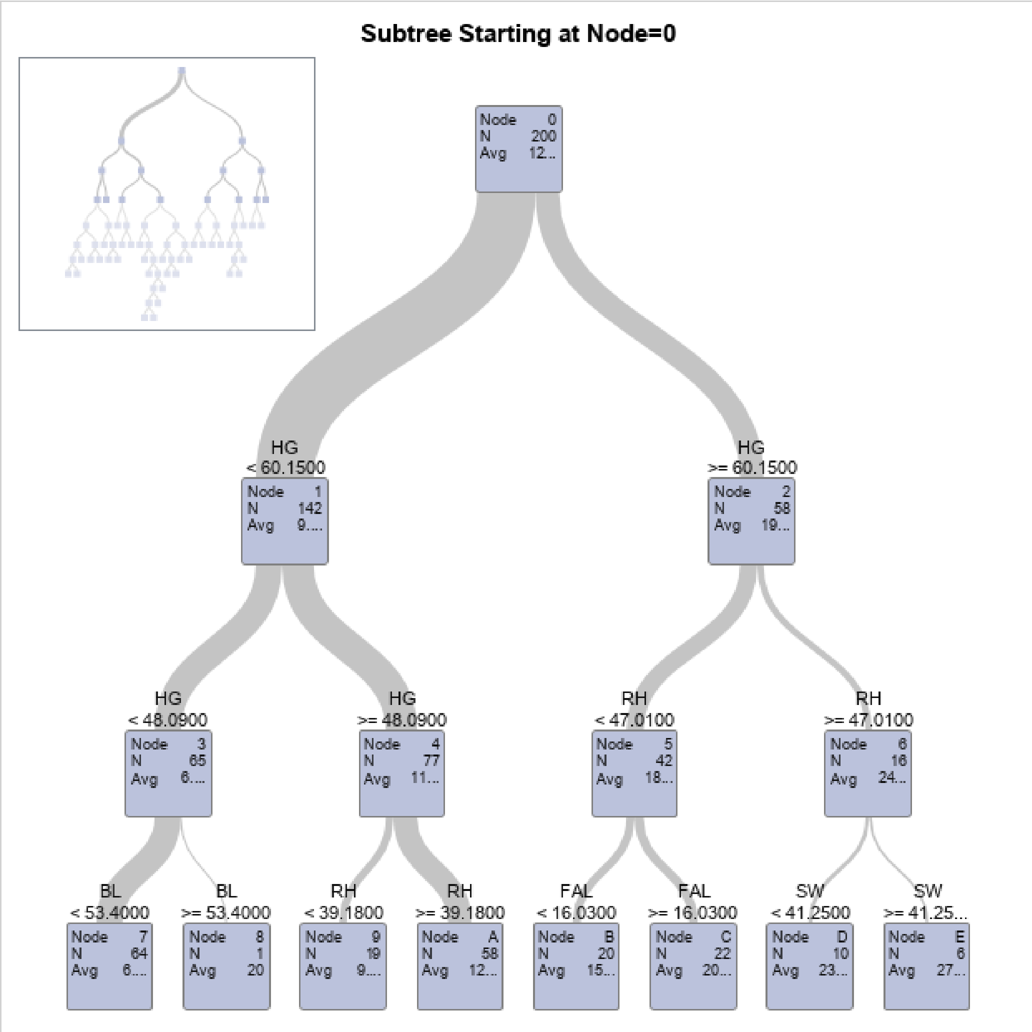

Figure 3. Classification and regression tree (CART) algorithmic model for West African Dwarf goats

HG: heart girth; RH: rump height; BL: body length; RH: rump height; FAL: face length; SW: shoulder width

Discussion

A viable alternative for the prediction of body weight (BWT) in goats among small-scale or rural farmers is morphological body measurements *(Norris et al., 2015; Eyduran et al., 2017; Tyasi et al., 2020)*. The classification and regression tree (CART) data mining algorithm is an effective tree-based statistical technique that can be used to identify morphological measurements that are important in predicting the BWT of live animals.

This study further cements the association between body weight and morphological measurements in goats, especially Red Sokoto (RS), Sahel (SH), and West African Dwarf (WAD) goats. Previous studies reported a positive and statistically significant correlation between BWT and body length (BL) in yearling Boer does *(Mathapo and Tyasi, 2021)*, Ganjam goats native to Odisha *(Karna et al., 2020)*, and Assam Hill goats *(Khargharia et al., 2015)*. Additionally, heart girth (HG) and BWT were observed to have a statistically significant correlation by *Tekin et al. (2019)*.

In Red Sokoto goats, there was a positive and statistically significant correlation between BWT and body morphological measurements. However, the degree of correlation with BWT varied among the morphological measurements. Heart girth (HG), rump length (RL), face length (FAL), and rump height (RH) were all highly correlated with BWT (that is, correlation coefficient, r, was > 0.5). The positive and significant correlation between HG and BWT by *Yakubu and Mohammed (2012)* agrees with this study. On the other hand, a low correlation coefficient (< 0.5) was observed in body length (BL), head width (HDW), height at wither (HTW), shoulder width (SW), and rump width (RW). Similarly, *Mokoena et al. (2022)* reported a low correlation coefficient between BWT and HDW in female Kalahari Red goats. In addition, *Yakubu and Mohammed (2012)* obtained a positive and statistically significant association between BWT and BL in Red Sokoto goats; *Tsegaye et al. (2013)* reported that the correlation between BWT and BL in Ethiopian Hararghe Highland goats was low.

High, positive and significant correlation coefficients was observed between BWT and morphological measurements in WAD goats. The correlation coefficients obtained for the association between BWT and morphological measurements in WAD goats was higher than the remaining two breeds, except for HDW. Likewise, *Yakubu (2009)* reported positive and statistically significant correlation between BWT and HTW, RH, BL and HG in WAD goats.

Unlike the other breeds, negative correlation was observed between BWT and HTW and RL, respectively, in SH goats. Positive and high correlation coefficients were estimated for the association between BWT and BL, HG, and RW. However, the correlation between BWT and HDW, FAL, SW, and RH, respectively, even though positive, was low. Except for HTW and RL in Sahel goats, there is a positive correlation between BW and other morphological measurements across the three breeds. This positive correlation posits that an increase in the designated morphological measurements is associated with an increase in BWT.

CART data mining algorithm was utilized to develop a prediction model for BWT in RS, SH and WAD goats using ten morphological body measurements (BL, HG, HTW, RW, RL, RH, HDW, SW, and FAL). The prediction model developed for this study indicates that, in Red Sokoto goats, HG played a critical role in predicting body weight, followed by RW, RL, HDW, and BL, respectively (Figure 1). In Sahel goats, BL was critical in predicting the body weight, then HG, FAL, RW, and RL, individually (Figure 2). In West African Dwarf goats, HG played the most important role in body weight prediction, followed by RH, BL, FAL, and SW, correspondingly (Figure 2).

The report of *Mokoena et al. (2022),* where HG and BL were found to be highly useful in predicting BWT of Kalahari Red goats, corroborates the findings of this study. In addition, *Tyasi et al. (2021)* reported that BL was the most critical parameter in body weight prediction of South African non-descript goats, followed by HG, sternum height, and RH, respectively. Similarly, *Celik (2019)* reported BL to be the most important morphological measurement in the body weight prediction of Pakistan goats. The relevance of certain morphological measurements, such as HG and BL – and the association between these morphological measurements and BWT – in the prediction of BWT is suggestive of these traits having the same monogenic effect.

Conclusion

Results from this study indicate that there is a positive and statistically significant correlation between BWT and morphological measurements in Red Sokoto and West African Dwarf goats. However, negative correlation was observed between BWT and HTW and RL in Sahel goats. This positive association observed among the breeds for most morphological measurements suggests that body measurements can be used as selection criteria in the body weight improvement of Red Sokoto, Sahel, and West African Dwarf goats. CART analyses indicated that HG plays an important role in predicting the BWT of Red Sokoto and West African Dwarf goats, while BL is crucial in the BWT prediction of Sahel goats. In other words, HG and BL are indicative of the body weight of certain goat breeds. Consequently, the results of this study will facilitate educating small-scale or rural farmers on how to predict the BWT of their livestock using simple morphological measurements, in the absence of weighing scales.

Predviđanje telesne mase autohtonih rasa koza na osnovu morfoloških merenja korišćenjem algoritma rudarenja podataka klasifikacijskog i regresionog stabla (CART)

Marvellous Olugbuyi Oyebanjo, Oluwakayode Michael Coker, Osamede Henry Osaiyuwu

Rezime

Stablo klasifikacije i regresije (CART) je algoritam za rudarenje podataka zasnovan na stablu koji razvija model za predviđanje ishoda. Cilj ovog istraživanja je bio da se stvori model za predviđanje telesne mase (BWT) koza rase crveni sokoto (RS), sahela (SH) i zapadnoafričkih patuljastih koza (WAD) koristeći morfološka merenja (kao što je dužina tela, BL; obim glave, HG ; širina glave, HDV; dužina lica, FAL; visina grebena, HTV; dužina karlice, RL; širina ramena, SV; širina karlice, RV; visina karlice, RH). Ukupno, 600 koza je korišćeno za ovo istraživanje (po 200 koza RS, SH i WAD). Pirsonova korelacija momenata je korišćena za procenu stepena povezanosti između BWT i svakog morfološkog merenja. Istovremeno, izvršena je CART analiza da bi se procenilo koja nezavisna varijabla (morfološko merenje) ima značajnu ulogu u predviđanju BWT (zavisne varijable). Kod koza RS i WAD uočena je pozitivna i statistički značajna ($p <$ 0,0001) korelacija između BWT i svakog morfološkog merenja. Međutim, kod SH koza, uočene su i pozitivne i negativne statistički značajne korelacije između BWT i morfoloških merenja. CART analiza je pokazala da je kod koza RS i WAD HG igrala značajnu ulogu u predviđanju BWT, dok se kod SH koza BL smatrala najkritičnijom nezavisnom varijablom u predviđanju BWT. Stoga, ovo istraživanje sugeriše da se HG može koristiti kao prognostički indeks za procenu BWT koza rase crveni sokoto i zapadnoafričkih patuljastih koza, dok se BL može koristiti za sahelske koze. Korišćeni SAS kodovi dostupni su preko GitHub repozitorijuma (https://github.com/Soullevram/CART).

Ključne reči: algoritam, telesna masa, rudarenje podataka, koze

Competing interests
The authors declare no competing of interest.

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PHYSIOLOGICAL RESPONSE OF NIGERIAN LOCALLY ADAPTED CHICKENS WITH DIFFERENT HEAT SHOCK PROTEIN 70 GENOTYPES TO ACUTE HEAT STRESS

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Original scientific paper

Abstract: Slow growth rate and acute heat stress are among the major constraints to indigenous chicken production in Nigeria. Characterization of heat-tolerance genes is a requisite for selective breeding of poultry for improved heat-tolerance and productivity. Therefore, this study investigated variation in HSP70 gene and its association with heat-tolerance traits of Nigerian locally adapted chickens. One-day old chicks comprising 118 Yoruba Ecotype Chicken-YEC and 138 FUNAAB Alpha Chicken-FAC were tagged and fed ad-libitum on starter (0 - 6 weeks) and grower (7-24 weeks) diets. At week 12, blood was sampled, DNA was extracted, amplified and electrophoresed following standard procedures. *HSP70* gene was genotyped using *Mme*I restriction endonuclease. At week 23, 36 chickens (six chickens per identified HSP70 genotypes) selected from each of YEC and FAC were exposed to 40±1°C for one hour. Cloaca temperature, respiratory rate (RR) and pulse rate (PR) were recorded and heat stress index (HSI) calculated. Data were analysed using descriptive statistics and ANOVA. Alleles A and B with genotypes: AA, AB, and BB were detected. After acute heat-stress, YEC with BB had higher RR value compared to those with AA and AB. The PR value of FAC with genotype BB was significantly higher ($p<0.05$) than those of AA, but similar to chickens with AB. Within FAC, HSI of BB-HSP70 was lower than AA-HSP70 but similar to AB-HSP70, while within YEC HSI of BB-HSP70 was similar to those of AA-HSP70 and AB-HSP70. The HSP70 gene was polymorphic in the studied chickens and genotype BB-HSP70 was associated with thermo-tolerance.

Key words: indigenous chickens, improved breed, heat stress, HSP70, heat stress index, PCR-RFLP

Introduction

The indigenous chickens are among the most adversely affected poultry species by the influence of high ambient temperatures and high relative humidity that characterize the tropical climate. This is because indigenous chickens are mainly reared under the extensive management system *(Ogundipe, 1990)*. Unlike the intensive commercial chicken management system where measures are always in place against adverse effects of heat stress, smallholder chicken producers usually provide temporary light shade and radiation shield which are often grossly inadequate to assuage heat stress effects *(Adedokun and Sonaiya, 2001)*. Recommended measures against the effects of heat stress are not only unaffordable to the smallholder farmers but also practically difficult to implement by smallholder chicken producers that dominate rural sub-Sahara Africa.

Physiological response to heat stress is a good indicator of measure of the degree of comfort or discomfort in farm animals. Pulse rate is widely considered as the simplest way to determine the physiological condition of an animal particularly under heat-stress. Increment in pulse rate builds blood spill out of the center to the surface and because of it more warmth is lost *(Marai et al., 2007).* The rectal temperature is recognized as an ideal indicator for heat stress valuation in animals and a significant increase or decrease in body temperature beyond the normal range of 41^{o}C will alter the homeostasis and consequently affects the normal body functioning and productivity of the poultry *(Franco-Jimenez et al., 2007; Adedeji et al., 2015)*. Changes in respiratory rate occasioned by increase in ambient temperature of the animal above its thermo-neutral range denotes heat stress condition in animals *(Lemerle and Goddard, 1986)*. The loss of heat often occurs through the respiratory tract when animals are trying to maintain the thermal equilibrium. An increase in ambient temperature above the thermo-neutral zone of the animals stimulates heat shock proteins (HSPs) production *(Nascimento et al., 2012; Mazzi et al., 2003)*. The predominant and temperature sensitive HSPs are HSP70 and HSP90. Both of them have protective roles during heat stress in farm animals *(Liang et al., 2016).* The HSPs are expressed during hyperthermic stress to aid the maintenance and prevention of protein degradation, regeneration of denatured proteins and contribute to the cell survival by eliminating the impaired polypeptides within cells *(Marai et al., 2007; Archana et al., 2017)*. Single nucleotide polymorphisms (SNPs) in chicken HSP70 gene have earlier been identified and its attendant effects on growth traits and egg production performance of acute heat stressed chickens have been evaluated *(Liang et al., 2016)*. Significant association have been reported between HSP70 genotypes and thermo-tolerance in chickens *(Mahmoud, 2000; Tamzil et al., 2014)*. However, information on the variation in HSP70 gene and their possible association with heat tolerance has been poorly documented in Yoruba and FUNAAB-Alpha chickens in Nigeria. Yoruba ecotype chickens are often found around Rainforest and Derived Savannah zones of Nigeria while, FUNAAB-Alpha chickens are crossbred between Nigerian indigenous chickens and an exotic breed of chicken *(Ajayi, 2010; Ilori et al., 2016).*

Material and Methods

Experimental birds

A total of 256 apparently healthy one-day-old chicks comprising 138 FUNAAB-Alpha and 118 Yoruba ecotype chicks were used for this study. The chicks were tagged at a one-day-old and managed on deep litter system for 24 weeks. They were fed chick starter (0-6 weeks) and grower (7-24 weeks) diets ad-libitum with unrestricted access to fresh clean water.

Blood sampling

At week 12, blood samples were collected via the jugular vein into sterilized tubes with EDTA as anticoagulant, kept immediately in icebox and transported to the laboratory for DNA extraction.

DNA extraction, PCR amplification and restriction digestion

Genomic DNA was extracted with the Zymo® Quick-DNA™ Mini Prep kit by following the manufacturer's protocols. The PCR was performed using primers designed by *Akaboot et al. (2012)* F: 5'-AACCGCACCACACCCAGCTATG-3' and R: 3'-CTGGGAGTCGTTGAAGTAAGCG-5' in a 50 µL total reaction volume. Each PCR tube contained 25 µL one taq quick® load 2X Master mix buffer (M0486S) (Biolabs; New England), 5 µL genomic DNA, 1 µL forward primer, 1 µL reverse primer and 18 µL nuclease free water. After an initial 5-minute denaturation at 94°C, 35 cycles denaturation for 30 s were performed at 94°C, annealed for 60s at 60 °C with 90 s extension at 72°C then, followed by a final 5-minute extension at 72℃. The amplified DNA were subsequently digested using *Mme*I restriction enzyme with the following reaction mixtures: 9 µL nuclease free water, 1 µL 10 x assay buffer, 0.5 µL restriction enzyme, 5 µL PCR product. The mixture was incubated at 37℃ for 15 minutes. The bands were separated using 1.5% agarose gel electrophoresis for 45 minutes at 100 V and then viewed with BIO-RAD gel documentation system (USA). The reaction protocols were as described by *Akaboot et al. (2012)* with slight modifications.

Acute heat stress exposure and data collection

At week 23, a total of 36 chickens (6 each of the identified HSP70 genotype) were purposefully selected from the flock and exposed to an acute heat stress at 40±1°C for 1.0 hour *(Tamzil et al., 2014)*. At 0 and 1 hour after heat stress the following physiological parameters were measured as follow: cloaca temperature (CT): inserting a clean clinical thermometer into the vent for one minute after which the readings were taken; respiration rate (RR): counting the number of movements of abdominal region or vent of each bird for a minute using a stopwatch; pulse rate (PR): placing the stethoscope under the wing vein and counting the number of beats per minute. Heat stress index (HSI): The heat stress index was calculated as described by *Isidahomen et al. (2012)* as follows:

$$\text{HSI} = \frac{\text{Average respiratory rate value}}{\text{Average pulse value}} \times \frac{\text{Normal pulse rate value}}{\text{Normal respiratory rate}}$$

Statistical analysis

Allele and genotype frequencies, test for Hardy-Weinberg equilibrium and Heterozygosity were obtained using POPGENE 1.32 software package *(Yeh, 1999)*. Data on physiological parameters were analysed using the generalized linear model (GLM) of SAS (2010). The following linear model was employed:

$Yij = \mu + Gi + Bj + (GB)ij + eij$

Where: Yij: is observed physiological, μ: is the overall mean, Gi: is the fixed effect of ith genotype, Bj: is the fixed effect of jth breed, (GB) ij is the interaction effect of ith genotype and jth breed, eij: is random error associated with each record.

Results and Discussion

The PCR amplification of HSP70 gene resulted in a single and specific band in all the studied chicken populations (360 bp), while restriction fragment analysis using *Mme*I restriction endonuclease resulted in three genotypes: AA (uncut 360 bp), BB (229 bp) and AB (360 bp and 229 bp) (Plate 1).

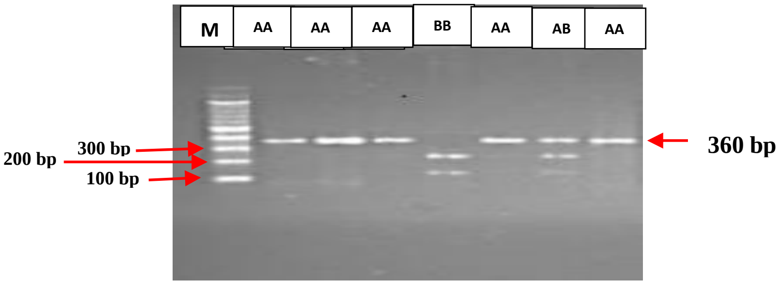

Plate 1. RFLP gel image of HSP70 gene in Nigerian Chickens
M: 100 bp DNA marker, Genotypes: AA, BB and AB.

The AA genotype (360 bp) had the highest frequency while BB genotype (229 bp) was the least frequent in the studied chicken populations (Table 1). Thus, using *Mme*I restriction enzyme; HSP70 gene can be safely concluded to be polymorphic in the studied Nigerian chickens. *Mahmoud (2000)* obtained three distinct allelic fragments from restriction digestion of chicken HSP70 gene with *Pst*I enzyme. Two polymorphic sites (A258G and C276G) and three genotypes (AA, AB and BB) were also reported in broiler and Taiwan native chickens, respectively *(Mazzi et al., 2003; Liang et al., 2016)*. Chi-square analysis of the observed and expected genotype frequencies showed deviation from the Hardy-Weinberg equilibrium (Table 1). This implies that, HSP70 locus in the studied birds has not been significantly affected by factors such non-random mating, mutation, genetic drift and or selection.

Table 1. Genotype frequencies of HSP70 gene in Nigerian chickens

		Genotype frequency			Heterozygosity		
Ecotype	N	AA	AB	BB	Observed	Expected	HWE (χ^2)
Yoruba	67	0.62	0.24	0.15	0.24	0.39	10.3*
FUNAAB Alpha	72	0.65	0.19	0.15	0.22	0.38	16.7*

Rectal temperature (RT), pulse-rate (PR) and respiratory rate (RR) are among the most important measure of physiological response of poultry to the heat stress. Following acute heat stress exposure, the observed average respiratory rate (RR) from this study ranged between 43.7±3.93 and 58.2±6.09 beat/min (Tables 2 and 3). This is closer to the earlier reported average RR value (44.6 and 51.07 beat/min) by *Adedeji et al. (2015)* in Nigerian chickens under heat stress. The higher RR value in FUNAAB-Alpha compare with the Yoruba chickens agreed with the reports of *Yalcin et al. (1997)* that body size of chicken influences the RR and pulse rate.

Table 2. Physiological responses of acute heat-stressed Yoruba chickens

Parameter	Duration of heat	AA	AB	BB
RR (beat/min)	0 hour	35.8±1.37[b]	38.5±1.38[ab]	39.7±1.37[a]
	1 hour	43.7±3.93	46.8±3.54	45.7±4.03
CT (^{O}C)	0 hour	40.3±0.82[b]	40.0±0.27[b]	41.1±0.82[a]
	1 hour	40.8±0.45	40.2±0.86	41.6±0.79
PR (breath/min)	0 hour	239.0±47.75	295.5±30.96	281.8±21.68
	1 hour	295.5±30.96	302.7±46.09	299.2±46.23
HSI	0 hour	1.30±0.19	1.13±0.12	1.16±0.11
	1 hour	1.44±0.51	1.37±0.26	1.42±0.17

CT: cloaca temperature, RR: respiration rate, PR: pulse rate, HSI: heat stress index.
Means ± SD with different superscript along the rows are significantly different ($p<0.05$)

Body size affects tolerance to heat stress and the exotic chickens are less tolerant to heat stress than the tropically adapted chickens. More so, crossbreeding might have led to reduced thermo-tolerance of the FUNAAB-Alpha chickens. In addition, *Defra (2003)* submitted that body weight, species and breed affected the heat production by poultry, thus, increase in ambient temperature led to increase panting rate consequently increases the respiratory rates. More so, the observed changes in physiological parameters by the acute heat stressed birds could indicate an attempt to maintain thermal equilibrium.

Table 3. Physiological responses of acute heat-stressed FUNAAB-Alpha chickens

Parameter	Duration of heat	AA	AB	BB
RR (beat/min)	0 hour	41.8±1.17	41.7±1.37	41.0±1.55
	1 hour	58.2±6.09	53.6±2.93	53.8±1.49
CT (^{O}C)	0 hour	40.0±0.72	40.5±0.58	40.3±0.26
	1 hour	40.4±0.64	41.7±1.29	40.6±0.53
PR (breath/min)	0 hour	284.6±8.66	296.9±23.22	312.4±34.27
	1 hour	316.9±24.81[b]	319.8±23.84[ab]	344.5±8.55[a]
HSI	0 hour	1.23±0.03	1.22±0.08	1.14±0.11
	1 hour	1.59±0.23[a]	1.46±0.14[ab]	1.35±0.10[b]

CT: cloaca temperature, RR (beat/min): respiration rate, PR (breath/min): pulse rate, HSI: heat stress index. Means ± SD with different superscript along the rows are significantly different ($p<0.05$)

The heat stress index of UNAAB-Alpha chickens with AB and BB genotypes were similar ($p>0.05$) but significantly different from their counterparts with AA genotype (Table 3), indicating that individuals having B allele as potential heat resistant candidate; since higher heat stress index indicates higher severity of the heat stress *(Isidahomen et al., 2012)*. In contrast with this observation, *Tamzil et al. (2014)* reported that chickens with heterozygote (AD) and homozygote (BB) genotypes of HSP70 as the most heat-tolerant and the least heat-tolerant, respectively. The discrepancies between the observed values from this study and reports of *Tamzil et al. (2014)* could be due to differences in chicken breeds studied (Nigerian indigenous chickens vs Indonesian native chickens) or varied laboratory protocols, as the authors utilised Polymerase Chain Reaction (PCR)-Single Stranded Conformation Polymorphism-(SSCP) as against the PCR-RFLP that was used in this study. In PCR-RFLP, a PCR amplicon is treated by a certain restriction enzyme that cleaves the DNA via the restriction sites to generate DNA fragments (thus, involves screening limited amplicons) while PCR-SSCP screens almost all amplicon sequences thereby capable of detecting more genotype or SNPs.

Conclusion

The RFLP analysis of HSP70 gene was polymorphic in the studied Nigerian chickens using *Mme*I endonuclease. The B allele was associated with lower heat stress index indicating that; chickens with B allele exhibited higher heat-tolerance. However, further investigations under long-term heat stress would be necessary to validate this observations.

Fiziološki odgovor nigerijskih lokalnih pilića različitih genotipova HSP70 na akutni toplotni stres

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Rezime

Spora brzina porasta i akutni toplotni stres su među glavnim ograničenjima za proizvodnju autohtonih pilića u Nigeriji. Karakterizacija gena otpornosti na toplotu je preduslov za selektivni uzgoj živine radi poboljšanja toplotne tolerancije i produktivnosti. Stoga je predmet ovog istraživanja bilo ispitivanje varijacije u genu HSP70 i njegove povezanosti sa osobinama otpornosti na toplotu nigerijskih lokalno prilagođenih pilića. Pilići uzrasta od jednog dana, i to - 118 pilića ekotipa Yoruba (Yoruba Ecotype Chicken – YEC) i 138 pilića FUNAAB Alpha - FAC su obeleženi i hranjeni, *ad-libitum* starter (0 - 6 nedelja) i grover (7-24 nedelje) obrocima. U 12. nedelji, uzorkovana je krv, ekstrahovana DNK, amplifikovana i podvrgnuta elektroforezi prema standardnim procedurama. Gen HSP70 je genotipizovan korišćenjem *Mme*I restrikcione endonukleaze. U 23. nedelji, 36 pilića (šest pilića po identifikovanim HSP70 genotipovima) odabranih iz svakog od YEC i FAC je izloženo temperaturi od 40±1°C tokom jednog sata. Temperatura kloake, brzina disanja (RR) i puls (PR) su zabeleženi i izračunat je indeks toplotnog stresa (Heat Stress Index - HSI). Podaci su analizirani korišćenjem deskriptivne statistike i ANOVA. Detektovani su aleli A i B sa genotipovima: AA, AB i BB. Nakon akutnog toplotnog stresa, YEC sa BB je imao veću RR vrednost u poređenju sa onima sa AA i AB. PR vrednost za genotip FAC sa genotipom BB bila je značajno viša (p<0,05) od vrednosti AA, ali slična kao kod pilića sa AB. U okviru FAC, indeks toplotnog stresa za BB-HSP70 bio je niži od AA-HSP70, ali sličan AB-HSP70, dok je unutar YEC, indeks toplotnog stresa za BB-HSP70 bio sličan onima za AA-HSP70 i AB-HSP70. Gen HSP70 je bio polimorfan kod proučavanih pilića, a genotip BB-HSP70 je bio povezan sa termo-tolerancijom.

Ključne reči: autohtona živina, poboljšana rasa, toplotni stres, HSP70, indeks toplotnog stresa, PCR-RFLP

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BODY THERMOREGULATION AND SERUM METABOLIC PROFILE OF KANO BROWN BUCKS FED *Pluerotus ostreatus* BIODEGRADED SUGARCANE SCRAPINGS

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Abstract: The study assessed if feeding of *Pleurotus ostreatus* biodegraded sugarcane scrapings (BSS) would have detrimental effects on body thermoregulation and serum metabolic profile of goats. Twenty-one healthy male Kano Brown bucks (6 – 7 months of age; 9.44 ± 0.39 kg mean body weight) were stratified based on their BW into three treatment groups containing 0 (T1), 15 (T2) and 30% (T3) of BSS in a completely randomised design. Serum total protein, albumin, globulin and albumin:globulin ratio were greater (P<0.05) in T2 than in T1 and T3. Serum urea was higher (P<0.05) in T3 relative to T1 and T2. While serum glucose was greater in BSS diets, cholesterol and alanine transaminase were higher in the T1 than in BSS diets (P<0.05). Alanine phosphatase decreased in the order: T1 > T2 > T3 (P<0.05). Serum creatinine, aspartate transaminase, total bilirubin, sodium, potassium, calcium and bicarbonate, rectal temperature, earlobe temperature, heart rate and respiratory rate were not influenced (P>0.05) by dietary treatments. Values of all serum metabolic indices and body vital signs were within normal ranges for goats. Results show that *Pleurotus ostreatus* biodegraded sugarcane scrapings can be used up to 30% in the diets of goats without negatively impacting their body thermoregulation, metabolic welfare and health.

Key words: sugarcane scrapings, solid state fermentation, *Pleurotus ostreatus*, metabolic welfare, vital signs, goats

Introduction

Lignocellulosic biomass from agricultural residues are abundantly available with about 73.9 Tg yearly production in the world *(Kim and Dale, 2014)*. The disposal of these wastes, which are often left on the field, constitutes a problem for the local producing agro-industries and thus causes environmental degradation. However, if properly harnessed and processed, these wastes have a great potential as livestock feed. Sugarcane scrapings, the bark of cane sugar stem after peeling, is one of such lignocellulosic materials in Nigeria. Like other lignocellulosic materials, its use in livestock feed is constrained by the poor nutritive value attributable to low protein and large contents of cellulose, hemicellulose and lignin. Lignin is, however, the major culprit because linkages between lignin and cellulose and hemicellulose inhibit accessibility of rumen microbial enzymes to the cell wall contents and, thus lock up appreciable amounts of potential energy and nutrients *(Tengerdy and Szakacs, 2003)*. Therefore, even ruminants with their effective digestive system for fibre degradability cannot extract sufficient energy and protein from lignocelluloses. Biological treatment such as solid state fermentation/biodegradation with white rot fungi, particularly *Pleurotus ostreatus*, has been used to improve the nutritional value of poor-quality roughages and develop unconventional ingredients at competitive prices *(Isroi et al., 2011; Atuhaire et al., 2016)*. Although there are many reports on the use of white rot fungi biodegraded lignocelluloses in ruminants, there is, however, a paucity of information on the effect of feeding these biodegraded materials on the body thermoregulation and blood metabolic profile of goats in the tropics. Examination of blood metabolic profile of ruminants fed fungal solid state fermented fibrous materials may be necessary to ascertain if the welfare of the animals is compromised or not. Blood metabolic profiles have been used for diagnosis and prognosis of diseases in animals and are also useful to assess the welfare condition of animals or to understand if some changes in a diet can affect animal physiology and health *(Olafadehan, 2011a; Olafadehan et al., 2014)*. The current study aimed to evaluate the body thermoregulation and serum metabolic profile of Kano Brown bucks fed diets containing *Pleurotus ostreatus* biodegraded sugarcane scrapings in their diets.

Materials and Methods

Experimental site

The experiment was conducted at the University of Abuja Teaching and Research Farm, Federal Capital Territory, Nigeria. The site is at 456 m altitude and lies between latitude 8º 55' N and 9º 00' E and longitude 7º 00' N and 7º 05' E. It has a tropical climate with temperature and annual rainfall ranging from 25.8 to 42°C and 1100 to 1650 mm respectively.

Substrate preparation and biodegradation process

Fresh sugarcane scrapings (SS) were collected from local processors of sugarcane, chopped into 1-2 cm lengths and air-dried at ambient temperature (25 – 30°C). The white rot fungus, *Pleurotus ostreatus*, used for the solid state fermentation of the SS was obtained from a reputable commercial producer in Nigeria. Prior to the inoculation with the *Pluerotus* for solid state fermentation, the inoculation containers were sterilised by being washed thoroughly and dried for 10 minutes at 100°C, while SS were autoclaved twice at 121°C for 15 minutes with cooling between cycles to eliminate any active microorganisms. Thereafter, the moisture content of the SS was adjusted to 67% by mixing the SS and distilled water in ratio 1:1.

After cooling under aseptic condition, the prepared SS were inoculated with the *P. ostreatus* spores in ratio 25:1 and kept in the inoculation room maintained at 30°C and 100% relative humidity until mycelia were formed. After 21 days of inoculation and solid state fermentation, the biodegraded SS (BSS) was autoclaved to terminate mycelia growth and biodegradation. The BSS were then dried to constant weight, bagged and kept until needed for feeding.

Experimental animals, management and diets

Twenty-one healthy intact Kano Brown male goats, about 6 to 7 months of age with an average initial body weight (BW) of 9.44 ± 0.39 kg, were used for the study. Two weeks prior to the arrival of the goats, the pens and its immediate surroundings were thoroughly disinfected with antiseptic (Morigad). The animals were quarantined for two weeks and administered prophylactic treatment comprising subcutaneous vaccination with *Peste Des Petits* Ruminants (PPR) live vaccine diluted at 2 ml per 10 kg BW, subcutaneous injection with Avomec® at 0.5 ml/25 kg of the BW for the control of endo and ecto parasites and injection with a long acting oxytetracycline HCl at 1 ml/10 kg BW. Goats were housed in individual cages measuring 1.2 m^2 each and kept in open sided pens measuring 3 m x 4 m x 4 m. Three complete diets were formulated, using varying levels of BSS meal: no sugarcane scrapings (control; T1), 15 (T2) and 30% (T3) inclusion levels to replace corn bran at 0, 50 and 100 % respectively on dry matter (DM) basis. Table 1 shows the ingredient and chemical composition of the experimental diets formulated to meet the requirements of growing goats according to the recommendations of *NRC (2007)*. Goats in each treatment group were randomly assigned to one of the experimental diets and fed at 5% of their BW on DM basis for a period of 12 weeks. Adjustments were made to feed given to the goats as the experiment continued to ensure collection of orts. Feeding was twice in a day at 08:00 h and 16:00 h. Clean water was supplied daily *ad libitum*. Feed intake was determined by subtracting the weight of the left over feed from the weight of the feed offered the previous day. Nutrient intake was determined by multiplying the feed intake (in DM) of an animal by the nutrient (in DM) from the chemical composition of the diets.

Body thermoregulation

Rectal temperature (RT), earlobe temperature (ET), respiratory rate (RR) and heart rate (HR) of each goat were measured twice a week at 11:00 h. Rectal temperature was measured using digital thermometer. The sensory tip was disinfected with an antiseptic, lubed with petroleum jelly (Vaseline) and then inserted into the rectum of individual animal, at the display of a constant temperature indicated by "C L0" on the thermometer's mini digital screen, at a uniform depth of 1.5 cm. After the beeping sound of the alarm signal of the digital thermometer, the thermometer was removed from the rectum and the displayed body temperature was recorded. The ET was also measured by placing the digital thermometer in the earlobe at the display of a constant temperature indicated by "C L0" on the thermometer's mini digital screen. The earlobe was then folded around the thermometer to ensure adequate contact and avoid temperature interactions. The digital thermometer was removed from the earlobe after the beeping sound of the alarm signal and the temperature recorded. RR was determined by counting the number of abdominal movement per minute using the seconds hand in an analogue wrist watch for one minute and the counts recorded. HR was determined using a stethoscope placed on the left side of the ribs (the anatomical location of the heart). The normal sound of the heart was the loob dope sound which indicated a complete heart beat for a minute using the seconds hand in an analogue wrist watch for one minute and the rates recorded appropriately.

Blood collection

Blood samples (5 ml) were taken from the jugular vein of each goat in the morning before feeding on the last day of the experiment into anti-coagulant free vacutainer tubes placed in ice-packed flasks and taken immediately to the laboratory for serum biochemical analysis. Serum for biochemical indicators was harvested by centrifugation of complete blood at 3000 rpm for 15 minutes in a laboratory centrifuge (NOP-350R, NOP medical instruments, Punjabi, India) at 4°C. The serum was analysed within four hours of collection.

Chemical analysis

Samples of the experimental diets were analysed for their proximate constituents in accordance with the procedures of *AOAC (2000)*. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were analysed using the procedures of *Van Soest et al. (1991)*. NDF was analysed using sodium sulphite and amylase and expressed with residual ash. Acid detergent lignin was determined by solubilisation of cellulose with sulphuric acid. Concentrations of hemicellulose and cellulose were calculated mathematically as the differences between NDF and ADF and ADF and lignin respectively. Non-structural carbohydrate (NSC) was calculated using the following formula NSC= 100 – (CP + EE + ash + NDF) %. Serum total protein (TP), albumin, glucose, urea, creatinine, cholesterol, alanine transaminase (ALT), alkaline phosphatase (ALP), aspartate transaminase (AST), calcium, sodium, potassium and bicarbonate were analysed by calorimetric methods using Bio Maxima reagent sets (Lublin, Poland) in a Metrolab 5.0, Norway, Oslo. Globulin was calculated as the difference between TP and albumin. Albumin/globulin (A/G) ratio was calculated by dividing albumin values by globulin values.

Data analysis

Data were subjected to analysis of variance in a completely randomized design using the SPSS (23.0). Duncan multiple range test of the same software was used to test the significant difference between the means at P≤0.05 level of significance. The statistical model is:

$Yij = \mu + ti + ei$

where Yij = the general response to the specific parameter under investigation, μ = the general mean peculiar to each observation, ti = the fixed effect of the dietary treatments on the observed parameters, and ei = the random error term for each estimate.

Results and Discussion

Chemical composition of experimental diets

The parallel CP of the diets implies that solid state fermentation of sugarcane scrapings upgraded the CP to a similar level to that of the corn bran being replaced. Earlier studies *(Belewu, 2008; Bento et al., 2014)* reported increased CP of fungal biodegraded lignocellulosic materials. The NSC decreased while the structural carbohydrates, except for hemicellulose, increased with increasing replacement of BSS for corn bran.

Table 1. Ingredient and chemical composition of the experimental diets (% DM)

Ingredient	Treatment		
	T1	T2	T3
Maize	25	25	25
Corn bran	30	15	0
Biodegraded sugarcane scrapings	0	15	30
Groundnut cake	17	17	17
Cowpea husk	25	25	25
Salt	0.5	0.5	0.5
Limestone	2	2	2
Premix	0.5	0.5	0.5
Chemical composition			
Organic matter	93.76	91.96	91.55
Crude protein	15.70	15.80	15.89
Non-structural carbohydrate	33.59	29.74	27.24
Neutral detergent fibre	38.69	40.62	42.60
Acid detergent fibre	20.95	23.10	25.24
Acid detergent lignin	2.89	3.21	4.45
Cellulose	18.06	19.89	20.79
Hemicellulose	17.74	17.52	17.36

T1, 0% biodegraded sugarcane scrapings; T2, 15% biodegraded sugarcane scrapings; T3, 30% biodegraded sugarcane scrapings inclusion

Serum biochemistry

Although some of the serum metabolites were altered by feeding BSS diets, all the measured parameters were within the optimum ranges for healthy goats *(Merck Veterinary Manual, 2010),* suggesting absence of metabolic disorder or health issue *(Olafadehan, 2017)*. Serum TP, albumin and A/G ratio were higher ($P<0.05$) in T2 than in other diets, indicating enhanced protein intake, availability, metabolism, absorption and utilisation, liver and kidney function, and nutritional and health status of the goats *(Olafadehan, 2011, 2017; Shoby et al., 2020)*. Whereas albumin, a negative acute phase protein *(Ceciliani et al., 2012)*, is a good index of health and nutritional status of an animal *(Olafadehan, 2017)*, A/G ratio reflects protein utilisation efficiency and the state of the liver, kidney and health of the goats *(Farver, et al., 1997; El-Sherif and Assad, 2001)*. Globulin concentration was greater in BSS diets than in control diet ($P<0.05$). Since globulin is produced in the liver and by immune system, the increased globulin of the BSS diets reveals enhanced liver function and immune response of the goats on these diets. Nevertheless, since the TP, albumin, globulin and A/G ratio of the treatments were within the established ranges, the results indicate adequate protein intake and utilisation, uncompromised nutritional and health status, and absence of liver and kidney dysfunctions, immunosuppression and metabolic disorders in the goats *(Olafadehan, 2017)*. Serum urea N was higher ($P<0.05$) in T3 than in T1 and T2.

The increased SUN in T3 compared to in T1 suggests higher ruminal proteolysis of BSS protein relative to corn bran protein.

In ruminant animals, serum glucose and creatinine are the most significant indices of energy metabolism. That these indicators of energy status in animals were within the physiological ranges indicate unimpaired energy availability and utilization. However, the higher (P<0.05) serum glucose of the BSS diets shows the glycogenic potential of the BSS as an ingredient in ruminant diets and suggests high nutritional and energy status *(Kholif et al., 2021a; Olafadehan, 2011a)*, organic matter digestibility *(Abd El Tawab et al., 2020b; Azazz et al., 2020)* and ruminal propionate concentration *(Olafadehan et al., 2018; Kholif et al., 2021b, c)*. Since nutrient digestibility and ruminal fermentation are not measured in the present study, more studies are required to further elucidate the effect of replacement of corn bran with BSS in the diets of growing goats on these parameters. Serum creatinine and total bilirubin were not (P>0.05) affected by the treatments. Normal creatinine concentrations also indicate proper renal function, as a direct relationship has been established between serum creatinine and kidney function *(Prvulovic et al., 2012)*. Bilirubin values were within the reference ranges for goats, indicating the absence of haemolysis. According to *Olafadehan (2011b)* bilirubin is an insoluble molecule derived from the breakdown of haemoglobin in the spleen. Whereas AST was not affected, ALP, ALT and cholesterol were lower (P<0.05) in BSS diets relative to the control diet. Though BSS diets reduced ALP and ALT concentrations, values were within the normal ranges, suggesting normal liver condition and function and absence of liver pathological lesions *(Olafadehan et al., 2014)*. The reduced serum cholesterol of BSS diets suggests that altered fat metabolism and absorption, although the normal values indicate the absence of hyper or hypocholesterolemia *(Olafadehan, 2011a; 2017)*, bile obstruction *(Silanikove and Tiomkin, 1992)* and liver dysfunction and fat malabsorption *(Zubcic, 2001)*.

Table 2. Serum biochemical indices of goats fed biodegraded sugarcane scrapings diets

Parameter	Treatment			SEM	RV
	T1	T2	T3		
Total protein (g/L)	67.81[b]	70.92[a]	68.44[b]	2.03	61 - 75
Albumin (g/L)	37.30[b]	38.91[a]	36.12[b]	1.71	23 - 36
Globulin (g/L)_	30.51[b]	32.02[a]	32.33[a]	0.63	27 - 44
Albumin/globulin ratio	1.22[a]	1.22[a]	1.12[b]	0.05	1.0 – 2.0
Urea nitrogen (mmol/L)	5.06[b]	5.18[b]	5.56[a]	0.10	4.5 - 9.2
Creatinine (mmol/L)	109	113	103	22.0	60 - 135
Glucose (mmol/L)	3.07[b]	4.01[a]	3.77[a]	0.16	2.7- 4.2
Cholesterol (mmol/L)	3.14[a]	2.58[b]	2.47[b]	0.10	1.7 - 3.5
Alkaline phosphatase (U/L)	209.52[a]	205.51[b]	200.68[c]	1.23	61- 283
Alanine transaminase (U/L)	16.23[a]	15.56[b]	15.32[b]	0.15	15- 52
Aspartate transaminase (U/L)	201.21	200.83	200.79	0.33	66 - 230
Total bilirubin (mmol/L)	1.88	2.05	3.40	0.68	1.7 - 4.3

Means with the different superscripts along the row are significantly ($P < 0.05$) different.
T1, 0% biodegraded sugarcane scrapings; T2, 15% biodegraded sugarcane scrapings; T3, 30% biodegraded sugarcane scrapings; RV, reference value as stated by *Merck Veterinary Manual (2010)*

Serum minerals

Serum sodium (Na), potassium (K), calcium (Ca) and bicarbonate were similar ($P>0.05$) across the three experimental groups.

Serum mineral concentrations were within the normal ranges for goats *(Merck Veterinary Manual, 2010)*. The normal serum K levels of the goats implies non-interference of the diets with K availability and absorption *(Olafadehan et al., 2014)*. *Busher (1990)* showed a positive relationship between low serum bicarbonate and chronic kidney disease; therefore, the normal serum bicarbonate level indicates the absence of incidence of chronic kidney disease. The within the physiological range Ca levels suggest absence of hyperparathyroidism, hypervitaminosis D, multiple myoloma and neoplastic disease or osteomalacia, rickets and renal failure reportedly engendered by calcuim imbalance in ruminants *(Olafadehan et al., 2014)*. *Cheesbrough (2004)* explained that maintenance of serum Na levels, as obtained in this study, suggests that the experimental diets were able to maintain cellular tonicity fluid balance and pH, regulate metabolic processes as well as involved in regulation of neural and muscular functions.

Normal values of the serum metabolic indices indicate no hepatic and renal damage, revealing that BSS can fed to goats with uncompromised metabolic welfare.

Table 3. Major serum minerals of goats fed biodegraded sugarcane scrapings diets

Parameter	Treatment			SEM	RV
	T1	T2	T3		
Sodium (mmol/L)	141.64	148.00	147.76	9.18	137 – 152
Potassium (mmol/L)	4.07	4.48	4.52	0.12	3.8 - 5.7
Calcium (mmol/L)	2.84	2.85	2.89	0.18	2.3 - 2.9
Bicarbonate (mmol/L)	23.41	24.93	23.83	0.11	20 – 27

T1, 0% biodegraded sugarcane scrapings inclusion; T2, 15% biodegraded sugarcane scrapings inclusion and T3, 30% biodegraded sugarcane scrapings inclusion; RV; reference values as stated *Merck Veterinary Manual (2010).*

Body thermoregulation

All the vital signs (RT, ET, HR and RR) were not ($P>0.05$) affected by diets and were with the reference ranges of 38.5 – 39.7°C, 70 – 90 bpm and 16 – 34 cpm for RT, HR and RR respectively for healthy goats *(Merck Veterinary Manual, 2010)*. The results indicate the diets did not compromise normal goat vital signs. Generally, normal vital signs of an animal depend on recent activity, feed and water consumptions and the physiological state of the animals *(Gado et al., 2016)*. The normal values obtained for the vital signs are a confirmation of the fact that the diets did not affect the body physiology and health of the animals, suggesting that that BSS can safely be used as a feedstuff in the diets of goats without posing any nutritional or health challenge because lower or higher values than the normal ranges indicate physiological or health problem.

Table 4. Body thermoregulation of goats fed biodegraded sugarcane scrapings diets

Parameter	Treatment			SEM
	T1	T2	T3	
Rectal temperature (℃)	38.73	38.45	38.69	0.17
Earlobe temperature (℃)	37.68	37.63	37.71	0.19
Heart rate (bpm)	83.63	82.31	83.11	0.91
Respiratory rate (cpm)	22.67	23.47	23.63	0.75

T1, 0% biodegraded sugarcane scrapings inclusion; T2, 15% biodegraded sugarcane scrapings inclusion and T3, 30% biodegraded sugarcane scrapings inclusion

Conclusion

Inclusion of biodegraded sugarcane scrapings in the diets of goats posed no harm to the animal body physiology, thermoregulation, serum metabolic profile and health. Biodegraded sugarcane scrapings can thus be included up to 30% to completely replace corn bran in the diets of goats without affecting goat metabolic welfare and health condition.

Termoregulacija tela i metabolički profil seruma kano smeđih jarčeva hranjenih strugotinama šećerne trske biorazgrađenim sa *Pluerotus ostreatus*

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Rezime

Cilj ove studije je bio da se proceni da li bi ishrana strugotinama šećerne trske (BSS), koje su biorazgrađene korišćenjem *Pleurotus ostreatus*, imalo štetne efekte na telesnu termoregulaciju i metabolički profil seruma koza. Dvadeset i jedan zdrav mužjak rase smeđi kano (Kano Brown) (6 – 7 meseci starosti; 9,44 ± 0,39 kg srednje telesne mase) je raspoređen na osnovu njihove telesne mase u tri tretmana koji su sadržavali 0 (T1), 15 (T2) i 30% (T3) BSS-a u potpuno slučajnom dizajnu.

Ukupni serumski protein, albumin, globulin i odnos albumin:globulin su bili veći (P<0,05) u T2 nego u T1 i T3. Urea u serumu je bila viša (P<0,05) u T3 u odnosu na T1 i T2. Dok je glukoza u serumu bila veća u BSS ishrani, holesterol i alanin transaminaza su bili viši u T1 nego kod životinja na BSS ishrani (P<0,05). Alanin fosfataza se smanjivala po redosledu: T1 > T2 > T3 (P<0,05). Prehrambeni tretmani nisu uticali na kreatinin u serumu, aspartat transaminazu, ukupni bilirubin, konjugovani bilirubin, natrijum, kalijum, kalcijum i bikarbonat, rektalnu temperaturu, temperaturu ušne resice, otkucaje srca i brzinu disanja (P>0,05). Vrednosti svih serumskih metaboličkih indeksa i telesnih vitalnih znakova bile su u granicama normale za koze. Rezultati pokazuju da strugotine šećerne trske biorazgrađene uz korišćenje *Pleurotus ostreatus* mogu da se koriste do 30% u ishrani koza bez negativnog uticaja na termoregulaciju njihovog tela, metaboličko blagostanje i zdravlje.

Ključne reči: strugotine šećerne trske, fermentacija u čvrstom stanju, *Pleurotus ostreatus*, metaboličko blagostanje, vitalni znaci, koze

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NUTRITIONAL EVALUATION OF COOKED SAUSAGES IN THE MARKET OF THE REPUBLIC OF SERBIA USING THE NUTRI-SCORE METHODOLOGY

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Abstract: Enhancing diet quality is a recognized strategy for reducing the burden of non-communicable diseases (NCDs), making it a primary focus of public health policies worldwide. The Nutri-Score, a front-of-pack labeling logo utilizing five color-coded letters (A, B, C, D, E), has been established as a means to assist consumers in promptly identifying healthier prepackaged foods within a given food category. It has a positive influence in terms of consumer awareness, perception, comprehension, and purchasing behavior and potentially contributes to a decrease in the prevalence of NCDs. The objective of this research was to assess the Nutri-Score of finely and coarsely ground cooked sausages available in the Serbian market. To accomplish this, a total of 189 packaged cooked sausages from the eight largest supermarket chains in the Belgrade region of the Republic of Serbia were analyzed. The sausages were evaluated using the Nutri-score method and categorized accordingly. The results of the Nutri-Score analysis revealed that only 1.1% of the cooked sausages fell into Group C, while 62.4% were classified as Group D, and 36.5% were classified as Group E. The reduction of sodium and saturated fatty acids, which are major contributors to unfavorable Nutri-scores in cooked sausages, is crucial due to their significant presence, often leading to their classification as "unhealthy foods."

Key words: Nutri-score, front-of-pack labelling, cooked sausages, saturated fatty acids

Introduction

The World Health Organization's Global Monitoring Framework on non communicable diseases sets an overall target of achieving a 25% relative reduction in mortality from cardiovascular disease, cancer, diabetes, and chronic respiratory diseases among individuals aged 30-70 years by 2025, with one of the specific goals includes prevention of any increase in the prevalence of diabetes and obesity in the population (*WHO*, 2013). As poor dietary quality is a leading modifiable risk factor for obesity and non-communicable diseases, the World Health Organization (WHO) emphasizes the importance of promoting healthier food choices (*Magnusson et al., 2019*). For this to happen, consumers need to be able to distinguish between healthier and less healthy products. Hence, it is critical that consumers are well-informed about the nutritional content of food products. Nutritional labeling is a vital intervention that can aid consumers in making informed decisions and improving their diets' healthfulness (*Bossuyt et al., 2021*). The popularity of Front-Of-Pack labeling is a direct result of the numerous studies showing that conventional nutritional information, including text and back-of-pack labels, which are now mandatory in most countries, including Serbia (*Official Gazette of RS, No. 23/2022)*, has little impact on consumers' dietary choices: they are frequently disregarded, misinterpreted, and do not result in action (*Grunert et al.,* 2010; *Chantal et al.,* 2017*)*. The primary objective of Front-of-pack nutrition labeling is to enhance consumers' comprehension of the nutritional value of foods, facilitate informed food selection by consumers, as well as to incentivize manufacturers to reformulate their products with healthier ingredients (*Delhomme, 2021*). The available literature suggests that in experimental environments, the majority of Front-Of-Pack nutrition labels have a favorable impact on consumers' ability to recognize the healthier option as compared to a scenario where no label is provided (*Ducrot et al., 2015; Roseman et al., 2018*; *Finkelstein et al., 2018*). Consumers tend to show a preference for Front-Of-Pack labels over traditional nutrition declarations in terms of their attractiveness, and further research indicates that evaluative schemes which utilize color-coding, particularly those with a color-coding graded indicator, are the most effective at helping consumers of different ages, cultural backgrounds, and socio-economic status identify healthier food choices, as demonstrated in an international study by *Egnell et al.* (*2018*), which was supported later on with numerous research (*Storcksdieck et al., 2020*; *Fondevila-Gascón et al*., *2022*). The Nutri-Score, a color-coded Front-of-pack indicator used to classify foods based on their nutritional value, is gaining popularity in Europe. Initially developed and implemented in France, it is currently being adopted by an increasing number of EU Member States, including Austria, Belgium, Germany, Luxembourg, The Netherlands, Portugal, and Spain (*Delhomme, 2021*). The Nutri-Score employs a five-point scale, ranging from the healthiest to the least healthy, which employs a combination of colors and letters to rank food items (Figure 1). The healthiest products are assigned an A score and are indicated by a dark green color, while the least healthy option receives an E score and is highlighted in red. It is based on a nutrient profiling system that considers both beneficial and unfavorable food components. Food products that contain ingredients such as fruits, vegetables, nuts, fiber, and proteins are given a better overall score, whereas those with high energy content, sugar, saturated fatty acids, and salt leads to a worse score (*Chantal et al., 2017*). The French Public Health Agency, which developed the Nutri-Score, has modified the algorithm to include olive oil, nuts, and rapeseed oils as favorable components, following scientific evidence, especially from recent intervention studies with olive oil (*Hercberg et al., 2021*). To summarize, the Nutri-Score represents a general food rating system that aims to assist people in making informed choices about their overall diet and to enable comparison of products within the same category (e.g., helping consumers to choose between two hot dogs).

Material and Methods

This study adopted a descriptive research design to analyze food labels provided by manufacturers of 189 pre-packaged cooked sausages. Data collection was conducted across 29 retail stores affiliated with the 8 largest supermarket chains in the Belgrade region of the Republic of Serbia, with the objective of maximizing the representation of available products. The data on the samples were collected based on the leading retailers currently present in the Serbian market, including LIDL Serbia, Delhaize Serbia (sales divisions: Maxi, Shop & Go, and Tempo Centar), Mercator S (sales divisions: Idea and Roda), DIS, Metro, Univerexport, Aman and Sunce (Qvattro company) containing a wide variety of domestic as well as foreign products manufacturers. The data collection period spanned from March to May 2023. Cooked sausages are classified by the Rulebook on the Quality of Minced Meat, Meat Products, and Semi-Processed Meat Products (*Official Gazette of RS, 50/2019; 34/2023*) into four separate categories: finely ground cooked sausages, coarsely ground cooked sausages, cooked sausages with meat pieces and meatloaf. Despite their efforts, the researchers were unable to find any products labeled as cooked sausages with meat pieces and meatloaf on the market shelves during the data collection time. The two remaining groups of meat products were classified by the researchers according to their common use from a consumer's perspective, with similar items being grouped together into categories, but also bearing in mind the classification provided by the *Official Gazette of RS (50/2019; 34/2023).* Classification was conducted as follows: finely ground cooked sausages – hot dog sausages, hot dog-type sausages (products marketed under a different name), frankfurter sausages, white sausages, Bologna sausage (original in

Serbian: *parizer*) and Bologna-type sausages (products marketed under a different name); as for the coarsely ground cooked sausages – mortadella, Srpska sausage, Tirolska sausage, Tirolska-type sausage (usually under the name of Alpska sausage; products marketed under a different name) and products marketed under a different name (some of the many product names include: Grill sausage, Smoked sausage, Domaća sausage, Kranjska sausage ect.).

Nutri-scores were calculated by the method proposed by *Chantal et al. (2017).* To summarize, the model employs a straightforward scoring system that assigns points based on the nutrient content of 100 grams of food. The points are allotted for both the A nutrients – positive points category (energy, saturated fat, total sugar, and sodium content) and category C nutrients – negative points category (fruit, vegetable, and nut content, walnut and olive oil, fiber, and protein content). The score for category C nutrients is then subtracted from the score for category A nutrients to generate the final nutrient score of the cooked sausage. Each nutrient/food component in category A nutrients can be awarded a maximum of ten points, while each nutrient/food component in category C nutrients can be awarded a maximum of five points. Once the final nutrient scores are obtained, they are compared with the cut-off values for the color-coded food grades from A to E. The score is determined on a scale that ranges continuously from < -1 (e.g. water), which represents the healthiest option, to +40, which represents the least healthy option (Figure 1). It is important to emphasize that if a meat product achieves a total score of 11 points or higher in category A, the deduction of negative points for protein will only occur if the product also obtains a score of 5 points or higher in other components of category C, such as fruit, vegetables, or fiber content.

These calculations were made by taking available information on nutritional labels from the products packages provided by manufacturers and required by *Official Gazette of RS (23/2022)* (which are in accordance with *Regulation (EU) No. 1169/2011* of the European Parliament and the Council on the provision of food information to consumers). This information included: energy (kJ/100g), total fats (g/100g), saturated fatty acid content (g/100g), total carbohydrate content (g/100g), protein content (g/100g), salt content (as NaCl, g/100g), fiber (if present, g/100g), and fruits, vegetables, pulses, nuts, and rapeseed, walnut and olive oils if present (g/100g). Total sodium content (mg/100g) was calculated using the formula: salt content (g/100g)/2.5*1000.

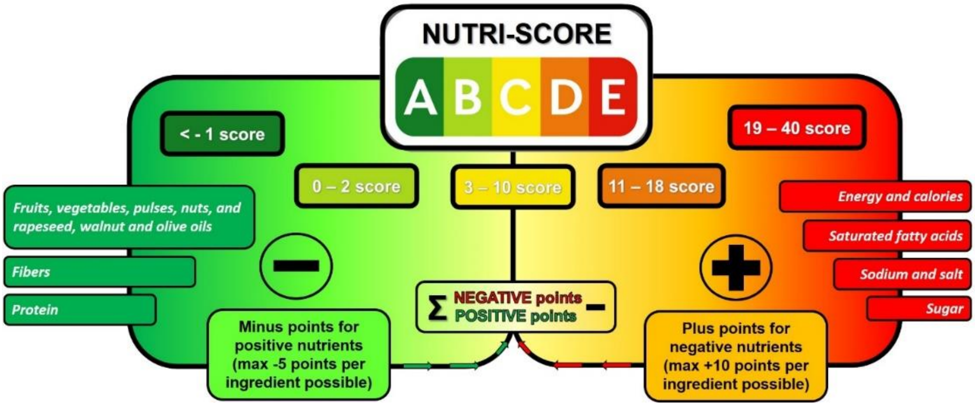

Figure 1. Nutri-score 5-color nutrition labeling system on the front-of-pack label of the products

In summary, when it comes to the nutrient content in 100 g, meat or meat products that are designated with a green label (A or B) are deemed to be of higher nutritional value, while those with an orange/red label (D or E) are regarded as being of lower nutritional quality. Additionally, researchers focused on following the percentage of products carrying Nutri-score in addition to conventional nutritional information.

Furthermore, determining the cumulative average amount of saturated fats consumed on a daily basis through consumption of cooked sausages was performed. Based on the recommended daily intake of 2,000 kcal/day and the guideline stating that no more than 10% of daily kcal should come from saturated fats (*EFSA, 2010*), it is estimated that a maximum of approximately 22 g of saturated fatty acids (SFA) can be consumed daily to meet this criterion. The calculation for determining total saturated fat intake involved dividing the average saturated fat content in finely and coarsely ground cooked sausages expressed per 100 g of a product by 22 g.

Results and Discussion

Table 1 showcases the summarized results of the color-coded Nutri-scores awarded to cooked sausages obtained from the retail outlets in the area of Belgrade, Serbia.

Table 1. Number of meat products distributed among distinct nutritional grades within groupings of cooked sausages that are commercially available at retail outlets in Belgrade region, Serbia

Cooked sausage group		Total number of samples evaluated	Nutritional grade				
			A	B	C	D	E
Finely ground cooked sausages							
1.	Hot dog sausages	10	-	-	-	5	5
2.	Hot dog-type sausages (products marketed under a different name)	43	-	-	-	35	8
3.	Frankfurter sausages	1	-	-	-	-	1
4.	White sausages	1	-	-	-	-	1
5.	Bologna sausages	1	-	-	-	1	-
6.	Bologna-type sausages (products marketed under a different name)	52	-	-	2	46	4
Total number of finely ground cooked sausages		**108**	**-**	**-**	**2**	**87**	**19**
Total number of finely ground cooked sausages (%)		**100**	**-**	**-**	**1.9**	**80.5**	**17.6**
Coarsely ground cooked sausages							
1.	Mortadella	12	-	-	-	8	4
2.	Srpska sausage	2	-	-	-	-	2
3.	Tirolska sausage	1	-	-	-	-	1
4.	Tirolska-type sausage (under the name of Alpska sausage; products marketed under a different name)	18	-	-	-	11	7
5.	Products marketed under a different name	48	-	-	-	12	36
Total number of coarsely ground cooked sausages		**81**	**-**	**-**	**-**	**31**	**50**
Total number of coarsely ground cooked sausages (%)		**100**	**-**	**-**	**-**	**38.3**	**61.7**
Total number of cooked sausages evaluated		**189**	**-**	**-**	**2**	**118**	**69**
Total number of cooked sausages evaluated (%)		**100**	**-**	**-**	**1.1**	**62.4**	**36.5**

As noted by *Vlassopoulos et al. (2022)*, category C is the considered best possible class for processed meat products that include added animal fats and, with that, higher levels of saturated fatty acids, such as cooked sausages. The Nutri-Score is in line with the principles of the Mediterranean diet and the nutritional guidelines of several European countries, which emphasize moderation in the consumption of animal fats and a preference for certain types of vegetable oils (*Hercberg et al., 2021*). According to *Vlassopoulos et al. (2022)*, for meat products, in general, coming from the Greek market, the most common Nutri-Score was D for all subcategories, including sausages, with 57.1% having Nutri-Score D, and 42.9% having a score E. This observation is in accordance with the results of this research (Table 1), with around a third of the cooked sausages having the Nutri-Score D, while Nutri-Score C was only at 1.1%. Interestingly enough, both products from the Group C came from the same subgroup (Bologna-type sausages, finely ground cooked sausages) and the same manufacturer who reformulated Bologna sausages by adding fiber, hence increasing the positive outcome for the calculated Nutri-Score. *Rašeta et al. (2020)* conducted a study in Serbia that examined a total of 110 meat, minced meat, meat products, and semi-processed meat products available in retail stores in the Republic of Serbia. The calculated Nutri-Scores of these products revealed that approximately 41% were classified as Group D and 41.5% were classified as Group E, with bacon, dried and semi-dried fermented sausages, and coarsely ground cooked sausages contributing almost two-thirds to Group E. Nutri-Scores A (2.9%), B (1.6%) and C (13%) groups were mainly given to the fresh and minced meat, smoked meat products, canned meat chunks and meat dishes and dishes with meat, respectively. From all of the examined finely ground cooked sausages D category was the most prevalent (66.7%), while the rest of the products were categorized as having Nutri-Score E. These results majorly differ from the ones we obtained, as in our research, only 17.6% were in the red zone (E score). Just like the study conducted by *Rašeta et al. (2020)* that investigated the labels of coarsely ground cooked sausages and discovered that 73.3% of products belonged to Group E while only 26.7% belonged to Group D, we also observed that the majority of these products fall under the E category (61.7%), with only 38.3% falling under Group D. In both cases (finely and coarsely ground sausages), the results may differ because of the two reasons – first being the difference in a total number of examined products of these type (60 vs. 188), and second that could indicate that shifts are slowly being made in the meat industry in Serbia towards creating healthier products for the consumers.

It is noticeable that the number of products in the group under the name of „products marketed under a different name" have risen in the last decade compared to the products which are regulated under the names provided by the official classification by the *Official Gazette of RS (50/2019; 34/2023)*. *Kurćubić et al. (2012)* were able to find 14 Srpska sausages on the market, while we were able to find only two. This could be attributed to the newer legislation of Srpska sausage, which, unlike the previous versions of this document *Official Gazette of RS (94/2015; 104/2015; 19/2017)* had much fewer demands surrounding the composition of this product. Nevertheless, since the composition of Srpska sausage is narrowly defined by the Rulebook (as it is a product made from pork meat, namely: 20% meat emulsion, (prepared as 40% meat, 40% solid fatty tissue, and 20% water), and 80% minced pork meat and fatty tissue in which the proportion of meat is 80%, and solid fat tissue 20%, salt, nitrites, phosphates, antioxidants, and defined spices), improving the Nutri-score of Srpska sausages cannot be achieved easily since it contains, by default, large quantities of fat content (> 20%), and hence high content of saturated fatty acids and energy (kJ/100 g) which are two of five contributors to plus points leading to “bad” nutritional score. Moreover, according to *Đorđević et al. (2017)*, Srpska sausage has the great potential to receive protection from The Intellectual Property Office of the Republic of Serbia through trademark registration as well as the indication of geographical origin s.c. “Serbian quality” trademark. The introduction of an EU evaluative label, such as Nutri-Score, on traditional products is viewed by certain individuals as a potential threat to the authenticity of these products and to the preservation of cultural customs. The European Union acknowledges several types of "quality products" that are safeguarded by schemes like the protected designation of origin, protected geographical indication, or traditional specialty guaranteed. These schemes aim to preserve and promote the unique characteristics of specific products that are linked to their geographical origin and traditional knowledge passed down through generations (*European Commission, 2020; Delhomme, 2021*). However, these types of products usually have a tendency to be high in energy and frequently contain high amounts of fat, salt, or sugar. In Serbia’s case, this would mean that Srpska sausage, due to its formulation, will always be considered in the most undesirable Group E, similar to Italian Parma ham. Critics of Nutri-Score argue that implementing the system may create a market divide between "traditional products" that are challenging, if not impossible, to reformulate due to their legal composition specifications, and are therefore considered non-healthy foods (*European Commission, 2020; Delhomme, 2021*). Modifications in recipes by manufacturers to enhance the Nutri-Score could potentially lead to an amplified use of additives aimed at preserving taste and texture and prolonging the product's shelf life. Nonetheless, these additives are not taken into consideration when calculating the Nutri-Score. This could misguide consumers in the way that "processed foods," which can be easily subjected to the reformulation process, could be preserved as „healthy foods” (*Fedde et al., 2022*).

The categorization of cooked sausages as predominantly unhealthy can be attributed to the inherent characteristics and formulations of the meat products themselves (Figure 2). Reducing components that largely contribute to “bad” Nutri-score through recipe corrections is an essential task that the meat processing industry, paired with the newest scientific knowledge, consistently tackles along.

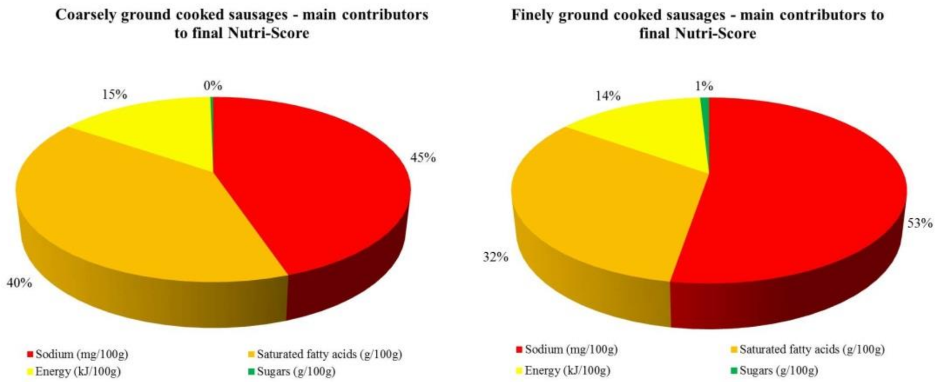

Figure 2. Major Nutri-score contributors of finely and coarsely ground cooked sausages

As shown in Figure 2, the need to reduce sodium and saturated fatty acids in meat products is crucial, given that cooked sausages are often categorized as "unhealthy foods" due to the significant presence of these components. In a previous study conducted by *Stamenić et al.* (*2021)*, it was highlighted that the reduction of sodium content is necessary for various categories of meat products, as the findings revealed that certain samples contained sodium chloride levels that approached the upper limit of the World Health Organization's recommended daily salt intake (5 g/day) per 100 g of meat product. Sodium chloride content among finely ground cooked sausages varied from 2.08 ± 0.36 g/100 g (expressed as Xsr ± Sd), whereas the average amount of NaCl found on nutritional labels as provided by manufacturers in coarsely ground cooked sausages was 2.27 ± 0.48g/100 g of product. The saturated fatty acid (SFA) content emerged as another significant nutritional parameter of concern. Multiple studies provide evidence of a positive association between the consumption of specific foods, particularly those high in cholesterol and SFA like meat products, and the incidence of various diseases such as cardiovascular diseases, dyslipidemia, obesity, and more (*Simopoulos, 2016; Patel et al., 2022*). As a result of these findings, the need to create new recommendations on fat intake has developed. Organizations such as the World Health Organization (*WHO, 2003*), and the European Food Safety Agency (*EFSA, 2010*) suggest an optimal intake (for healthy adults) of total fat that constitutes 20-35% of the total daily energy input (average Dietary Reference Intakes for healthy adults is 8400kJ/day or 2000 kcal/day), whereby less than 10% of energy should come from saturated fatty acids. In finely ground cooked sausages, the average SFA content was calculated to be 6.00 ± 2.29 g/100 g of products. Out of 108 products analyzed, nine of them (of which five belonging to the category of hot dog sausages) contained ≥10 g of SFA per 100 g of meat product. Similarly, for coarsely ground cooked sausages, the results indicated a variation in SFA content of 8.54 ± 0.48 g/100 g of products, with 19 products containing ≥10 g of SFA per

100 g of meat product. Cumulative average amount of saturated fats consumed on a daily basis through consumption of finely ground cooked sausages was calculated to be contributing from 17 – 40%/100 g of consumed product, with a maximum reaching 13 g of SFA/100g contributing with 65% of maximum recommended SFA daily intake. Similarly, for coarsely ground cooked sausages, the results indicated a variation in SFA content of 8.54 ± 0.48 g/100 g of products, with 19 products containing ≥10 g of SFA per 100 g of meat product, contributing to 37 – 41 % of maximum recommended SFA daily intake. Furthermore, the highest SFA content observed in this group of products was 14 g per 100 g of consumed cooked sausage, contributing to a substantial 67% of the maximum recommended daily intake of SFA.

Conclusion

The utilization of Nutri-Score and Front-of-Pack labels is not widely adopted in the food retail industry of the Republic of Serbia, particularly within the meat processing industry. Since the application of the Nutri-score represents a voluntary initiative of manufacturers, it does not come as a surprise that only 8 products, all coming from one large food retailer chain – Delhaize Serbia, including its two retailer-branded trademarks (Maxi and Premia) of the 189 examined pre-packaged cooked sausages had Nutri-Scoring scheme on the Front-Of-Pack label. The manufacturers' limited adoption of Front-Of-Pack labels and Nutri-Score on meat products can be attributed to various factors, including the additional resource requirements associated with implementing such labeling. Given that the adoption of the Nutri-Score system in EU countries represents a significant measure in facilitating consumer choices towards healthier options, the Serbian domestic meat industry must undertake targeted and dedicated endeavors to enhance the production of cooked sausages. This urgency arises from the fact that a substantial 98.9% of retail cooked sausages were categorized within Groups D and E (falling in the dark orange and red zones) according to the Nutri-Score method, signifying their classification as unhealthy. The meat industry is recommended to focus on adopting newer technological solutions and giving special consideration to reducing the levels of challenging nutritional aspects, such as sodium, saturated fatty acid content, and overall energy balance in meat products like finely and coarsely ground cooked sausages, which offer great potential for nutritional score enhancement through relatively straightforward reformulation methods. To date, as to the authors' knowledge, no survey has been conducted in Serbia regarding consumers' attitudes towards Front-of-food labeling and incorporation of the Nutri-Score scheme and its impact on their decision-making process when purchasing products.

Nutritivna evaluacija barenih kobasica dostupnih na tržištu Republike Srbije primenom nutri-score metodologije

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Rezime

Poboljšanje nutritivnog kvaliteta hrane predstavlja priznatu strategiju u cilju prevencije hroničnih nezaraznih bolesti (HNB), i predstavlja jedan od primarnih fokusa politike javnog zdravlja širom sveta. Nutri-skor predstavlja logo lociran na prednjoj strani pakovanja i predstavlja kobminaciju boja i slovnih obeležja (A, B, C, D, E), pri čemu služi kao sredstvo za pomoć potrošačima za brzu identifikuju zdravijih alternativa u okviru iste grupacije hrane. Ima pozitivan uticaj u smislu svesti potrošača, percepcije, razumevanja i ponašanja prilikom kupovine i potencijalno doprinosi smanjenju prevalencije HNB. Cilj ovog istraživanja je bio da se proceni nutritivni skor fino i grubo usitnjenih barenih kobasica dostupnih na tržištu Srbije. Analizirano je ukupno 189 upakovanih barenih kobasica iz osam najvećih lanaca supermarketa u beogradskom regionu Republike Srbije. Barene kobasice su ocenjene primenom metodologije Nutri-skor sistema i shodno tome, smeštene u odgovarajuće kategorije. Rezultati skrininga tržišta su ustanovili da samo 1,1% barenih kobasica spada u Nutri-skor grupu C, dok je 62,4% klasifikovano u grupu D, a 36,5% u grupu E. Smanjenje natrijuma i zasićenih masnih kiselina, činioca koji u najvećoj meri doprinose nepovoljnim nutritivnim karakteristikama barenih kobasica, predstavlja ključni korak usled njihovog znčajnog prisustva u ovim proizvodima koja dovodi do njihovog obeležavanja kao „nezdrave hrane".

Ključne reči: nutritivni skor, deklarisanje namirnica, barene kobasice, zasićene masne kiseline

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DETECTION OF NON-MILK FAT ADDITION IN KAJMAK PRODUCTION

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Abstract: Kajmak is a very popular dairy product in Balkan countries, however, it is often a subject of adulteration. The aim of this study was to investigate the effects of kajmak adulteration on its yield and composition, and determine the possibility of detecting the adulteration using the iodine and acid values and sensory evaluation of kajmak quality. In this study, experimental kajmak samples were produced from milk with increased fat contents (by 18 % and 36 %), via the addition of refined palm oil, pig fat and margarine to milk.
The experimental kajmak samples had increased yield (21-32 %), changed composition and sensory quality. The control had the lowest iodine value (35.60), while the experimental kajmak with pig fat had the highest value of this constant (49.95), so the chemical constant - iodine value can be useful for detecting of adulterated milk fat in kajmak with refined palm oil, pig fat and margarine. However, these adulterants did not lead to a significant change in the acid value of the kajmak samples. The adulterants - refined palm oil and margarine in experimental kajmak samples could not be detected by sensory analysis.

Key words: adulteration, milk fat, kajmak, iodine value, acid value

Introduction

Kajmak is a specific fat based dairy product which is produced in regions of Southeast Europe, and some Asian regions such as Turkey, Iran, Afghanistan and India. According to its composition and characteristics, kajmak is classified as somewhere between cheese and butter (*Pudja et al., 2008*). Due to the distinct diversity of production methods and conditions in different regions a very large variations in the composition, quality and properties of kajmak often are found (*Cakmakci and Hayaloglu, 2011; Akarca et al., 2014; Mirecki et al., 2017*). In Balkan countries, kajmak is highly valued product and consider as an autochthonous dairy product, which is usually manufactured from cow milk by the traditional method (*Dozet et al., 2017*).

Kajmak production begins with heat treatment and pouring of the hot milk in shallow vessels followed by spontaneous cooling of milk by surrounding air for 12 or more hours (*Dozet et al., 2004*). The kajmak formation process can be split into two phases: hot and cold phase (*Pudja et al., 2008*). At the beginning of the hot phase, milk fat and proteins are concentrated on the top of the milk forming a kind of thin matrix layer. During the cold phase, the kajmak skin layer is growing up, due to the incorporation of milk components, mostly milk fat (*Radovanovic et al., 2013*). When the cold phase completes, a layer on top of milk, known as kajmak is collected. The kajmak formation process and quality are affected by several factors such as composition and heat treatment of milk; and ambient air parameters - temperature, relative humidity and air circulation dynamic (*Pudja et al., 2008*).

Milk products based on milk fat such as ghee and butter, are highly prone to milk fat adulteration with cheaper oils and fats (*Derewiaka et al., 2011; Kala, 2013; Uysal et al., 2013; Gandhi et al., 2015; Nedeljkovic, 2019*). Adulteration of milk fat with cheaper oils and fats has been often followed without labeling the product appropriately. According to *Ntakatsane et al. (2013)*, such a situation poses a risk to human health and decreases functional value of the product.

The specific process of kajmak manufacture and the growing demand placed it in the premium price group of products, making it attractive for adulteration. Milk fat, as the paramount constituent of the kajmak, possesses good flavour, pleasant aroma, high caloric value, and represents a source of valuable nutrients such as fat-soluble vitamins and essential fatty acids (*Truong et al., 2020*). Cheaper vegetable oils and animal fats (ex. pig fat) are often used as admixtures to replace a part of the milk fat and reduce the cost of kajmak production. However, the legislation requires that this product not contain any oils or fats of non-milk origin *(Official Gazette of the Republic of Serbia No. 34/2014).*

Studies related to the detection and quantification of foreign oils and fats in dairy products have been conducted for many decades. Detection of the adulterants in milk fat includes number of methods such as: Methods based on physical properties (melting point, opacity test, differential scanning calorimetry etc.); Spectroscopic methods (tests based on visible, infrared and Raman spectroscopy); Methods based on chemical properties (tests based on chemical constants - iodine value, Reichert-Meissl value, Polenske value; tests based on fatty acid profile using gas chromatography and tests based on the identification of DNA in milk fat) etc. (*Sharma et al., 2020*). Some of the methods require expensive equipment and trained staff, especially infrared and Raman spectroscopy, which have been widely used for years to characterize oils and fats. In contrast, the determination of chemical constants is fairly simple and relatively inexpensive tests are still used in practice. Furthermore, according to *Dyminska et al. (2017)*, there is a good correlation between spectral parameters and the iodine value.The number of grams of iodine absorbed by 100 g of fat under specified conditions represents the iodine value i.e. iodine number (*Sharma et al., 2020*). This constant is a measure of unsaturated linkages present in a fat. The iodine value for milk fat is lower in comparison to most of the other fats and oils except coconut oil (6-10) and palm kernel oil (10-18) (*Hazra et al., 2017*).

The acid value (acid number) is the quantity of base, expressed in milligrams of potassium hydroxide, which is required to neutralize all acidic constituents present in 1 g of sample (*ISO 660, 2009*). The acid value is a common parameter in the specification of fats and oils (*Koczon et al., 2008*). According to *Nielsen (2017*), acid value of oils and fats are dependent on different factors such as free fatty acids (FFA), acid phosphate and conditions of the storage. The acid value of pure milk fat is 0.48 (*Park et al., 2007*). According to the *Codex Standard for edible fats and oils (1999)* and to the current Serbian legislation *(Official Gazette of the Republic of Serbia No. 43/2013),* the acid value of refined oils should be up to 0.6.

The aim of this study was to investigate the effects of the kajmak adulteration with non-milk fats (vegetable oil, animal fat and margarine), on its yield, composition, chemical constants (iodine and acid values) and sensory quality, as well as, to determine whether or not it is possible to detect the adulteration of kajmak using the chemical constants and sensory quality evaluation of the kajmak samples.

Material and Methods

Production of control and experimental kajmak samples

For each trial, 21 kg of raw milk (3.6% fat and 3.4% proteins, obtained from dairy farm "PIK Zemun", Serbia) was portioned into seven 3 kg lots. Each lot was heat treated at 85°C for 10 min followed by 95°C for 10 min treatment – for production of both control and adulterated kajmak (experimental samples). The control sample was prepared without adding non-milk fats.

Six experimental samples were prepared by adding non-milk fats to milk at two different levels: 20 g and 40 g per lot, i.e. 6.7g/kg and 13.3 g/kg that represent an increase in fat content of 18% and 36% respectively (Tab. 1).

Table 1. Production of control and experimental kajmak samples

Kajmak	Non-milk fats	
	Amount added to milk, (g/kg)	
MM	Control sample	0.0
PU1	Palm refined oil	13.3
PU2		6.7
SM1	Pig fat	13.3
SM2		6.7
MG1	Margarine	13.3
MG2		6.7

The following non-milk fats were added: refined palm oil imported from Malaysia (d.o.o. "Uvita", Debeljaca, Serbia); pig fat ("Backa mast" Univerexport group Backa ad, Backa Palanka, Serbia); and margarine ("Vital", Vrbas, Serbia) (Tab. 2).

Table 2. Iodine and acid values of non-milk fats used for experimental kajmak production

Non-milk fats	Iodine value	Acid value
Palm refined oil ("Uvita")	55.82	0.11
Pig fat ("Backa mast")	62.99	1.91
Margarine ("Vital")	58.30	0.22

During the production of experimental samples - non-milk fats were added to milk at a temperature of 85°C in order to evenly melt and incorporate. After that, the milk was gently mixed for 3 minutes with a mixer and heated up to 95°C to complete the heating regime. The heat-treated milk samples were poured into round vessels where kajmak formation took place (dimensions 0.24 x 0.07 m) over 24 hours at room temperature imitating typical conditions in the traditional kajmak production, after which the kajmak was collected from the surface of the milk. The temperature of milk during collecting kajmak was 15°C. The kajmak samples were weighed and analyzed 5 min after drainage on the grid. The experiments were carried out in triplicates.

Analytical analyses
Kajmak composition

Kajmak samples were analyzed for fat by butyrometric method (*FIL-IDF, 1986)*, total nitrogen content was determined by Kjeldahl method *(AOAC, 1990*) on a Kjeltec System (Tecator 1002, Sweden), and total protein content was calculated by multiplaying it with 6.38. Dry matter (DM) was determined by drying method at 102±2ºC *(FIL-IDF, 1982).* Fat in dry matter (FDM), protein in dry matter (PDM), absolute amounts of fat and proteins in kajmak and the relative ratio of fat to protein (fat x 100/(fat + protein) were also calculated. All analysis were carried out at least in duplicate.

Determination of chemical constants (iodine and acid values)
Sample preparation

Approximately 100 g of kajmak was melted at 70ºC and centrifuged at 2400 g/10 min (centrifuge model 5430; Eppendorf AG, Hamburg, Germany). Then the fat layer was removed and the residue was filtrated using cheesecloth. Finally, the cheesecloth was gently squeezed to extract every drop of liquid. The filtrate was reheated to 70ºC and centrifuged at 2400 g/10 min. The clear fat supernatant was subjected to further examinations (iodine and acid values).

Determination of iodine value

Iodine value of the kajmak samples was determined by Wijs method *(ISO 3961, 2009)* and performed according to *Dyminska et al. (2017).* The prepared kajmak sample was filtered through anhydrous Na_2SO_4 (Merck, Germany). The filtered kajmak sample was weighed (0.5500 – 0.6000g) into an erlenmeyer flask and 20 ml of reagent (cyclohexane: glacial acetic acid, 1:1) (Sigma-Aldrich, France) was added and the solution was mixed well. Then 25 ml of 0.1 mol/l Wijs-solution (Carl Roth, Germany) was added. Erlenmeyer was closed with a lid and placed in the dark for 60 minutes. After the reaction time, 20 ml of KJ (10% solution) was added. The sample was diluted with 150 ml of deionized water and titrated with 0.1 mol/l $Na_2S_2O_3$ until the solution became light orange. Then 10 drops of 2% starch solution were added and the titration was considered completed once the blue color disappeared. The Blank probe was prepared by the same way, but without the kajmak sample, and the blank value was determined as well.
Calculation:
Iodine value, (g/100g) = $((V_0-V_1) \times C \times 12.69)/m$

Where:

- $(V_0\text{-}V_1)$ is the difference between the volumes, in ml, of $Na_2S_2O_3$ required for the Blank probe and for the sample, respectively;
- C is the molarity of $Na_2S_2O_3$ solution, (mol/l);
- 12.69 is the conversion factor from mol $Na_2S_2O_3$ to grams of iodine (the molecular weight of iodine is 126.9 g/mol);
- m is the weight of the sample, (g).

Determination of acid value

Determination of acid value of kajmak was done with KOH ethanol solution and was performed according to method of *Koczon et al. (2008)*. The solvent (diethylether: ethanol absolute, 1:1) (Honeywell, Germany) was prepared by titrating it with 0.1 mol/l KOH in the presence of phenolphthalein as an indicator.

The sample of kajmak was weighed (about 10g) into an erlenmeyer flask and dissolved in 70 ml of the prepared solvent. After complete dissolution, the sample is titrated with 0.1 mol/l KOH. For the Blank probe, 70 ml of solvent was placed in an erlenmeyer flask and titrated with 0.1 mol/l KOH.

Calculation:

Acid value (mg KOH/g) = $((V_1 – V_0) \times 56.1 \times C)/m$

Where:

- V_1 is volumes of KOH required for the sample titration, (ml);
- V_0 is volumes of KOH required for the Blank probe, (ml);
- C is the molarity of KOH solution, (mol/l);
- 56.1 is the conversion factor from 1 ml of solution to mol/l KOH (the molecular weight of KOH is 56.1 g/mol);
- m is the weight of the sample, (g).

Sensory quality evaluation

Sensory quality evaluation was performed according to the slightly modified method of *Koca and Metin (2004)* using a five-level quality scoring method as follows: excellent quality (quality score > 4.5); very good quality (3.5 <

score ≤ 4.5); good quality (2.5 < score ≤ 3.5); poor/unsatisfactory quality (1.5 < score ≤ 2.5); very poor quality (0.5 ≤ score ≤ 1.5); spoiled product/not for human nutrition (0 ≤ mean score < 0.5). The five-level quality scoring was conducted by a sensory panel consisting of six members from the University of Belgrade, Faculty of Agriculture who were experienced in dairy product quality judging.

Overall sensory quality was assessed by evaluating four selected characteristics: appearance (including color), smell, flavor and texture. According to the individual impact on overall quality, the selected characteristics were assigned appropriate coefficients of importance: 3, 2, 6, and 9, respectively.

The quality of kajmak was expressed as a percentage of the total quality (from 0 to 100%) and was obtained by adding up the multiplied scores of every assessed characteristic of kajmak and appropriate coefficient of importance.

Statistical analysis

Data were analyzed in order to investigate the influence of palm oil, pig fat and margarine on the composition and yield of the produced experimental kajmak samples by using STATISTICA 6.0 (StatSoft, USA) data analysis software. Mean comparisons of the parameters were performed by LSD-test, with the levels of significance at 0.05.

Results and Discussion

Kajmak yield and composition

The increase in the content of fat in milk by adding non-milk fats (for 18% and 36%) led to an increase in the yield of kajmak (Tab. 3). Higher amounts of added non-milk fats (PU1, SM1, MG1) increased the fat content of milk by about 36%. These amounts of the fats resulted in an increase in the yield of kajmak by about 30% in the kajmak samples made with added pig fat and margarine ($P < 0.05$), while the addition of palm oil resulted in a slight increase in the yield of kajmak, which was not statistically significant ($P > 0.05$) (Tab. 3).

Lower amounts of added non-milk fats (PU2, SM2, MG2) increased the fat content of milk by about 18%. In these kajmak samples, the yield was statistically significantly increased ($P < 0.05$) in all samples with added the fats compared to the control. The yield increase was about 22% for all experimental samples – 21% with palm oil; 24% with margarine; 25% with pig fat (Tab. 3).

Table 3. Yield of kajmak samples

Kajmak	Yield, (g)	Kajmak	Yield, (g)
MM	136.79[a]	MM	136.79[a]
PU1	139.88[a]	PU2	165.50[b]
SM1	180.58[b]	SM2	170.66[b]
MG1	177.03[b]	MG2	169.70[b]

Values with different letters within the same column are significantly different ($P < 0.05$)

Milk fat was the dominant component in all kajmak samples. Increasing of fat content in milk (from 18% to 36%) contributed to the increase of % fat and % DM, as well as % FDM of kajmak samples. At same time, the increase in fat content in milk resulted in a slight decrease in % protein of the kajmak samples as well as % PDM (Tab. 4).

Table 4. Kajmak samples composition

Kajmak	Fat, (%)	Protein, (%)	DM, (%)	FDM, (%)	PDM, (%)
MM	57.50	3.93	63.97	89.26	6.10
PU1	64.00	3.89	73.87	90.91	5.53
PU2	60.75	4.03	71.25	89.72	5.95
SM1	65.00	3.75	71.77	93.30	5.38
SM2	57.00	3.90	63.08	86.42	5.91
MG1	60.50	3.95	67.79	91.23	5.96
MG2	59.00	3.92	67.27	89.84	5.97

The addition of the non-milk fats improved fat separation and increased kajmak yield. Experimental kajmak samples prepared with lower amounts of the added fats (PU2, SM2, MG2), had the percentages of fat and protein practically unchanged compared to the control sample. However, the absolute amounts of fat and protein were higher, so the yields were higher compared to the control sample of kajmak (Tab. 4, Fig. 1). The relative ratios of fat to protein were identical for all kajmak samples and ranged from 93 to 94 % for both percentage and absolute amounts (Fig. 1 C, D). With added higher amounts of the fats (PU1, SM1, MG1), this regularity was not established (Fig. 1 A, B).

The obtained kajmak samples were not compact, which was quite pronounced in kajmak produced with the added refined palm oil. In this case, during the kajmak collection, a considerable amount of the lower layer of kajmak was separated and lost, which affected the reduction of the fat content and the yield of the final kajmak. It is likely that a lower temperature when removing the kajmak from milk, would reduce these defects to a certain extent and contribute to a higher yield and compactness of the lower layer of the formed kajmak.

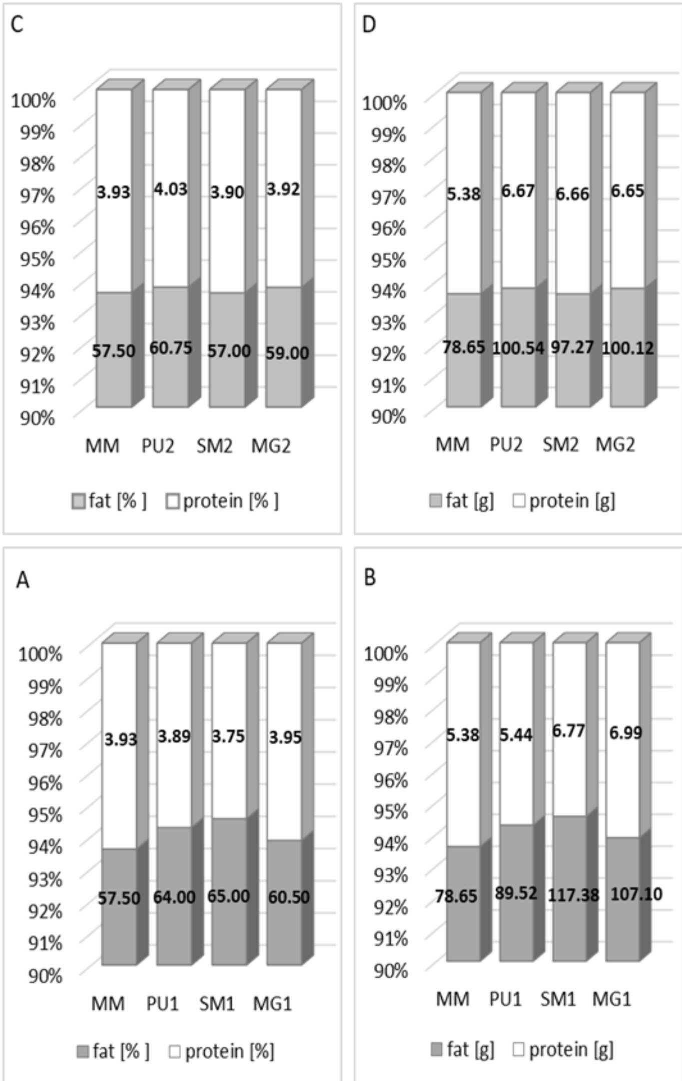

Figure 1. The relative ratios of fat to protein of kajmak samples

(The relative ratios of fat to protein for their percentage (A, C) and absolute amounts (B, D))

Iodine and acid values

The iodine value of milk fat in the control kajmak sample was 35.60, which is in accordance with the literature data for the iodine value of pure milk fat *(Gandhi et al., 2015; Salem et al., 2019; Sharma et al., 2020).* According to *Bolton (1999)* pure milk fat contains about 35% of total unsaturated acids, most of which consists of oleic acid. Because of that the iodine value for milk fat ranges from 26 to 35, which is lower in comparison to most of the other fats and oils *(Hazra et al., 2017*).

Animal body fats show slightly higher iodine values ranging from 36 to 49. However, for vegetable oils, the iodine value is very high (74-145), while for hydrogenated oil fats, it lies in the range of 70 to 79 *(Hazra et al., 2017)*. According to *Tarmizi et al. (2008)* iodine value of refined palm oil is about 53, while for margarine it is about 56.88 *(Kahyaoglu and Cakmakci, 2016).* In accordance with our findings for obtained kajmak data in Table 6, it could be observed that the control kajmak sample had the lowest iodine value (35.60) among all the kajmak samples. On the other hand, the kajmak adulterated with pig fat had the highest iodine value (49.95) compared to the other kajmak samples.

Based on these results, it can be concluded that the iodine value is easily derived and can be successfully used as a screening test to detect the adulteration of milk fat in kajmak with refined palm oil, pig fat and margarine.

In agreement with our findings for kajmak, *Kahyaoglu and Cakmakci (2016)* reported that iodine value could be reliably used to discriminate pure butter from butter adulterated with the two margarine types in various mixing ratios. Also, according to *Metin (2008)* the iodine value of the milk fat increases with the addition of vegetable fats, except coconut oil *(Salem et al., 2019).* Additionally, it was found that iodine values in adulterated ghee are increasing proportionally to the level of added palm oil *(Kauser et al., 2022).*

According to *Salem et al. (2019),* acid value of pure milk fat is 0.45, while for butter it is in interval from 0.45 to 0.70 *(Koczon et al., 2008).* Our result of acid value for milk fat in the control kajmak sample was 0.61. However, there were no significant differences in acid values between differently adulterated kajmak samples (Tab. 5), so the chemical constant - acid value is not suitable for determining adulterated milk fat in kajmak with oils and fat used in this study.

Table 5. Iodine and acid values of kajmak samples

Kajmak	Iodine value	Acid value
MM	35.60	0.61
PU1	40.76	0.64
SM1	49.95	0.61
MG1	45.49	0.57
PU2	38.16	0.54
SM2	42.67	0.69
MG2	40.07	0.64

Sensory quality evaluation

Samples of kajmak adulterated with palm oil and margarine (PU2, MG2) were as high evaluated as the control sample MM. On the other hand, the lowest sensory quality was assigned to the samples adulterated with pig fat for all quality parameters, due to the prevalence of the untypical smell of the pig fat (Tab. 6).

Determination of authenticity and detection of adulteration of dairy products have been performed by a number of analytical methods previously, and sensory analysis is recognized as one of them *(Kamal and Karoui, 2015).* Several authors considered the possible detection of adulterated dairy products on the basis of their sensory properties. The results of a research conducted to detect and discriminate fresh cheeses adulterated with different ratios of vegetable fat using sensory analysis, showed that the power of discrimination was high ($P<0.05$) in each panelist and panel level for all attributes evaluated *(Juarez-Barrientos et al., 2019).* In our study, the fact that samples adulterated with palm oil or margarine (PU2, MG2) were highly evaluated as the control sample suggests that these adulterants may not be detectable by consumers.

Table 6. Quality of kajmak samples

Kajmak	Quality, (%)	Description of kajmak samples
MM	87	Typical pale-yellow color; typical smell; typical structure with a little separated serum
PU1	55	Typical pale-yellow color; typical smell; the kajmak is not compact, it is disintegrated; a larger amount of serum is extracted compared to other kajmak samples
PU2	86	Typical pale-yellow color; typical smell; the kajmak is compact, a larger amount of serum is released, but less compared to PU1
SM1	57	The color is lighter compared to the other samples; the smell is not typical, it smells like pig fat; the kajmak is compact during extraction, a larger amount of serum is separated compared to the control sample
SM2	53	The color is lighter compared to the other samples; the smell is not typical, it smells like pig fat; the kajmak is compact during extraction, a typical amount of serum is separated
MG1	80	Pronounced yellowish color of the kajmak; typical smell; the kajmak is compact and firm; a small amount of serum is separated;
MG2	86	Pronounced yellowish color of the kajmak; typical smell; the kajmak is compact and firm; a small amount of serum is separated;

Conclusions

Kajmak samples were prepared by adding non-milk fats to milk at two different levels, representing an increase in milk fat content by 18% and 36%. The kajmak samples produced from milk with an increased fat content of 18% had an increased yield (21-25%) and an increased content of absolute amounts of fat and proteins while their relative ratios remained unchanged. The kajmak produced from milk with an increased fat content of 36% had an increased yield of max. 32%. It was demonstrated that the chemical constant - iodine value can be useful for the detection of the adulterated milk fat as a screening test in all kajmak samples.

The kajmak samples adulterated with palm oil and margarine were sensory highly scored indicating that these adulterants could not be detected by consumers. In the further research, it would be interesting to investigate the effects of the range of concentrations considering these adulterants to establish the sensory detectable sensitivity threshold.

Detekcija dodavanja ne-mlečnih masti u proizvodnji kajmaka

Mira Radovanovic, Marina Hovjecki, Sanja Djurdjevic, Andjelija Stanojevic, Jelena Miocinovic

Rezime

Kajmak je veoma popularan mlečni proizvod na području Balkana i Bliskog istoka, mada je često predmet falsifikovanja. Cilj ovog rada je bio da se ispita uticaj falsifikovanja mlečne masti kajmaka na randman i hemijski sastav kajmaka, kao i da se ispita mogućnost detekcije falsifikovanja pomoću jodnog i kiselinskog broja, kao i senzorne analize kvaliteta kajmaka. Eksperimentalni uzorci kajmaka su pripremljeni dodavanjem ne-mlečnih masti (rafinisano palmino ulje, svinjska mast i margarin) u mleko za proizvodnju kajmaka, u količinama od 18% i 36%. Rezultati su pokazali da su eksperimentalni uzorci kajmaka imali povećan prinos (21-32%), izmenjen sastav i senzorni kvalitet. Kontrolni uzorak kajmaka je imao najnižu vrednost jodnog broja (35,60), dok je eksperimentalni uzorak sa svinjskom mašću imao najvišu vrednost (49,95), te se može zaključiti da se jodni broj može koristiti za detekciju falsifikovanja mlečne masti kajmaka. Vrednosti kiselinskog broja ispitivanih uzoraka kajmaka nisu bile značajno različite. Senzornom analizom kvaliteta kajmaka nije se moglo ustanoviti falsifikovanje mlečne masti rafinisanim palminim uljem i margarinom.

Ključne reči: falsifikovanje, mlečna mast, kajmak, jodni broj, kiselinski broj

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ASSOCIATIVE EFFECTS OF A MIXED BRACHIARIA DECUMBENS AND PENNISETUM PURPUREUM GRASS FEED ON THE ENSILING PROPERTIES

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Abstract: This study aimed to evaluate the effects of the association between two tropical grasses, Signal grass (*Brachiaria decumbens*) and Napier grass (*Pennisetum purpureum*) on the proximate composition, in vitro digestibility, and fermentation characteristics of ensiled material. A complete randomized design was used on three treatments namely Signal grass, Napier grass, and Signal-Napier grass combination. Silage was made using molasses applied in proportions of 1:2 with water and mixed with silage at the rate of 5% for a 5kg bag of 2.5cm cut grass. Signal-Napier grass combination silage was superior in almost all parameters to the two sole crop silages. After ensiling, a significant difference ($p<0.05$) in most parameters was recorded. A significant difference ($p=0.0004$) in pH was determined where pH was lower in the sole Napier grass silage than that of the Signal-Napier combination and Signal grass. A similar outcome ($P<0.05$) was recorded for crude protein, ash, ether extracts, nitrogen-free extracts, and neutral detergent fiber. However, no significant difference ($p>0.05$) was obtained in dry matter ($p=0.1524$), crude fiber ($p=0.5924$), and ADF ($p=0.1168$). Although having poor digestibility values in all treatments, Signal grass proved to be better than the rest. Organoleptic characteristics were promising, with normal color, smell, and texture changes observed. These results indicated that the association of grasses had an impressive positive effect on the nutritional value and quality of silages. Therefore, the use of mixed grass silages is encouraged.

Key words: Napier grass, Signal grass, molasses, Signal-Napier grass, silage, forage

Introduction

Climate change has led to higher-than-normal temperatures, inconsistent rainfalls, and shorter rainfall seasons (*Rama et al., 2022*). Because of poor plant productivity arising from these extreme weather conditions and a shift in seasonality, good-quality pastures are rapidly declining as productivity is inversely related to consumption (*FAO, 2015*). Such is the case of Napier grass which livestock heavily depend upon. Against this background, it is therefore important to consider alternative feed sources like silages for the animals.

Silages are any crop residues, agricultural or organic industrial by-products, preserved in the absence of air by artificial or natural acidification and used for animal feed, especially in winter (*Moran, 2005*). Fermentation improves the quality characteristics of the plant which includes acceptability and palatability (*FAO, 2012*). Silage is an important food source for farm animals especially during the dry season contributing over 50% of nutrition on the farm (*Khan et al., 2021*). Its use is spurred by the significant negative impacts caused by climate change on plant productivity worldwide (*Lone et al., 2017*).

In modern animal husbandry, harvesting of forage crops is done when they are at maximal yield and nutritional value. Grasses are also used in the manufacture of silages. Signal grass (*Brachiaria decumbens*) and Napier grass *(Pennisetum purpureum)* are perennial grasses found in most semi-arid and humid areas and are readily available resources that can be used for the provision of livestock feeds *(Cook et al., 2005)*. Furthermore, these grasses are easy to establish, making them beneficial in smallholder communities.

Napier grass is a high-yielding green fodder providing an excellent nutrition for cattle. Besides yield, Napier has crude protein ranging from 17 to 18 percent (*Orodho, 2006*). It is very palatable because the stalks are tender. The leaves are smooth and hairless and animals enjoy their sweet juice (*Randa et al., 2017*). On the other hand, Signal grass is aggressive and has the ability to grow well under varying soil and moisture conditions (*Muniandy et al., 2019*). It is a low-growing leafy grass with an erect or trailing habit. Despite the excellent productivity of Signal grass, it contains chemicals that can damage the liver and cause skin photosensitization in cattle and sheep if consumed in excess (*Awad et al., 2012*). Due to its low crude protein of around 8% (DM), it becomes imperative to improve the nutritional status of Signal grass through association with highly nutritious grasses or legumes (*Fisher and Kerridge, 1996; Muniandy et al., 2019*).

This study, therefore, tested the hypothesis that mixing Signal grass and Napier grass leads to better proximate composition, in vitro organic matter digestibility, and fermentation characteristics of ensiled material than fermenting the same species individually.

Materials and Methods

Study area

The research was carried out at the Grasslands research station in Marondera. The Research station is located in the Highveld and its latitude is 18^{o} 11’, longitude 31^{o}28’ E, and altitude 1630m. The mean day length is around 13 hours during the summer down to 11 hours during winter. The area receives an average annual rainfall of 873 mm and the hot summer days stand between September and December with October identified as the hottest month of the year having maximum temperatures above 30^{o}C. Soils are mostly acidic (pH 4.5) deep brown, fine-loamy kaolinitic thermic derived from granite (*Mapiye et al., 2006*). Large and small ruminant production is practiced with cattle, goats, and sheep being the most dominant.

Experimental design and layout

For this study, Signal grass and Napier were left to grow under natural pasture rangeland conditions. To ensure optimal nutritional quality, the grasses were harvested during their vegetative growth stage (2 months from establishment), while still juicy and prime (Table 1) as recommended by *Johansson (2010)*. The grasses were harvested at random across the field, pre-wilted for 24 hours, cut into 2.5 cm particles using a machete, and then put into micro-silos for ensiling.

Table 1. Chemical composition of the different forage grasses before ensiling

Variable	Treatments			SED	Contrasts Anova (P- value)
	Signal grass	Napier grass	Signal-Napier combination		Trt[2]
Dry matter	86.40	87.00	87.33	0.7694	0.5096
Crude protein	8.10^{b}	7.40^{c}	9.90^{a}	0.7462	0.0017
Crude fiber	29.07^{b}	31.85^{a}	33.52^{a}	0.8824	0.0066
Ash	7.62	6.82	7.69	0.3272	0.0676
Ether Extracts	1.25^{b}	2.32^{a}	2.55^{a}	0.0577	0.0001
pH	6.30	6.10	6.10	0.0667	0.0787
NFE	40.30^{a}	45.20^{a}	46.60^{a}	0.9549	0.9322
NDF	65.50	67.90	67.20	0.7126	0.9929
ADF	23.60	24.50	24.70	0.3382	0.9983

Means within a row followed by a different superscript are significantly different ($P < 0.05$)
[1]SED=Standard Error of Difference.[2]Trt = Treatment

Three treatments were under study: Napier grass, Signal grass, and a Signal-Napier grass combination. For each treatment, 5kg of forage was used, and molasses was added at a rate of 5%, diluted with water at a rate of 1 part molasses to 2 parts water, and mixed thoroughly with the forage. To make micro-silos, the forage mixed with molasses was then placed in polythene bags, carefully avoiding any holes, and gently squeezed to let the air out before tightly sealing the bags with strong duct tape to create an anaerobic environment. All bags containing each treatment were stored in a storeroom at room temperature for 8 weeks. After 8 weeks, the grass in the bags was premixed independently, and silage samples were collected for silage quality and proximate analysis.

Sampling procedures

The silage bags were opened 62 days after ensiling. Two samples weighing 200g were taken from each ensiling bag for each replicate. The first sample was used for pH analysis, and the second was for dry matter, crude protein, crude fibre, and ash.

Data collection

Proximate analysis was done on the samples from each treatment before ensiling and after 8 weeks of ensiling to obtain the chemical properties of the feed. The samples were analysed for dry matter (DM). Ash, crude protein (CP), and crude fibre (CF) according to *AOAC (2016)* procedure. ADF and NDF were obtained through the Van Soest nutrient determination (*Van Soest and Wine, 1967; Van Soest, 1990*). The pH of the silage was measured using a digital pH meter. Other qualities which include, smell, texture, and colour were obtained through the organoleptic test (*Randa et al., 2017*). Percentage spoilage was recorded upon opening the silage bags.

Chemical Analysis

The pre-dried original samples and ensiled samples were analyzed for dry matter content, crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), and ether extracts (EE). Dry matter (DM) determination samples were oven dried for 72 hours at 65^{o}C until constant weight. Ash determination samples were burned at 600^{o}C until constant weight using a muffle furnace. Burnt samples were then used to determine individual minerals. The Kjeldahl method was used to analyze crude protein (CP). Samples were digested in hot concentrated sulphuric acid and N was liberated as ammonia. Neutral detergent fiber (NDF) was tested by boiling the forage in a neutral detergent solution and measuring the insoluble residue (cell wall contents). Acid detergent fiber (ADF) determination samples were digested in an acid detergent solution hemicellulose was dissolved leaving cellulose and lignin. Ether extracts (EE) were determined by passing hot petroleum ether through a feed sample, dissolving crude fat, the solvent evaporated, and the remainder being Ether Extracts.

Statistical analysis

The data for a randomized complete design was analysed using the one-way ANOVA incorporating the multiple comparisons tests on GraphPad Prism software (2020 version). Treatment means were compared to the Signal-Napier combination and the means were separated using Tukey's studentized Range at a 0.05 significance level (P<0.05).

Results

Chemical composition after ensiling

Statistical analysis of the dry matter showed that there was no significant difference among the three treatments, Napier grass, Signal grass, and Signal-Napier grass combination. However, the result (Table 2) shows that the Signal-Napier grass combination had 1.01% and 0.35% higher dry matter than both Napier and Signal grass respectively. CP content of both sole silages (Napier and Signal grass) was lower than that of the Signal-Napier grass combination.

Table 2. Chemical composition of the different silages after ensiling

Variable	Treatments			SED	Contrasts Anova (P- value)
	Signal grass	Napier grass	Signal-Napier combination		Trt[2]
Dry matter	83.69	83.03	84.04	0.4452	0.1524
Crude protein	9.47[b]	8.51[c]	10.40[a]	0.1834	0.0001
Crude fiber	28.24	29.96	28.09	0.8709	0.5924
Ash	9.67 [b]	12.00[a]	12.00[a]	0.3175	0.0005
Ether Extracts	1.21[c]	1.91[b]	2.14[a]	0.0361	0.0001
pH	4.30[a]	3.80[b]	3.90[b]	0.0882	0.0004
NFE	33.65[c]	35.52[b]	37.73[a]	0.6206	0.0018
NDF	61.33[b]	63.62[a]	64.19[a]	0.8557	0.0342
ADF	22.78	23.62	23.88	0.4590	0.1168

Means within a row followed by a different superscript are significantly different (P < 0.05)
[1]SED=Standard Error of Difference.[2]Trt = Treatment

Table 2 shows that the Signal-Napier grass combination had a CP that is 0.93% higher than Signal grass and 1.89% higher than Napier grass. The statistical analysis shows that there was a significant difference among the three treatments ($p<0.05$).

No significant difference ($p>0.05$) was observed in the CF content of the three treatments with differences of 1.72%, 1.87%, and 0.15% between Signal and Napier grass, Napier and Signal-Napier combination and Signal and Signal-Napier combination respectively. Ash content also showed that there was a significant difference in the composition of the three treatments ($p<0.05$). Tabular values of ash composition show that more content was obtained in both the Napier and Signal-Napier grass combination which is 2.33% higher than Signal grass (Table 2). The pH from the three treatments also showed that there was a significant difference ($p<0.05$). Table 2 indicates that Napier grass silage had a lower pH value difference of 0.1 when compared to the Signal-Napier combination and a greater difference of 0.5 when compared to Signal grass.

The nitrogen-free extract content of Napier grass and Signal grass differed by 2.21 and 4.08, respectively, when compared to the Signal-Napier grass combination. The study found a significant difference ($p<0.05$) among the three treatments. Signal grass had a significantly higher nitrogen-free extract content than the other silage grasses, with both Napier and Signal-Napier grass combinations having a difference of 2.29% and 3.29%, respectively, when compared to Signal grass. However, there was no significant difference in the ADF content of the three treatments, with an average of 23.42% as shown in Table 2.

Physical Evaluation

No spoilage was recorded in all treatments at 8 weeks. Color change ranged from light green to moderately yellow in all treatments before and after ensiling. There was no significant difference in color amongst the three treatments as they all were moderately yellow after ensiling. Differences in smell were observed amongst ensiled Napier grass, Signal grass, and Signal-Napier combination. A slightly sour smell was the final result for ensiled Napier whilst both Signal grass and Signal-Napier combination smelt moderately sour. All treatments smelt slightly sweet before ensiling. Change in texture was observed in all three treatments as they shifted from dry and coarse before ensiling to slightly moist and coarse after ensiling. This is highlighted in Table 3.

Table 3. Physical evaluation of Silage quality characteristics

Silage	Before ensiling			After ensiling		
	Color	Smell	Texture	Color	Smell	Texture
Napier grass	Bluish-green	Slightly sweet	Dry and course	Moderately yellow	Slightly sour	Slightly moist and course
Signal grass	Light green	Slightly sweet	Dry and course	Light green/yellow	Moderately sour	Slightly moist and course
Signal-Napier grass Combination	Bluish-green	Slightly sweet	Dry and course	Moderately yellow	Moderately sour	Slightly moist and course

Discussion

The research focussed on the investigation of the associative effects of mixing *Brachiaria* and *Pennisetum* grass species on the ensiling properties. No spoilage was observed in any of the treatment samples after ensiling. This is mainly an indicator of the presence of desirable species of bacteria in the silage. Desirable species such as Lactic acid bacteria (LAB) only proliferate in the absence of oxygen which shows that the bags were tightly sealed (*Mugoti et al., 2022*). This is in agreement with *Kiczorowski et al. (2022),* who stated that LAB are known to produce various natural compounds such as diacetyl, ethanol, hydrogen peroxide, reuterin, acetaldehyde, acetoin, carbon dioxide, and bacteriocins. These compounds act as bio-preservatives and help in inhibiting the growth of different types of microorganisms, including pathogenic, non-pathogenic, and spoilage microorganisms.

There were no significant differences in the dry matter (DM) content of the three treatments. However, the tabulated values mean otherwise as they show a higher DM content in the combined Signal-Napier grass combination than all the other treatments. The high DM content in the Signal-Napier grass combination could be a result of the differences in the dry matter content of the two grass species in which Napier grass provides more dry matter than Signal grass making the combination slightly superior. This is supported by *Cook et al. (2005)* who noted that Napier grass provided more DM than Signal grass as it is a leafier plant than Signal grass. However, the values tend to range above the normal range of DM content and this may be a result of human error when preparing the samples for analysis. A simple error in weighing the samples can also affect the data obtained.

Crude protein results after ensiling indicated that there was a significant difference among the three treatments. Values from the results of the three treatments show that the two sole types of grass namely Napier and Signal grass had a lower crude protein composition than the Signal-Napier grass combination. This can also be explained by the fact that associating the grasses was more advantageous as the differences in crude protein content of the two feeds, when combined, give a higher value than the sole silage treatments. These results were in disagreement with work done by *Mtengeti et al. (2013)* in which crude protein content of different grass silage feeds had no significant difference, with Signal grass having a slightly higher CP value than Napier.

The crude fibre (CF) content of the three grass treatments (Napier, Signal, and Signal-Napier combination) had no significant difference after ensiling. This may be a result of the low variances exhibited by the grasses before ensiling and also the microbial action to slightly lower the CF values. This is in agreement with work done by *Desta et al. (2016)* who attested that presence of lactic acid bacteria during fermentation has led to a slight reduction in the crude fibre content of the feed. They also, indicated that the low decline of CF could also be a result of the inhibition of undesirable bacteria during fermentation due to molasses availability which enhances the formation of formic acid and rapid development of Lactic acid bacteria which ultimately lowers DM loss in silage.

Results on the ensiling properties of mixed grasses showed that associating different grasses had a significant impact on the ash content, with a statistical difference observed compared to using independent grasses. Interestingly, the combined Signal-Napier grass treatment had the highest ash content compared to the other two treatments. This finding is consistent with previous research by *Rambau et al. (2022)* and *Santos et al. (2016)* that have shown that Napier grass tends to have more ash than Signal grass, indicating that it is slightly richer in minerals, even though the data is from separate studies with varying environmental conditions. By associating these two types of grass, their unique properties were also combined, resulting in a superior mix that is rich in minerals. These findings are consistent with previous studies conducted by *Mtengeti et al. (2013)* and *Kebede et al. (2016)*, which have also demonstrated the benefits of associating different grasses for improved ensiling properties.

The significant difference obtained in ether extract (EE) content of the three treatments after ensiling is ultimately a result of the higher values obtained in Napier grass before ensiling. The association of Napier and Signal grass made this combination superior in EE content which outpaced signal grass. This may be because of the heat that is produced during fermentation which affects the state of EE. This was highlighted by *Baer et al. (1998)* who stated that high temperatures building off during fermentation affected EE composition by affecting membrane fluidity. In addition, the production of ethanol, a product of fermentation affects EE as ethanol can dissolve some of the freely available fatty compounds (*Baer et al., 1998; Han and Zhou, 2013*). *Han and Zhou (2013)* suggested that the reduction in the fat content of silage is due to the oxidation of unsaturated fatty acids during the early stages of ensiling.

Nitrogen Free Extract (NFE) is the estimate of water-soluble polysaccharides within the grasses. An improvement in NFE after ensiling with the highest quantities obtained in the Signal-Napier grass combination shows that fermentation can assist in breaking down fibre and making it available as NFE. This is in agreement with *Miksusanti et al. (2019)* who state that presence of moisture and microbial populations during fermentation can improve nutrient availability and increase the organic matter rations in a diet.

Neutral Detergent Fibre (NDF) results showed that there was a significant difference in the treatments, with both Napier and Signal-Napier grass combination possessing a higher value as compared to Signal grass. This signifies that Signal grass has higher digestibility. However, all of the grasses had a percentage greater than 50% indicating low digestibility. Ensiling helped reduce the NDF levels to show that microbial action can improve feed digestibility (*Dehghani et al., 2012*). *Halik et al. (2014),* also indicated that microbial and enzymatic actions help to improve the digestibility of the feed as these act on the cell walls and other cell components making them available as water solubles. In this case, associating the grasses diminished the feed digestibility as Napier possesses a higher NDF content. Similar sentiments by *Kebede et al. (2016)* indicated an overall high NDF content of the Napier grass in their assessment of digestibility values in the different genera of Napier grasses.

Acid detergent Fibre (ADF) results indicated no significant differences amongst the three ensiled treatments. Since ADF value is inversely related to digestibility, the low ADF values indicate a much more desirable grass feed. Ensiling further lowered the ADF content though it was not statistically significant. This reduction may be a result of fermentation, microbial action, and enzymatic action on the non-digestible components of the grasses. The results favour Signal grass to indicate its low lignin content as compared to Napier grass. The association of types of grass grasses resulted in a higher ADF value which is not desirable, even though the value is not significant. These results are however in disagreement with *Solvita et al. (2015)* who indicated that grasses tend to have a higher ADF when compared to crop residues like that of maize with an average of 26% ADF in grasses.

After ensiling, the pH of the three treatments had a significant difference having the lowest pH from the Napier grass silage and the highest from Signal grass. Lower pH is an indication of good quality silage as it is a representative of lactic acid, the requirement in silage to assure its preservation *(Moran, 2005)*. The low pH status could be a result of a high energy status of the silage which is readily available to facilitate the formation of lactic acid responsible for lowering the pH (*Sarwatt et al., 1992*).

No significant differences were observed in the organoleptic parameters which may be a result that proves that all the grasses had an almost similar fermentation environment and not too many differences in the outward appearance, smell, and texture before ensiling. This is in agreement with *Randa et al. (2017)* who had almost similar results in the Napier silage. Signal grass had, however, the slowest change in colour which may be a result of the slightly lower temperature (room temperature of 25^0C). This was explained by *Zhu et al. (2022)* who stated that colour change during fermentation is mainly due to the moderately high temperatures of about 35^0C which can lead to a complete colour change in leaves from green to yellow. Interestingly, the experiment showed progress toward a yellow colour in all treatments.

From all these observations, it is highly crucial to implore the new practice of combining feeds as it seemed a little more beneficial than the old-fashioned way of using sole forages in silage making as proven by the statistical differences in most outcomes. This however gives evidence that there will be positive effects on implementing this new initiative and it is advantageous to the animals as they benefit from the associative effects on a nutritional basis.

Conclusion

Associating silages had a beneficial output as compared to the sole independent grass silages as indicated by the good physical characteristics and improved nutrient composition of the silage. The study, therefore, aligns with the alternative hypothesis which states that there is a significant difference in the ensiling properties of the grasses associated with the test.

Recommendations

The adoption of association of the grasses when making silages is highly recommended as a few negative effects were observed and the nutritive value of the feed would not be compromised. Also, further studies need to be undertaken to know the keeping quality of the mixed feeds and further carrying this research to the fodder crops such as maize. Analysis of the anti-nutritional factors needs to be done to determine if there are any effects with this method when silage-making. Tests to determine acceptability and palatability need to be done to have an all-inclusive conclusion to the use of mixed grass silages in livestock diets.

Asocijativni efekti mešanja trave *Brachiaria decumbens* i *Pennisetum Purpureum* na svojstva siliranja

Alban Mugoti, Nation Chikumba, Anderson Munengwa, Lenin Dziwanyika, Sizo Moyo, Chiedza Mgumba

Rezime

Cilj ovog istraživanja je bio da se procene asocijativni efekti dve vrste trave, tropske (*Brachiaria decumbens*) i peraste oštre trave (*Pennisetum purpureum*) na približni sastav, in vitro svarljivost i karakteristike fermentacije siliranog materijala. Kompletan randomizovani dizajn je korišćen za tri tretmana, a to su tropska trava, perasta oštra trava i njihova kombinacija. Silaža je napravljena korišćenjem melase nanešene u proporcijama 1:2 sa vodom i pomešane sa silažom u količini od 5% za vreću od 5 kg pokošene trave od 2,5 cm. Kombinovana silaža tropske i peraste oštre trave bila je superiornija u skoro svim parametrima u odnosu na dve silaže pojedinačnih useva. Nakon siliranja zabeležena je značajna razlika ($p<0,05$) u većini parametara. Utvrđena je značajna razlika ($p=0,0004$) u pH, gde je pH bio niži u silaži peraste oštre trave, u poređenju sa silažom od kombinacije trava, kao i samo tropske trave. Sličan ishod ($P<0,05$) je zabeležen za sirove proteine, pepeo, etarske ekstrakte, ekstrakte bez azota i NDF. Međutim, nije dobijena značajna razlika ($p>0,05$) u suvoj materiji ($p=0,1524$), sirovim vlaknima ($p=0,5924$) i ADF-u ($p=0,1168$). Iako ima slabu svarljivost u svim tretmanima, tropska trava se pokazala boljom od ostalih. Organoleptičke karakteristike su bile obećavajuće, uz normalne promene boje, mirisa i teksture. Ovi rezultati su pokazali da je udruživanje trava imalo impresivan pozitivan efekat na nutritivnu vrednost i kvalitet silaže. Zbog toga se podstiče upotreba mešanih travnatih silaža.

Ključne reči: perasta oštra trava, tropska trava, melasa, kombinacija tropske i peraste oštre trave, silaža, krma

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Declaration of Interests

The authors declare that there is no conflict of interest surrounding the publication of this research.

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Milan M. Petrović[1], Stevica Aleksić[1], Milan P. Petrović[1], Milica Petrović[2], Vlada Pantelić[1], Željko Novaković[1], Dragana Ružić-Muslić[1]

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Corresponding author: Milan M.Petrović, **e-mail address**
Review paper

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EFFECTS OF REARING SYSTEM AND BODY WEIGHT OF REDBRO BROILERS ON THE FREQUENCY AND SEVERITY OF FOOTPAD DERMATITIS

Zdenka Škrbić, Zlatica Pavlovski, Miloš Lukić, Veselin Petričević

Institute for Animal Husbandry, Autoput 16, 11080 Belgrade, Serbia
Corresponding author: Zdenka Škrbić, **e-mail address**
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Accommodation at SPECIAL RATES is possible for reservations before **August, 31st 2023.**

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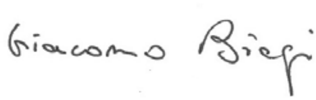
On behalf of
Organizing Committee

Dr Čedomir Radović,
Senior Research Associate
Serbia

On behalf of
International Scientific Committee



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