

# Effects of using indigenous heritage sheep breeds in organic and low-input production systems on production efficiency and animal welfare in Romania

Dinu Gavojdian\*, Ioan Padeanu\*\*, Maria Sauer\*, Neculai Dragomir\*\*, Elena Ilisiu\*\*\*, Szilvia Kusza\*, and Gerold Rahmann\*\*\*\*

## Abstract

Romania ranks third in the EU28 in terms of sheep numbers (9.8 millions), having a self-sufficiency for sheep meat of 150 % and therefore exports are important at national level. Objectives of the current research trials were: i) to evaluate the potential of the Turcana breed to produce organic lamb meat under highland pasture-based conditions; ii) to evaluate the overall resistance of the breed and fitness indicators, in comparison with two native breeds, reared under low-input conditions; iii) to study the genetic base using molecular assisted selection in order to produce hypoallergenic milk and derived milk products. The conventional pasture from the current project had a potential of production of 189 kg/ha for Turcana purebreds, and of 236 kg/ha for F<sub>1</sub>German Blackheaded x Turcana (GBxTA) crossbreed lambs. While for the organic pasture, it was estimated a production potential of 191 kg of meat per hectare while rearing Turcana purebred lambs, and of 240 kg/ha for the F<sub>1</sub>GBxTA dual-breeds. In the current preliminary study, the genetic polymorphism of ovine  $\beta$ -casein gene in the Romanian Zackel breed was highlighted, using TaqMan assay.  $\beta$ -casein in sheep is strongly correlated to economically important milk quantitative traits, being a first step in introducing genomic selection for the production of organic hypoallergenic sheep milk and derived products.

**Keywords:** *Romanian Zackel sheep, Turcana sheep, organic lamb production, animal welfare,  $\beta$ -casein*

## Zusammenfassung

### Bedeutung lokaler Schafrassen für die Produktionseffizienz und das Tierwohl in ökologischen und Low-input-Produktionssystemen in Rumänien

In Rumänien gibt es rund 9,8 Millionen Schafe und damit steht das Land an Platz drei in der EU28. Der Selbstversorgungsgrad liegt bei Schaffleisch bei 150 % und Exporte sind erforderlich. Qualitätsverbesserungen sind die Herausforderungen der Schafhaltung, um auf internationalen Märkten wettbewerbsfähig zu bleiben. Ökologisch zertifiziertes Schaffleisch und Schafmilchprodukte können eine Möglichkeit sein, die Schafhaltung ökonomisch nachhaltig zu betreiben. In aktuellen Versuchen wurde untersucht, wie i) sich die verbreitete Turcana Schafrasse auf Dauergrünland im Hochland für eine ökologische Schaffleischproduktion eignet, ii) sich die Rasse in Widerstandsfähigkeit und Fitness im Vergleich zu anderen lokalen Rassen verhält und iii) welche Rolle die Genetik in der Produktion von anti-allergener Schafmilch und deren Produkten spielt. Es konnten auf konventionell bewirtschafteten Weiden pro Hektar und Jahr 189 kg Lammfleisch mit reinrassigen Turkana und 236 kg mit F<sub>1</sub>-Kreuzungen aus Deutsches Schwarzkopf x Turcana produziert werden. Auf ökologisch bewirtschafteten Weiden lagen die Werte in ähnlicher Höhe (191 kg bzw. 240 kg Lammfleisch). Es konnte festgestellt werden, dass  $\beta$ -Kasein in der rumänischen Zackel-Schafmilch besonders hoch ist. Dieses ist ein wertgebender Inhaltstoff und wichtig für die Verarbeitung zu Schafkäse.

**Schlüsselwörter:** *Rumänisches Zackelschaf, Turcanaschaf, Ökologische Lammfleischproduktion, Tierwohl,  $\beta$ -Kasein*

\* Romanian Academy for Agricultural and Forestry Sciences, Research and Development Station for Sheep and Goats Caransebes, Resitei Street, 325400 Caransebes, Caras-Severin County, Romania

\*\* Banat's University of Agricultural Sciences and Veterinary Medicine 'King Michael I of Romania', Faculty of Animal Science and Biotechnologies, 119 Aradului Street, 300645 Timisoara, Timis County, Romania

\*\*\* Romanian Academy for Agricultural and Forestry Sciences, Research and Development Station for Sheep and Goats Reghin, 11 Dedradului Street, 545300 Reghin, Mures County, Romania

\*\*\*\* Johann Heinrich von Thünen Institute, Institute of Organic Farming, Trenthorst 32, 23847 Westerau, Germany

## 1 Introduction

In Europe, sheep production systems vary greatly among countries and regions. In most Mediterranean countries, the production of dairy sheep is predominant (Todaro et al., 2015; Rahmann, 2006), while in North, Central and Eastern Europe, meat has become the main product (Sargison, 2008). The substantial diversity among sheep breeds, production environments and management systems requires systematic and comprehensive breed evaluations, in order to allow producers to identify genotypes that are best suited for their production systems and meet current market demands (Leeds et al., 2012). Furthermore, exotic breeds are often being introduced into various rearing systems without adequate knowledge about the acclimatization ability to those specific conditions (Browning et al., 2011), which can lead to poor efficiency in sheep production and reproduction, and have detrimental impacts on the environment and animal welfare (Hristov et al., 2013; Alemseged and Hacker, 2014; Rahmann, 2013).

Currently 85.5 million sheep are being reared in the EU (Eurostat, 2016), with a total annual production of 925.000 tonnes of sheep meat, having a self-sufficiency rate of 88 %. The EU exports around 8 % of its total production, while imports are around 212.000 tonnes/year, mainly coming from New Zealand and Australia (94 %), which represents 23 % the EU's own consumption (DG-AGRI, 2013). Moreover, the EU sheep numbers have decreased continuously during the last 10 years, reaching 85.2 % in 2014, compared to year 2004.

In Eastern and Southern Europe, majority (over 85 %) of the sheep and goats are being reared in mountainous and disadvantageous areas, called Less Favoured Areas (LFAs) as defined in Dir.75/268/EEC, having an important economic, social and ecological role, and also contributing to the conservation of the environment (Sossidou et al., 2013; Rahmann et al., 2016). With most of the local breeds reared belonging to the Zackel and Tsigai strains (Kusza et al., 2008; Gavojdian et al., 2013).

Romania ranks third in the EU in terms of sheep numbers (9.8 millions), having a self-sufficiency for sheep meat of 150 % and therefore exports are important at national level (DG-AGRI, 2013). Over the last years, exports of live animals increased significantly, with exports of live lambs going mainly to the Middle East and North Africa. Moreover, the country has a pasture surface of 4.9 million ha, which could sustain up to 16 million sheep (Dragomir, 2009). With more than 95 % of sheep being reared under extensive low-input production systems, and the breed structure being dominated by indigenous, unimproved breeds (Turcana and Tsigai).

Romanian indigenous Turcana, accounting for over six million breeding ewes (Ilisiu et al., 2010), is one of the most representative breed belonging to the Zackel group, reared in 14 countries from Central, Eastern and South-Eastern Europe. With adult body weights of 30 to 50 kg in ewes and 60 to 80 kg in rams, growth rates in unweaned lambs of 110 to 180 g/day (Kusza et al., 2015), milk yields estimates of 60 to 150 kg/lactation and prolificacy rates (litter rate) ranging

between 105 to 130 % (Budai et al., 2013). Overall, the meat production potential of the breed is modest, making the breed uncompetitive for the current market demands.

Objectives of the current research trials were: i) to evaluate the potential of the Turcana breed to produce organic lamb meat under highland pasture-based conditions; ii) to evaluate the overall organic resistance of the breed and fitness indicators, in comparison with two native breeds, reared under low-input conditions; iii) to study the genetic base using molecular assisted selection in order to produce hypo-allergenic milk and derived milk products.

## 2 Material and methods

### 2.1 Organic lamb meat production conditions

The trial was initiated starting autumn 2015 at the Research and Development Station for Sheep and Goats from Caransebes (45°25'N/22°13'E). Caransebes region has an elevation of 280 m above sea level and a total annual precipitation of 737.2 mm, with a mean annual temperature of 12.9 °C. The project herd consisted of 90 multiparous purebred Turcana ewes, managed under organic rearing conditions as stipulated by the Council Regulations of the European Commission (EEC, 1991/2092 and EEC, 1999/1804; Rahmann, 2013) on standards of organic animal husbandry. Two breeding herds were set-up, with half of the ewes (n = 45), randomly selected being mated with Tucana purebred rams, while the second group of ewes were exposed to German Blackheaded Mutton (Deutsches Schwarzköpfiges Fleischschaf) (Rahmann et al., 2001; Rahmann, 2000). Ram/ewe ratio was of 1:25, for three consecutive oestrous cycles (51 days), with the reproduction season starting in mid September. Nutritional flushing was practiced for three weeks before the mating seasons, in addition, all animals had free access to potable water and mineral blocks year around. Animals were housed indoors during winter for a period of 120 days, on deep straw bedding, with a space allowance of 1.8 m<sup>2</sup> and 0.5 m<sup>2</sup> per ewe and lamb, respectively. Ewes received high-quality clovers and pasture hay *ad libitum*, with an additional 200 g of concentrates in late gestation and during suckling period. Hay and concentrates were organically produced on farm. Creep feeding of lambs was not practiced, they were solely reliant on the dams milk production. Lambs were weaned at 65 ± 5 days of age.

After weaning (April, 2016), lambs were divided into two groups of 40 individuals each (20 Turcana purebreds and 20 F<sub>1</sub> German Blackheaded x Turcana), balanced for body weights and sex among groups and genotypes. Group I was managed under conventional conditions, on a cultivated pasture (control group). Group II was managed under organic-pasture conditions (experimental group). Both groups were kept exclusively on pasture for a period of four months, with a gradual transition from indoor housing to pasture of 10 days. Rotational fenced grazing was practiced, each of the two pastures having 6 identical in size areas (1600 m<sup>2</sup>). Lambs were provided on pasture with shelter and shade, and had non-restricted access to water and mineral blocks.



Figure 1

Turcana ewe (photo: Gavojdian D.)



Figure 2

Tsigai ewe (photo: Gavojdian D.)

The conventional pasture was fertilized initially in two stages, first in early spring 2012 with a dose of  $N_{100}P_{70}K_{70}$  and secondly, after the first harvest with a dose of  $N_{50}$ . In 2013, fertilization was made by administering  $N_{100}$  in early spring and  $N_{50}$  after the first harvest. During both years, the pasture was used to produce bailed hay, and was not used for grazing.

The organic pasture was fertilized in early-spring 2012 by direct grazing with the animals, at a stocking rate of 1 adult sheep/1.5 m<sup>2</sup>, kept for four consecutive nights. After the animals removal from the pasture, minimum tillage works were done and the pasture was over-seeded using a mix of grasses and legumes having the following structure: *Lolium perenne* 40 % (8 kg/ha); *Festuca pratensis* 30 % (6 kg/ha); *Festuca arundinacea* 10 % (2 kg/ha); *Lotus corniculatus* 10 % (2 kg/ha) and *Trifolium repens* 10 % (2 kg/ha). Following a conversion period of two years (between 2013 and 2014), the pasture was used exclusively to produce hay, and was not directly grazed by animals. Data on the current experimental design, data collection and pasture management was previously published in more detail by Sauer et al., 2015.

## 2.2 Health indicators and fitness outputs

The study was carried out in three commercial farms, as follows: *i*) the first commercial sheep farm was rearing Turcana purebred ewes (Sanpetru Mare, Timis county, 46°5'14.14"N,20°47'26.69"E); *ii*) the second commercial farm was rearing Tsigai ewes (Chisineu Cris, Arad county, 46°31'21"N,21°30'57"E) and *iii*) third commercial farm rearing Transylvanian Merino ewes, as gene reserve (Sanislau, Satu Mare county, 47°38'16"N,22°19'32"E). All three farms were applying the traditional semi-intensive production system, with ewes being managed under communal natural pastures between April till November, and then indoor housing the ewes during winter, with stock feeding hay (1.5 to 2 kg of alfalfa and pasture hay) and 200 to 300 g/day of concentrates. Lambing season started in mid-February and lasted until end of March.

Ewes were between 1.5 and 8 years old, with age and parity balanced across genotypes and representing a diverse sampling of genetic lines within each flock and breed. The study herds consisted of 226 Turcana, 260 Tsigai and 560 Transylvanian Merino breeding ewes.

Data was collected from the veterinarian records and the farms own records for a period of 12 months (August 2015 to September 2016). All three flocks were included in the official performance recording schemes.



Figure 3

Transylvanian Merino ram (photo: Gavojdian D.)

Occurrence rates of the following health disorders were recorded: mastitis, lameness, pneumonia and abortion. Annual attrition rates were determined by identifying ewes in the herd at the start of the production year not present in the herd at the end of the production year. Death and culling because of all reasons were included when evaluating attrition rates. Data on the reproductive performance of ewes (conception rate, litter size and survival rates of unweaned lambs) were recorded for all three breeds.



In order to assess the effect of the breed on the above mentioned health disorders, as well as on the reproduction performance of the ewes, the STATISTICA StatSoft® Tulsa OK software was used (Hill and Lewicki, 2007). The Main Effect ANOVA analysis of variance was applied. Given that data was recorded in three farms, the model included this as correction factor. The model used for statistical analysis is presented below:

$$y_{ijk} = \mu + PC_i + g_j + e_{ijk}$$

where

- $y_{ijk}$  is the studied reproduction or health trait;  
 $\mu$  is the overall mean;  
 $PC_i$  represents the fix effect of the production system with three levels: farm 1, 2 and 3;  
 $g_j$  represents the random effect of the genotype with three levels: Turcana, Tsigai and Transylvanian Merino;  
 and  
 $e_{ijk}$  is the residual effect.

When significant effects of the genotype were observed, the comparison among populations was tested by performing contrast analysis, using Tukey test.

### 2.3 Genomic selection for hypoallergenic milk

Turcana purebred animals ( $n = 111$ ) were sampled in the present study to detect genetic polymorphism at the *CSN2* loci. Hair follicles were collected from adult breeding ewes, between 1.5 and 8 years old, with balanced age and parity within the flock and representing a diverse sampling of genetic lines. All animals included into the study, were part of the official performance recording system, with ancestry of the animals known for at least two generations.

Extraction of genetical DNAs from hair follicles were performed using method of FAO/IAEA (2004) and they were stored on  $-20$  °C until further analysis. Concentration and quality of the extracted DNAs were measured and checked by NanoDrop Spectrophotometer (Thermo Scientific, USA).

Taqman (Applied Biosystems, USA) genotyping probe for ovine  $\beta$ -casein was designed for the fast genotyping of the A>G mutation in part of an exon 7 at position 183 (Met183QVal183). Concentration of the DNAs used for the genotyping assay were 50 ng.

The following primers and probe were designed and used: forward primer: 5'- CGTGTGTCCCTTTCTCA-3', reverse primer: 5'-TTTTGTAGGGCTCTTAATTACTCAA-3', probe A/G: CCCAGAGAGAT[A/G]TGCCCATCC.

After the PCR was finished, allelic discrimination analysis was performed using Applied Biosystems software. Samples were automatically grouped according to their genotypes. The results of the TaqMan allelic discrimination assay were graphically interpreted and all of the samples were correctly assigned the right genotype.

The research activities were performed in accordance with the European Union's Directive for animal experimentation (EU-Directive 2010/63/EU).

## 3 Results and discussion

### 3.1 Lamb meat production under organic and conventional systems

Under pasture conditions, the Turcana lambs had growth rates of  $206.0 \pm 0.14$  g/day and  $182.7 \pm 0.13$  g/day, under conventional and organic systems, respectively (Table 1). Production system did influence ( $p \leq 0.05$ ) the growth rates in the purebred Turcana lambs. As for the F<sub>1</sub>German Black-headed x Turcana lambs (F<sub>1</sub>GBxTA), the production system influenced significantly ( $p \leq 0.05$ ) the growth rates, with average values of  $258.0 \pm 0.12$  g/day and  $227.3 \pm 0.10$  g/day being recorded in the conventional and organic systems, respectively.

Based on lamb growth rates and the pasture production estimates, the potential of meat production for the two genotypes and two pasture types was assessed. The conventional pasture from the current project had a potential of production of 189 kg/ha for Turcana purebreds, and of 236 kg/ha for F<sub>1</sub>GBxTA crossbred lambs. While for the organic pasture, it was estimated a production potential of 191 kg of meat per hectare while rearing Turcana purebred lambs, and of 240 kg/ha for the F<sub>1</sub>GBxTA dual-breeds. Current results are in accordance with previous reports of Ghita et al. (2010), which studied the fattening aptitudes of local Romanian sheep breeds, reared under various production systems. Thus, nutritional strategies in low input and easy to apply in pasture based systems can improve the productive performance of sheep and have the potential to lead to profitable and successful commercial enterprises.

Table 1

Mean ( $\pm$  SEM) for performances of Turcana lambs reared under conventional and organic production systems

Trait	Conventional	Organic
Growth rates in Turcana lambs (g/day)	206.0 $\pm$ 0.14 <sup>a</sup>	182.7 $\pm$ 0.13 <sup>b</sup>
Growth rates in F <sub>1</sub> GBxTA lambs (g/day)	258.0 $\pm$ 0.12 <sup>a</sup>	227.3 $\pm$ 0.10 <sup>b</sup>
Meat production estimates in Turcana lambs (kg/ha)	189 kg	191 kg
Meat production estimates in F <sub>1</sub> GBxTA (kg/ha)	236 kg	240 kg
Row means with different superscript differ significantly at $p \leq 0.05$		

The technical efficiency and viability of organic and conventional lamb meat production were investigated (Table 2). Overall, the production costs in the organic production system were greater, when compared with the conventional management of the pasture. The pasture seeding related costs being higher with 70.6 %, and those of price/tones of pasture of 31.5 % for the organic pasture, compared to the conventional system. Current results are in accordance with previous reports by Sauer et al. (2015) on organic kid meat production in Romania.

Estimated production costs per kg of lamb meat were higher by 68.2 % when rearing Turcana purebreds, and by 68.3 % when F<sub>1</sub>GBxTA crossbreeds were concerned. Altogether, higher production costs should be reflected in the economic returns (EUR/kg of sold live lamb), in order for the farmers to adopt the organic production systems, or for those who chosen this production system to respect the additional regulations. This could be achieved either throughout niche marketing of organic lambs meat products (e.g. development of PDO or PGI labelled food products), or by governmental policies and introduction of subsidies schemes into the sector to aid its development.

**Table 2**

Technical efficiency of organic and conventional production for lamb meat

Costs categories (in EUR)	Conventional	Organic	Differences
Pasture seeding costs/ha	185.55	316.66	131.11 EUR/ha
Production costs/t of pasture	7.77	10.22	2.45 EUR/t
Annual pasture maintenance costs/ha	105.88	180.47	74.59 EUR/ha
Production costs/kg of Turcana lamb meat	1.51	2.54	1.03 EUR/kg
Production costs/kg of F <sub>1</sub> GBxTA lamb meat	1.20	2.02	0.82 EUR/kg

Introduction and use of the German Blackheaded Mutton meat specialized breed into crossbreeding schemes with local unimproved sheep populations has led to a significant increase of both growth rates and lamb meat production per hectare. Taking advantage of the German Blackheaded Mutton meat producing potential, the local low-input breeds high levels of adaptation and organic resistance, and also of the heterosis effects.

### 3.2 Health and fitness indicators in Romanian native breeds

Comparison of the three main Romanian indigenous sheep breeds for their reproductive performance and organic resistance under European temperate conditions were considered necessary knowledge for the sheep industry as sheep breeds adaptation and welfare tend to be matters of concern. This comparative study was the first attempt to provide information on the reproductive efficiency and health traits in Turcana, Tsigai and Transylvanian Merino breeds under temperate climate conditions found in Eastern Europe.

Health, reproductive and fitness indicators in indigenous heritage breeds are of importance under extensive production systems, especially under organic production, where the use of antibiotics and several treatments are being restricted by the regulations. Health of the flock influences decidedly the economic returns of sheep farms, and genotypes which

possess a high organic resistance are desirable. However, health indicators and the fitness related traits have been found to have low heritability rates (0.02 to 0.08), making them unsuitable/unattractive for direct selection in the genetic improvement schemes.

Conception rates for the Turcana, Tsigai and Transylvanian Merino ewes are consistent with those estimated by Krupova et al. (2009) and Padeanu (2001) for the breeds. Significantly lower conception rates were found between the Tsigai and Turcana ( $p \leq 0.05$ ) and the Transylvanian Merino ( $p \leq 0.01$ ), respectively (Table 3).

For the Turcana and Transylvanian Merino breeds, the litter size is similar to reports of Ilisiu et al. (2010) and Padeanu (2010). Considerable lower values for litter size in Tsigai were previously reported by Krupova et al. (2009) and Padeanu et al. (2012). Higher litter size in Tsigai ewes during current trial might be attributed to the good feeding and management conditions. In addition, during previous studies, ewes were reared for both meat and milk productions, while in the current study, the reference flock ewes were lactating for only a short period of time ( $80 \pm 10$  days), until the lambs were weaned. This might have led to a better body condition of ewes during mating season and thus to higher ovulation rates.

Findings on lambs weaning rates are consistent with reports for the Turcana and Tsigai lambs reared under European temperate conditions (Padeanu et al., 2012; Gavojdian et al., 2013). Breed disparities were found for the lambs survival rates until weaning, with Tsigai lambs having the highest weaning rate ( $p \leq 0.05$ ), when compared to Turcana and Transylvanian Merino lambs.

Under commercial sheep production systems, the importance of fitness relates to the attrition of breeding ewes. The slight genotype disparities in attrition rates could be explained by the considerable lower selection pressure applied for the unimproved Turcana, and as a result, decisions on voluntary culling of ewes were made only in extreme cases for the genotype. In Tsigai and Transylvanian Merino breeds, the voluntary culling of ewes occurred especially based on traits such as milk yield, fertility, weaning ability, body condition, wool production and health disorders. Data on attrition rates for the three breeds are consistent with estimates reported for commercial sheep flocks (Mekaway et al., 2009; Gavojdian et al., 2015).

**Table 3**

Mean ( $\pm$  SEM) for reproductive performance and attrition rates in Turcana, Tsigai and Transylvanian Merino ewes

Breed	Conception rate (%)	Litter size (lambs)	Lambs weaned (%)	Attrition rate (%)
Turcana	97.7 $\pm$ 0.98 <sup>a</sup>	1.17 $\pm$ 0.02 <sup>a</sup>	96.9 $\pm$ 1.06 <sup>a</sup>	14.2 $\pm$ 2.33 <sup>a</sup>
Tsigai	94.1 $\pm$ 0.11 <sup>b</sup>	1.40 $\pm$ 0.23 <sup>b</sup>	98.7 $\pm$ 1.38 <sup>b</sup>	24.4 $\pm$ 0.28 <sup>b</sup>
Transylvanian Merino	97.2 $\pm$ 0.14 <sup>a</sup>	1.14 $\pm$ 0.29 <sup>a</sup>	96.1 $\pm$ 1.44 <sup>a</sup>	20.8 $\pm$ 0.37 <sup>b</sup>

Column means with different superscript differ significantly at  $p \leq 0.05$

Results for the three studied populations regarding the mastitis occurrence rates (Table 4) are consistent with estimates of Giadinis et al. (2011), which report occurrence rates for mastitis of over 10 % for commercial sheep flocks. Furthermore, occurrence rates of over 30 % in European dairy flocks are being frequently mentioned in the literature (Bergonier et al., 2003; Bishop et al., 2010).

Mastitis has a negative impact on ewe stay-ability, milk yield, lambs growth and survival, veterinary costs and represents a major concern for animal welfare. Importance of clinical mastitis in the Europe was highlighted by Ligda et al. (2003), which found mastitis to be the main cause for culling in the Greek sheep industry, accounting for 46 % of the attrition cases for the Chios dairy breed.

The current study highlights the low resistance to clinical mastitis of the Tsigai breed. As a result, measures such as including the genetic resistance to mastitis as a trait into the breed's selection indexes should be taken. Somatic cell count (SCC) has been found to be a reliable selection trait (Barillet et al., 2001), which can be used as an indicator to enable selection for increased resistance to subclinical mastitis in the French Lacaune breed (Rupp et al., 2003). For SCC in dairy sheep, heritability estimates reported range between 0.03 and 0.24 (Barillet et al., 2001; Bishop et al., 2010; Makovicky et al., 2014; Rahmann, 2013), depending on the breed. The incidence of clinical mastitis in small ruminants is generally lower than 5 % (Arranz et al., 2012), whereas the prevalence of subclinical mastitis ranges from 10 to 50 % (Sechi et al., 2009). If treated, ewes with subclinical mastitis were found to produce less milk with 15 to 20 %, and in the case of a reinfection, the milk yield to drop with 30 % (Petrovic et al., 2005).

Lameness occurrence rates in the Turcana and Transylvanian Merino breeds were in accordance with previous estimates for the English flocks (FAWC, 2011), which range from 8 to 10 %. In the current study, the Tsigai ewes were found to be more susceptible to lameness, with an incidence of 15.4 %. Therefore, selection should be given to future design of genetic improvement schemes for the breed.

Given the low winter temperatures reached in Romania, pneumonia incidence in Merino flocks is of concern to both scientists and breeders, being a lowland fine wool breed originating from Spain. Higher pneumonia incidence in the Transylvanian Merino population could be attributed to the upgrading of the breed with the Australian Merino, which is not adapted to the colder climate during winter found in Central and Eastern Europe. No available data for comparison on pneumonia occurrence in European countries was

found, except for information on flocks infected with Maedi-Visna (Benavides et al., 2013).

According to previous reports, in healthy flocks abortion accounts for less than 2 %, with 5 % occurrence rate being considered as an alarm threshold (Menziez, 2011). Out of the four health disorders studied, abortion has to have the most significant economical and welfare implications, given that abortion will most likely result in culling or death of the ewe.

**Table 4**

Mean ( $\pm$  SEM) for occurrence rates of mastitis, lameness, pneumonia and abortions in Turcana, Tsigai and Transylvanian Merino ewes

Genotype	Mastitis (%)	Lameness (%)	Pneumonia (%)	Abortion (%)
Turcana	3.11 $\pm$ 1.16 <sup>a</sup>	2.6 $\pm$ 1.08 <sup>a</sup>	1.3 $\pm$ 0.76 <sup>a</sup>	1.7 $\pm$ 0.88 <sup>a</sup>
Tsigai	4.8 $\pm$ 1.18 <sup>b</sup>	15.4 $\pm$ 1.43 <sup>b</sup>	1.8 $\pm$ 1.29 <sup>a</sup>	3.4 $\pm$ 1.46 <sup>b</sup>
Transylvanian Merino	2.14 $\pm$ 0.23 <sup>a</sup>	8.7 $\pm$ 1.22 <sup>c</sup>	3.1 $\pm$ 1.03 <sup>b</sup>	1.4 $\pm$ 1.05 <sup>a</sup>

Column means with different superscript differ significantly at  $p \leq 0.05$

### 3.3 Molecular marker assisted selection for hypoallergenic sheep milk

Aim of the current trial was to identify the genetic polymorphism of  $\beta$ -casein gene in Turcana breed, throughout the use of TaqMan genotyping assay, in order to provide information for future selection schemes of the Romanian indigenous sheep breeds, with the ultimate purpose to produce hypoallergenic sheep milk and derived dairy products (Załęcka et al., 2014).

In the studied population the A variant had a significant higher frequency of 0.97, compared to the G variant of 0.03, thus indicating that variant A is more characteristic of Turcana – Zackel sheep population compared to variant G (Table 5). With similar results being previously reported in other European Zackel breeds, with variant A detecting a higher frequency (50 to 80 %) compared with the G variant, as described by Sztankoova et al. (2011) in Czech Sumatra and Valachian sheep and by Ceriotti et al. (2004). The most frequent genotype was AA (94.59 %) followed by AG (5.41 %). Genotype GG was not found in the Turcana breeds. The distribution of genotypes is in agreement with previous results obtained for other European sheep breeds (Ceriotti et al., 2004; Sztankoova et al., 2011).

**Table 5**

Frequency of genotype and alleles of Turcana sheep

Breed	Genotype	Number	Frequency of genotype	Allele	Frequency of allele
Turcana	GG	0	0	A	0.97
	AG	6	5.41	G	0.03
	AA	105	94.59	0	0

In sheep and goat  $\alpha$ S1-casein ( $\alpha$ S1-CN), coded by the CSN1S1 gene, is characterized by extensive qualitative and quantitative polymorphisms. Some alleles are associated with null (i.e., CSN1S1\*01) or reduced (i.e., CSN1S1\*F) expression of the specific protein (Ballabio et al., 2011).

Almost 50 genetic variants have been described in bovine milk protein genes, and, in the sheep and goats, the number of genetic variants is continuously rising (Marletta et al., 2007). The current preliminary trial is the first attempt to produce hypoallergenic sheep milk throughout the use of genomic selection in Romania, in order to aid consumers affected by food-related allergies. Furthermore, given the rising numbers of persons (especially infants, incidence estimates of 5 to 8 %) affected by this type of allergies, the current research could help sheep breeders to produce and market hypoallergenic products, which to benefit both producers and consumers safety.

## 4 Conclusions

Current results have proved to be encouraging regarding the potential of the sheep extensive farms from Romania to produce lamb meat under organic rearing systems. However, both farmers and policy makers should be aware and keep in mind both the unconsolidated markets for organically sheep meat found in Eastern Europe, as well as the volatility of the small ruminants meat markets and demand. Further in more depth studies concerning the markets need and potential should be advised, in order for the policy makers to have a more consolidated perspective of the sector in order to formulate proper development strategies of the sector.

For the Tsigai and Transylvanian Merino breeds it would be advisable as to include fitness traits into the future breeding selection schemes, with special focus on mastitis genetic resistance, lameness and ewe stay-ability, in order to improve animal welfare and the overall flock productivity.

In the current preliminary study, the genetic polymorphism of ovine  $\beta$ -casein gene in the Romanian Zackel breed was highlighted, using TaqMan assay.  $\beta$ -casein in sheep is strongly correlated to economically important milk quantitative traits, being a first step in introducing genomic selection for the production of organic hypoallergenic sheep milk and derived products.

## Acknowledgments

This work was supported by a grant of the Romanian National Authority for Scientific Research and Innovation, CNCS – UEFISCDI.

## References

- Alemseged Y, Hacker RB (2014) Introduction of Dorper sheep into Australian rangelands : implications for production and natural resource management. *Rangeland J* 36:85-90
- Arranz JJ, Gutierrez-Gil B (2012) Detection of QTL underlying milk traits in sheep : an update [online]. To be found at <<http://www.intechopen.com/books/milk-production-advanced-genetic-traits-cellular-mechanism-animal-management-and-health/detection-of-qtl-underlying-milk-traits-in-sheep-an-update>> [quoted 12.12.2016]
- Ballabio C, Chessa S, Rignanese D, Gigliotti C, Pagnacco G, Terracciano L, Fiocchi A, Restani P, Caroli AM (2011) Goat milk allergenicity as a function of alphas-casein genetic polymorphism. *J Dairy Sci* 94:998-1004
- Barillet F, Marie C, Jacquin M, Lagriffoul G, Astruc JM (2001) The French Lacaune dairy sheep breed : use in France and abroad in the last 40 years. *Livest Prod Sci* 71:17-29
- Benavides J, Fuertes M, Pariente CG, Otaola J, Delgado L, Giraldez J, Marin JFG, Ferreras MC, Perez V (2013) Impact of maedi-visna in intensively managed dairy sheep. *Vet J* 197(3):607-612
- Bergonier D, Cremoux R, Rupp R, Lagriffoul G, Berthelot X (2003) Mastitis of dairy small ruminants. *Vet Res* 34(5):689-716
- Bishop SC, Axford RFE, Nicholas FW, Owen JB (2010) Breeding for disease resistance in farm animals. Wallingford : CABI, 362 p
- Browning R, Leite-Browning ML, Byars M (2011) Reproductive and health traits among Boer, Kiko, and Spanish meat goat does under humid, subtropical pasture conditions of the southeastern United States. *J Anim Sci* 89:648-660
- Budai C, Gavojdian D, Kovacs A, Negrut F, Olah J, Csiszter LT, Kusza S, Javor A (2013) Performance and adaptability of the Dorper sheep breed under Hungarian and Romanian rearing conditions. *Scientific Pap Anim Sci Biotechnol* 46:344-349
- Ceriotti G, Chessa S, Bolla P, Budelli E, Bianchi L, Duranti E, Caroli A (2004) Single nucleotide polymorphisms in the ovine casein genes detected by polymerase chain reaction : single strand conformation polymorphism. *J Dairy Sci* 87:2606-2613
- DG-AGRI (2013) Study on mandatory origin labelling for pig, poultry and sheep & goat meat, AGRI-2012-EVAL-01 [online]. To be found at <[http://ec.europa.eu/agriculture/external-studies/2013/origin-labelling/fulltext\\_en.pdf](http://ec.europa.eu/agriculture/external-studies/2013/origin-labelling/fulltext_en.pdf)> [quoted 12.12.2016]
- Dragomir N (2009) Multifunctional utilization of pastures in Romania. *Scientific Pap Anim Sci Biotechnol* 42(1):191-194
- EEC (1991) Council Regulation (EEC) No 2092/1991 of 24 June on organic production of agricultural products and indications referring thereto on agricultural products and foodstuffs [online]. To be found at <<http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=URISERV%3A121118>> [quoted 12.12.2016]
- EEC (1999) Council Regulation (EC) No 1804/1999 of 19 July supplementing regulation (EEC) No 2092/91 on organic production of agricultural products and indications referring thereto on agricultural products and foodstuffs to include livestock production [online]. To be found at <<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:1999:222:0001:0028:EN:PDF>> [quoted 12.12.2016]
- EU (2010) Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes [online]. To be found at <<http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32010L0063>> [quoted 12.12.2016]
- Eurostat (2016) Sheep population annual data [online]. To be found at <[http://ec.europa.eu/eurostat/search?p\\_auth=BUeVU4jM&p\\_id=estat-searchportlet\\_WAR\\_estatsearchportlet&p\\_lifecycle=1&p\\_p\\_state=maximized&p\\_p\\_mode=view&\\_estatsearchportlet\\_WAR\\_estatsearchportlet\\_action=search&text=sheep+population](http://ec.europa.eu/eurostat/search?p_auth=BUeVU4jM&p_id=estat-searchportlet_WAR_estatsearchportlet&p_lifecycle=1&p_p_state=maximized&p_p_mode=view&_estatsearchportlet_WAR_estatsearchportlet_action=search&text=sheep+population)> [quoted 12.12.2016]
- FAO/IAEA (2004) Agriculture Biotechnology Laboratory - Handbook of Laboratory Exercises. Seibersdorf: IAEA Laboratories, p. 18
- FAWC - Farm Animal Welfare Council (2011) Opinion on lameness in sheep [online]. To be found at <[https://www.gov.uk/government/uploads/system/uploads/attachment\\_data/file/325039/FAWC\\_opinion\\_on\\_sheep\\_lameness.pdf](https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/325039/FAWC_opinion_on_sheep_lameness.pdf)> [quoted 12.12.2016]



- Gavojdian D, Csiszter LT, Sossidou EN, Pacala N (2013) Improving performance of Zackel sheep through cross-breeding with prolific Bluefaced Leicester under semi-intensive and extensive production systems. *J Appl Anim Sci Res* 41(4):432-441
- Gavojdian D, Budai C, Csiszter LT, Csizmar N, Javor A, Kusza S (2015) Reproduction efficiency and health traits in Dorper, White Dorper, and Tsigai Sheep Breeds under temperate European conditions. *Asian Austral J Anim Sci* 28(4):599-603
- Ghita E, Lazar C, Pelmus R, Ropota M (2010) Comparative studies on the aptitudes for meat production of the fattened lambs from local Romanian breeds. In: Book of abstracts of the 61st Annual meeting of the European Association for Animal Production : Heraklion, Crete Island, Greece, August 23<sup>rd</sup> – 27<sup>th</sup>, 2010. Wageningen : Wageningen Acad Publ, pp 106
- Giadinis ND, Panousis N, Petridou EJ, Siarkou VI, Lafi SQ, Pourliotis K, Hatzopoulou E, Fthenakis GC (2011) Selenium, vitamin E and vitamin A blood concentrations in dairy sheep flocks with increased or low clinical mastitis incidence. *Small Rumin Res* 95(2):193-196
- Hill T, Lewicki P (2007) *Statistics : methods and applications*. Tulsa : StatSoft, 800 p
- Hristov N, Ott T, Tricarico J, Rotz A, Waghorn G, Adesogan A, Dijkstra J, Montes F, Oh J, Kebreab E, Oosting SJ, Gerber PJ, Henderson B, Makkar HPS, Firkins JL (2013) Mitigation of methane and nitrous oxide emissions from animal operations : III. A review of animal management mitigation options. *J Anim Sci* 91:5095-5113
- Ilisiu E, Daraban S, Gabi-Neacsu M, Ilisiu V-C, Rahmann G (2010) Improvement of lamb production in Romania by crossbreeding of local Tsigai breed with high performance breeds. *Landbauforsch* 60(4):259-265
- Krupova Z, Wolfova M, Wolf J, Oravcova M, Margetin M, Peskovicova D, Krupa E, Dano J (2009) Economic values for dairy sheep breeds in Slovakia. *J Anim Sci* 22:1693-1702
- Kusza S, Nagy I, Sasvari Z, Stagel A, Nemeth T, Molnar A, Kume K, Bosze Z, Javor A, Kukovics S (2008) Genetic diversity and population structure of Tsigai and Zackel type of sheep breeds in the central-, eastern- and southern-European regions. *Small Rumin Res* 78:13-23
- Kusza S, Zakar E, Budai C, Csiszter LT, Padeanu I, Gavojdian D (2015) Mitochondrial DNA variability in Gyimesi Racka and Turcana sheep breeds. *Acta Biochim Pol* 62(2):273-280
- Leeds TD, Notter DR, Leymaster KA, Mousel MR, Lewis GS (2012) Evaluation of Columbia, USMARC-Composite, Suffolk, and Texel rams as terminal sires in an extensive rangeland production system : I. Ewe productivity and crossbred lamb survival and preweaning growth. *J Anim Sci* 90:2931-2940
- Ligda C, Papadopoulos T, Mavrogenis A, Georgoudis A (2003) Genetic parameters for test day milk traits and somatic cell counts in Chios dairy sheep. *Options mediterraneennes A* 55:55-59
- Makovicky P, Makovicky P, Nagy M, Rimarova K, Diabelkova J (2014) Genetic parameters for somatic cell count, logsc and somatic cell score of breeds : improved Valachian, Tsigai, Lacaune and their crosses. *Acta Vet Beograd* 64(3):386-396
- Marletta D, Criscione A, Bordonaro S, Guastella AM, D'Urso G (2007) Casein polymorphism in goat's milk. *Lait* 87:491-504
- Mekkawy W, Roehe R, Lewis RM, Davies MH, Bunger L, Simm G, Haresign W (2009) Genetic relationship between longevity and objectively or subjectively assessed performance traits in sheep using linear censored models. *J Anim Sci* 87:3482-3489
- Menzies PI (2011) Control of important causes of infectious abortion in sheep and goats. *Vet Clin North Am Food Anim Pract* 27:81-93
- Padeanu I (2001) Tehnologia cresterii ovinelor si caprinelor. Timisoara : Mirton, 388 p
- Padeanu I (2010) Productia de carne la ovine. Timisoara : Mirton, 189 p
- Padeanu I, Voia SO, Gavojdian D, Mircu C, Pascal C, Sauer M, Rau V, Fratila I (2012) Effect of using melatonin implants on postpartum reproductive indices in Tsigai sheep breed. *Scientific Pap Anim Sci Biotechnol* 45(2):462-465
- Petrovic MP, Mekic C, Ruzic D, Zujovic M (2005) Genetic principles relating to improvement of milk yield in sheep and goats. *Biotech Anim Husb* 21(5/6):73-78
- Rahmann G (2000) Vermarktungsanalyse für „Fleisch aus dem Naturschutz“ im Biosphärenreservat Rhön. *Ber Landwirtschaft* 78(2):248-261
- Rahmann G (2006) Do endangered sheep breeds have an advantage in organic farming? *Asp Appl Biol* 79:247-251
- Rahmann G (2013) Ökologische Schaf- und Ziegenhaltung : 100 Fragen und Antworten für die Praxis. Braunschweig : Johann Heinrich von Thünen-Institut, 281 p
- Rahmann G, Ashworth SW, Boutonnet JP, Brunori G, Papadopoulos I (2001) Opportunities and barriers for niche marketing of lamb in European LFAs based on consumer attitudes to product quality. *Agrarwirtschaft* 50(5):293-301
- Rahmann G, Ardakani MR, Barberi P, Boehm H, Canali S, Chander M, David W, Dengel L, Erisman JW, Galvis-Martinez AC, Hamm U, Kahl J, Köpke U, Kühne S, Lee SB (2016) Organic Agriculture 3.0 is innovation with research. *Organ Agric* 2016:1-29, DOI 10.1007/s13165-016-0171-5
- Rupp R, Schibler L, Cribru E, Amigues Y, Boscher MY, Mura L, Sechi T, Fraghi A, Casu S, Barillet F, Carta A (2003) Evidence of chromosomal regions controlling somatic cell counts in dairy sheep from two QTL detections projects. In: Bodin L (ed) *Third International Workshop on Major Gens and QTLs in Sheep and Goats*, 8-11 December 2003, Toulouse, France
- Sargison N (2008) *Sheep flock health : a planned approach*. Oxford : Blackwell, 465 p
- Sauer M, Padeanu I, Dragomir N, Ilisiu E, Rahmann G, Sauer W-I, Voia S-O, Gavojdian D (2015) Organic goat meat production in less favoured areas of Romania. *Landbauforsch Appl Agric Forestry Res* 65(1):59-64
- Sechi S, Salaris S, Scala A, Rupp R, Moreno C, Bishop SC, Casu S (2009) Estimation of (co)variance components of nematode parasites resistance and somatic cell count in dairy sheep. *Ital J Anim Sci* 8(2):156-158
- Sossidou E, Ligda C, Mastranestasis I, Tsiokos D, Samartzis F (2013) Sheep and goat farming in Greece : implications and challenges for the sustainable development of less favoured areas. *Scientific Pap Anim Sci Biotechnol* 46(2):446-449
- Sztankooova Z, Kyselova J, Rychtarova J, Czernekova V (2011) Technical note: A novel method for routine genotyping of the G allele of b-casein (CSN2) and T allele of k-casein (CSN3) in a sheep population using LightCycler. *J Anim Sci* 89:3843-3845
- Todaro M, Dattena M, Acciaioli A, Bonanno A, Bruni G, Caroprese M, Mele M, Sevi A, Trabalza Marinucci M (2015) A seasonal sheep and goat milk production in the Mediterranean area : physiological and technical insights. *Small Rumin Res* 126(1):59-66
- Załęcka A, Bügel S, Paoletti F, Kahl J, Bonanno A, Dostalova A, Rahmann G (2014) The influence of organic production on food quality – research findings, gaps and future challenges. *J Sci Food Agric* 94(13):2600-2604