

GENETIC STRUCTURE OF SEMI-FAT TAILED GREEK SHEEP BREEDS: I. GENE FREQUENCIES, GENETIC VARIATION AND F-STATISTICS

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Summary. The aim of this study was to develop objective criteria for the conservation and genetic improvement of indigenous semi-fat tailed sheep breeds. For this purpose, ninety-six fresh heparinized blood samples were collected, from 4, 4 and 2 flocks from the Argos, Lesvos and Chios breeds, respectively. The samples were genotyped for 12 microsatellite loci in order to assess their genetic variation and structure. All microsatellites were highly polymorphic. In total, 154 allelic variants were found with 101, 108 and 92 allelic variants within the Argos, Lesvos and Chios breeds, respectively. The greatest number of alleles ($n = 20$) was found at locus MAF70, while locus BM1824 had the smallest number ($n = 6$). PIC values ranged from 0.088 to 0.882. Mean number of alleles N_a was 8.42 ± 1.00, 9.00 ± 0.95 and 7.67 ± 0.87 for the Argos, Lesvos and Chios breeds, respectively. Mean effective allele number N_e was 4.27 ± 1.72, 4.69 ± 2.07 and 3.59 ± 1.69 for the Argos, Lesvos and Chios breeds, respectively. Mean observed and expected heterozygosity were: 0.612 ± 0.070 and 0.711 ± 0.062 for the Argos breed, 0.648 ± 0.062 and 0.760 ± 0.031 for the Lesvos breed, 0.590 ± 0.047 and 0.673 ± 0.045 for the Chios breed, respectively. Mean observed heterozygosity values were always lower than the expected Hardy-Weinberg, but there are no significant differences between these estimations within each breed, nor among breeds. Deviations from Hardy-Weinberg Equilibrium (HWE) expectations were found for four loci ($P < 0.05$ or lower), towards an excess of homozygotes. F-statistics analysis revealed significant heterozygote deficiency in the total population ($F_{IT} = 0.253 \pm 0.059$, $P < 0.001$) and within breeds ($F_{IS} = 0.143 \pm 0.041$, $P < 0.001$) as well as significant genetic differentiation among the three breeds ($F_{ST} = 0.129 \pm 0.035$, $P < 0.001$). F-statistics analysis within each breed, considering the breed as the whole population subdivided into its flocks, showed that F-values were found to be significant. F_{IT} and F_{IS} values were high, suggesting the incidence of inbreeding in each breed. For the Argos

breed, the value F_{ST} was higher, suggesting that 7.2% of the total genetic variation in the breed could be explained by differences among flocks of the breed and the remaining percentage among individuals. The relative F_{ST} values for the Lesvos and Chios breeds were found to be lower (2.7 and 3.0%, respectively), suggesting that a greater gene flow is possible among flocks of these breeds.

Keywords: *sheep breeds, microsatellites, gene frequencies, genetic variation, F-statistics*

RESEARCH ON THE MORPHOLOGICAL CHARACTERISTICS AND HEALTH STATUS OF THE GREEK SHEEPDOG IN THE PREFECTURE OF SERRES

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ABSTRACT

Ninety-five sheepdogs from 20 farms (19 sheep farms, one goat farm and one cattle farm) were recorded from 1/1/2002 until 31/8/2004 in the prefecture of Serres. The aim of this survey was to collect information on the presence of the Greek sheepdog within the prefecture of Serres as well as to identify individuals that match the morphological model of the specific breed. Information was collected concurrently on the life of this breed along with the recording of the parameters concerning temperament and health status.

In the prefecture of Serres there are 21,000 dogs, 11,075 of which are identified as sheepdogs (52%). The average number of dogs per farm is 4.5 (3-9); this number varies according to the number of animals on the farm, the season of the year and the region of the farm as far as altitude is concerned.

Survey questionnaires were completed for the 95 recorded sheepdogs and contained information about the farm (size, type (sheep, goat, cattle)) and about the dog itself (origin, colour, etc.).

Only 56 sheepdogs of various age and gender were selected from the farms and recorded in the questionnaires. The remaining 39 sheepdogs were excluded from any further analysis since they deviated from the morphological model of the Greek sheepdog. The 56 dogs were weighed, photographed and assessed morphologically and excrement samples were also taken. In their assessment, special attention was given to parameters involving their physical size (height, physical weight, etc.), head morphology, tail structure and shape as well as hair quality and length.

From the 56 dogs, 23 (41.1%) originated from lowlands and partly mountainous regions and 33 (58.9%) from mountainous regions with 26 being identified as male (46.4%) and 30 (53.6%) as

female. The average height of males was 60.6 (55-64) cm and that of females 57.4 (54-60) cm. The average physical weight was 29.2 (21-35) kg for males and 24.8 (21-27) kg for females. There was a lack of homogeneity in the morphological features of the breed and there were deviations in height and physical weight. As far as the fur is concerned, 31 dogs (55.4%) appeared to have hair of medium length while 25 (44.6%) appeared to have long hair. Concerning their colouring, we have noticed the presence of multicoloured animals; 28 dogs were black-white (50%) and 17 dogs were brown-white or light brown- white (30.35%). In addition, single-colour animals were recorded, three black (5.35%), six brown (10.71%) and two striped brown (3.57%).

The previously mentioned features are not sufficient to offer a definitive description of a specific type of sheepdog since there were deviations in the morphology and temperament from the primitive Greek sheepdog as described in the literature. Only 15 dogs out of the 56 appear to be morphologically interesting, partly resembling the morphological model of the Greek sheepdog.

In the present survey, excrement samples collected from the 56 Shepherd dogs were analyzed in order to identify possible parasites (eggs, larvae, etc.) with 11 of the dogs (19.64%) having been found to be infected with one to three species of parasites. Seven species of parasites (three protozoa and four nematodes) were identified in the excrement samples. Simple kind of infection (one parasite) was observed in the six (10.71%) excrement samples and mixed kind of infection (more than one parasite) in five (8.93%).

Keywords: sheepdog, sheep, goat, cattle, parasites, Serres

**PERFORMANCE AND EGG QUALITY TRAITS OF LAYERS FED
DIETS CONTAINING INCREASING LEVELS OF CHICKPEA
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SUMMARY

This experiment was conducted in order to evaluate the effect of supplementation of “Amorgos” chickpea in layers’ diets on performance and egg traits. The “Amorgos” chickpea is a local variety which grows usually early in the spring in northern Greece and which is distinguished by yellow coloured flowers and seeds. The “Amorgos” variety contains 92.3, 19.5, 6.7, 9.35 and 3.22% of dry matter, crude protein, ether extract, crude fibre and ash, respectively and 1.60 and 3.0 mg/g of trypsin and hymotrypsin inhibitors, respectively. One hundred and twenty Löhmann hens (80-wks-old) were allocated at random into five dietary treatments (M, P₁₁, P₂₁, P₃₁, P₄₁), according to randomized completely blocks (RCB) experimental design. Each treatment was comprised of four replicates of six layers each. Each replicate was comprised of two adjacent cages containing three layers each. The experiment lasted 70 days. The layers of the control (M) diet were fed on a typical diet based on corn-soybean meal. The layers of the remaining treatments were fed the same diet in which soybean meal was replaced by “Amorgos” chickpea on an isonitrogenous basis, at 25, 50, 75 and 100% for treatments P₁₁, P₂₁, P₃₁ and P₄₁, respectively. All the diets were deemed isocaloric through the use of soybean oil. Feed and water were supplied ad libitum. Total egg production for each replicate was calculated daily. Feed intake was calculated for each replicate weekly and changes in mean Live Weight (LW) of layers every 15 days. Once weekly, two eggs

per replicate were taken at random (a total of 40 eggs) which were weighed individually. From these 40 eggs, 20 were chosen (one per replicate) and were used for the estimation of egg quality traits. No statistically significant differences were noticed between treatments in the performance and egg quality traits except yolk colour which was statistically ($P < 0.05$) lower in treatment P_{41} in comparison to that of control diet (M). The results of the present study showed that the “Amorgos” chickpea may be used in layers’ diets (80-wks-old) up to a level of 410g/kg by adding the necessary amount of methionine without causing any adverse effects on the performance and eggs traits of layers except in yolk colour, which was significantly ($P < 0.05$) worse in the eggs of layers with the high level of chickpea in the feed (410g/kg), versus those of the control.

Key words: layers, chickpea, performance, egg quality, traits

FATTY ACID COMPOSITION OF SUBCUTANEOUS AND PERIRENAL ADIPOSE TISSUES IN LAMBS OF A MOUNTAINOUS GREEK BREED UP TO THE AGE OF 120 DAYS

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Abstract

Animal fat is characterized by large amounts of saturated fatty acids and a low concentration of PUFA, both of which are undesirable for the human consumer. For this reason there have been many experiments and much research in the last few years in order to investigate the possibility of modification of animal fat. In some of these experiments, an attempt was made to modify animal fat by changes in the diet of the animals, and in other experiments an attempt was made at modification either by changes in the genetic potential of the animals or the weight of slaughter.

In ruminants there is no correspondence between the fatty acids from the diet and the fatty acids that are incorporated into the tissues of the animal. In order to modify the composition of fatty acids in the ruminants through the diet, it is recommended offering the fat of the diet under “protected” form. Other factors which affect the composition of fatty acids are the deposit site, the age and the degree of fattening.

In Greece, as also happens in other Mediterranean regions, all lambs that are intended for marketing are slaughtered whether just after weaning (at 45 days approximately) or after a period of fattening up to 120 days after birth in which they may be conducted in pasture but also very frequently under intensive feeding systems.

The aim of this study was to determine the proportion of fatty acids of the subcutaneous and perirenal adipose tissues in lambs of a mountainous Greek breed slaughtered at different ages. Fifty six lambs of this breed were distributed into seven groups of eight lambs and were slaughtered at the ages of 30, 45, 60, 75, 90, 105 and 120 days.

The proportion of fatty acids was different between the two adipose tissues and as age increased. Important relations between tissues and ages for the proportion of saturated and unsaturated fatty acids were also observed. The proportion of long chain fatty acids (C₁₈–C₂₀) was significantly higher in the perirenal tissue, (P<0.001) for the ages of 30, 45 and 120 days; (P<0.01) for the age of 60 days, and (P<0.05) for the ages of 90 and 105 days. The proportion, also, of unsaturated fatty acids was considerably higher in the subcutaneous adipose tissue at the ages of 75, 90, 105 and 120 days (P<0.05). At the age of 30 days the lambs were solely fed on milk and the fatty acids of which are incorporated directly into the fatty tissue. It result that the proportion of fatty acids in suckling animals depends upon the composition of the

milk. With the increase of age the intake of solid food begins and after the age of 45 days is the sole food for the lambs. At the same time, in the rumen we have the production of a significant amount of volatile fatty acids and especially the acetic which is used in the composition of long chain fatty acids in the fatty tissue. Thus we have the gradual increase of long chain fatty acids in both tissues.

The proportion of fatty acids of small and medium length chain (C_6 – C_{16}) was decreased while that of long chain fatty acids (C_{18} – C_{20}) was increased with the increase of age of slaughter in both subcutaneous and perirenal adipose tissues ($P < 0.05$). From long chain fatty acids, the proportion of C_{18} was increased ($P < 0.01$) only in perirenal and the $C_{18:1}$ (n-9) was increased ($P < 0.001$) only in the subcutaneous adipose tissue and this difference may explain the different melting point between subcutaneous and perirenal tissue, while the $C_{18:2}$ (n-6) increased in both tissue depots ($P < 0.05$). Finally, there was an obvious increase ($P < 0.05$) in the proportion of fatty acid trans- $C_{18:1}$ (n-7), $C_{18:3}$ (n-3) and CLA in both tissues as the age of slaughter increased. Particularly, in perirenal adipose tissue the observed values for the seven ages were for $C_{18:2}$ (n-6) %: 2.08, 2.43, 3.58, 2.66, 3.02, 2.91 and 3.60 ($P < 0.05$), for $C_{18:3}$ (n-3) %: 0.26, 0.36, 0.54, 0.41, 0.40, 0.38 and 0.56 ($P < 0.05$) and for CLA %: 0.03, 0.11, 0.18, 0.10, 0.11, 0.11 and 0.13 ($P < 0.05$) and in subcutaneous adipose tissue were for $C_{18:2}$ (n-6) %: 1.07, 2.01, 2.87, 2.78, 2.83, 2.77 and 3.00 ($P < 0.001$), for $C_{18:3}$ (n-3) %: 0.21, 0.35, 0.45, 0.45, 0.31, 0.45 and 0.44 ($P < 0.05$) and for CLA %: 0.02, 0.17, 0.19, 0.17, 0.17, 0.10 and 0.13 ($P < 0.05$).

The results of this study show that an increase in the age of slaughter has a positive effect on the proportion of unsaturated fats as well as on the proportion of polyunsaturated fatty acids. Also, the subcutaneous adipose tissue can be characterized, after the age of 75 days, as the most desirable for the consumer, due to an increase in the proportion of the unsaturated fatty acids in relation to the perirenal adipose tissue. To determine the most appropriate date for slaughter, however, other factors which concern the slaughter have to be taken under consideration.

GENETIC STRUCTURE OF SEMI-FAT TAILED GREEK SHEEP BREEDS II. GENETIC RELATIONSHIPS AMONG BREEDS AND USE OF MICROSATELLITE GENOTYPES FOR BREED ASSIGNMENT

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Summary. The aim of this study was to investigate the genetic relationships among Greek semi-fat tailed and two foreign sheep breeds. The analysis of genetic relationships was performed according to two different procedures: A) Firstly, using allele frequencies from all microsatellites for the entire population, chord distance (D_C) of Cavalli - Sforza and Edwards (1967) was estimated and dendrograms were constructed with the PHYLIP (3.5 version) computer package (Felsenstein, 1989). Calculated genetic distances D_C (chord distance of Cavalli - Sforza and Edwards (1967)) were found to be smaller between the Argos and Lesvos breed (0.0496) and larger between the Friesian and Awassi (0.1282). Dendrograms based on D_C distances were constructed by Neighbour - Joining and UPGMA methods and clearly showed that the Argos and Lesvos breeds are more closely related to each other than to Chios and foreign breeds. B) Secondly, by treating the animals of each breed as a taxonomic unit, "individual" trees were constructed by using as the distance measure between individual animals the quantity $D_{ps} = 1 - P_s$, where P_s is the proportion of shared alleles summed over loci / (2 x number of loci compared) (Bowcock et al., 1994). "Individual" trees were constructed by Neighbour - Joining and UPGMA methods. In these trees, definite clusters can be distinguished and the grouping of individuals to their breed of origin was clear with few exceptions, where an admixture of some animals from the Argos and Lesvos breeds can be observed. Breed assignment of individuals based on multilocus genotype data identify the correct breed with high incidence using two different approaches. The first approach (programme WHICHRUN4.1) assigned individuals from the Argos, Lesvos, Chios, Friesian and Awassi breeds to their breed of origin with percentages of 90.6, 90.6, 100.0, 100.0 and 95.0%, respectively ($P \leq 0.01$), while the second approach (programme GENECLASS2) also gave high percentages of correct assignments varying from 80.0

to 100.0%, with the best scores (100.0%) being computed with Bayesian methods using the algorithm of Paetkau et al. (2004).

Key words: sheep breeds, genetic distances, dendrograms, breed assignment

**EFFICIENCY OF MILKING MACHINES FOR DAIRY EWES
IN CENTRAL MACEDONIA, GREECE***

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SUMMARY. The sheep production is the main sector of livestock production in Greece. During the last decade machine milking of ewes was used in many flocks mainly in the lowland regions. The aim of this work was to study the efficiency of different types of milking machines for dairy ewes in the region of Central Macedonia, Greece. Thirty-nine dairy sheep farms were used. The average size of the farms was above 200-300 ewes in milk. In each farm 5 visits were carried out during the first 5 months of the milking period of ewes (one visit per month). All the visits were realized during the evening milking. Two questionnaires were used for registering different measures and technical data in the milking parlors such as the time of starting and finishing of milking, the time of entering and exit of ewes in the parlors, the time of milking of each ewes' group (machine milking and machine stripping), the time of hand stripping of ewes' groups (in cases that was applied), the quantity of milk milked per hour, the farm size, the ewes breeds, the milking machines type and size etc. Of the total of milking parlors 46.15% was of «Casse system» (1 x 24, 2 x 24, 1 x 30 and 2 x 30), 43.59% of modern «Casse system» or «Fast exit system» (1 x 24, 1 x 32, 2 x 32 and 2 x 33), 5.13% were of Rotary type (Carrousel) and 5.13% of buckets system. The average parlor throughput per hour in the Rotary milking machines was varied from 279.3±18,6 to 388.2 ±29.3 ewes, while the number of ewes milked per hour and per milker was varied from 64.7±2.3 to 93.1±1.2. In the fast exit milking machines the average parlor throughput per hour was varied from 127.5±7.5 to 256.9±18.5 ewes. The number of milked ewes per hour and per milker was varied from 62.8±2.4 to 127.5±7.5. In the «Casse system» parlors the average throughput per hour was varied from 117.9±10.1 to 258.4±19.8 ewes per hour. The number of milked ewes per milker and per hour was varied from 57.0±1.6 to 130.8±6.8. In

the buckets system milking parlors the average throughput per hour was varied from 44.7 ± 1.7 to 56.8 ± 1.2 ewes. The number of milked ewes per milker and per hour was varied from 28.4 ± 0.6 to 44.7 ± 1.7 . The main causes of low or medium efficiency of milking machines for dairy ewes in Central Macedonia, Greece are: the great variability inside the flocks and the breeds of ewes as regard the milk yield, the function of milk emission reflex, the conformation and anatomical and morphological characteristics of the udders and the teats. The above factors lead to overmilking of some ewes and oblige operators to apply the hand stripping that is a very time-consuming procedure. Hand stripping suppression in some cases increased milking machines' efficiency by 45.3% and decreased the total milking time by 33.6%. Furthermore, the ability and skills of operators during the milking procedure, the habituation of the animals to the milking machines, the regulation and the regular service of milking machines influenced their efficiency. The total milking time of ewe flocks was varied from 1.2 to 2.2 hours. This parameter is in normal limits having in mind that the farmers are applying time-consuming milking routines. The average throughput of milking parlors was increased significantly by the increase of the number of milking places of machines. Also, this parameter was improved significantly by using milking machines with the ratio milking places:milking units 1:1 instead of 2:1.

Key words: milking machines, efficiency, parlors, ewes, average throughput

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MONTHLY VARIATION OF MINERALS IN ABOVEGROUND BIOMASS OF MOUNT VARNOUDAS PASTURELANDS - NW GREECE

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SUMMARY

This study was conducted to evaluate herbage production and mineral status in three different altitudinal zones in Mount Varnoudas pastures, northwestern Greece. Samples were collected during the period from May to October of the years 2004 and 2005. In order to study the effects of altitudinal zone, harvest month and sampling year on aboveground biomass production and its mineral concentration (K, Na, Ca, P, Mg, Fe, Zn, Cu and Mn), twenty-four constant experimental cages 4 m x 5 m fenced with metallic net 1.5m high in order to obstruct free – range grazing, were put in place in various openings of forest and subalpine grasslands. In each of the three altitudinal zones (first altitudinal zone: 900-1300m, second altitudinal zone: 1301 – 1700m, third altitudinal zone: 1701-2100m) eight experimental cages were placed. Each experimental cage was divided into 36 equal parts. In the beginning of each month, from May to October, aboveground biomass was collected from 6 of the 36 equal parts. Sample collection was accomplished by cutting aboveground biomass in imitation of the way in which small ruminants graze. The results showed that herbage production and trace minerals of grazable material were significantly affected ($P < 0.001$) by sampling year, growing season and altitudinal zone respectively, while macrominerals were not affected ($P < 0.05$) by altitudinal zone (except Mg). The interactions between sampling year, harvest month and altitudinal zone had no effect on both macro and trace minerals (except Fe). Mean herbage production was higher during the first year of the experimental period in all altitudinal zones. It was negatively correlated ($P < 0.05$) with Na ($r = -0.205$) as well as ($P < 0.01$) with Zn ($r = -0.326$) and Cu ($r = -0.341$) concentrations, but exhibited no significant relation between herbage production and the other mineral concentrations assayed. Concentrations of potassium (K), calcium (Ca), magnesium (Mg) and copper (Cu) were higher than the critical level suggested for deficiency in beef cows in all altitudinal zones during the whole experimental period in both sampling years. To the contrary, a significant deficiency in the concentration of sodium (Na), phosphorus (P), iron (Fe), zinc (Zn) and manganese (Mn) was found. Cattle can possibly extend their periods of adequate mineral nutrition by selectively grazing in pastures. However, supplementing minerals to ruminants has been shown to have positive effects on reproduction, immune status, disease resistance and feed intake. We suggest that a mineral supplement of Na, P, Fe, Zn and Mn should be administered during the grazing period in the experimental area.

Key words: Herbage production, mineral status, pasturelands, Mount Varnoudas, Greece