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Lineage as source of specific variance of productive traits in autochthonous sheep breeds

Genoveva Staykova*, Pencho Penchev

Agricultural Institute - Shumen, 3 Simeon Veliki Blvd., 9700 Shumen, Bulgaria

*E-mail: staikova666@abv.bg

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SUMMARY

315
() 303
2003-2012
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6 (5+1).
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(2003).
(Harvey, 1990)
1,5 (< 0,05)
(< 0,05)

Subject of the study were 315 ewes of the Copper-Red Shumen (CRS) breed and 303 ewes of the Karakachan breed (KK) borne in the period 2003-2012 on the state farm Kabiuk, near Shumen. The traits live weight at weaning, at 9 months, at 1.5 yr and at 2.5 yr, as well as yield and length of wool at 1.5 yr have been studied in 4 line groups in CRS (3 breeding lines + 1 undetermined) and 6 in KK (5 +1). The information was extracted from the pedigree books of the breeds. The data were obtained in conformity with the standard methods and guidelines in the Instruction Codex for Breeding for Preservation of the Local (Indigenous) Sheep Breeds in Bulgaria (2003). Analysis of variance was conducted on the basis of multi-factor linear models (Harvey, 1990) per each age. It was established that lineage significantly affects wool yield in CRS (< 0.05) and weaning weight in KK (< 0.05). No effect of line was observed for live weight and staple length in CRS and for weaning live

($h^2=0,211$, $h^2=0,259$,
 $h^2=0,402$, $h^2=0,168$),
 ($h^2=0,245$ $h^2=0,069$)

($h^2=0,031$, $h^2=0,059$,
 $h^2=0,315$, $h^2=0,065$),
 ($h^2=0,045$ $h^2=0,014$)

(Balevska and Petrov, 1970, 1972;
 Baulov, 1992),

ammon musimon (

weight and wool yield in KK.

The heritability of live weight at different age ($h^2=0.211$, $h^2=0.259$, $h^2=0.402$, $h^2=0.168$), wool yield and length ($h^2=0.245$ $h^2=0.069$) have low to moderate values in KK. In CRS the coefficients of live weights ($h^2=0.031$, $h^2=0.059$, $h^2=0.315$, $h^2=0.065$), wool yield and length ($h^2=0.045$ $h^2=0.014$) are low, suggesting narrowing genetic diversity in the population.

Key words: Copper-Red Shumen sheep, Karakachan breed, breeding lines, live weight, wool productivity, heritability

Abbreviations: CRS – Copper-Red Shumen sheep, and denotation of its breeding lines; KK – Karakachan breed and its lines

INTRODUCTION

Preservation of biological diversity is a chief priority of European Union and a part of the strategy against the threatening climatic changes. In this context, autochthonous breeds are an essential genetic reserve. Bulgaria possesses one of the richest gene banks in livestock husbandry, compared to the other European countries. Breeding indigenous sheep breeds is based on age-old traditions and has utmost importance in ecological, economical and social aspect for the development of many regions of the country. Bulgarian authors (Balevska and Petrov, 1970, 1972; Baulov, 1992), using different methods, have proved the high degree of genetic linkage between Copper-Red, Karakachan and other sheep breeds and confirm their common origin from the ancient Tzakel sheep breed with a common wild ancestor *Ovis ammon musimon* (European mouflon).

The Copper-Red Shumen sheep (CRS) is a local breed, its areal being the Northeast of Bulgaria, while the

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Karakachan breed (KK) is endemic for the Balkans and well adapted to the severe conditions of the mountain and semi-mountain areas. For the preservation of their genetic diversity, the only approach in these animals is pure breeding on the basis of supporting selection.

Prerequisite for the preservation of valuable local breeds is to avoid selection pressure for productivity improvement, and this necessitates testing the genetic and non-genetic effects on the main production traits. In this way, this affords the existing genetic diversity of the breed to be preserved, in the same time attaining the highest levels of productivity, as upper limit of the genetic potential of the breed.

The optimal live weight within the different physiological statuses is of utmost importance, as it directly affects profitability and the other productive traits.

Marked with relatively low economical significance but exotic in its nuances is the wool production from the Copper-Red sheep.

The wool of the Karakachan breed is specific in its fiber structure, suitable for traditional folk-style articles only – handicraft fleecy rugs and carpets, etc. In normal balance in the population, 40-50% of the animals bear the Nd gene and that renders it unique with respect to the specific fleece structure and the rug type of the wool. The peculiarities, the productivity and the status of the CRS and KK populations in Bulgaria have been research topics for a number of authors on national scale (Hlebarov, 1940; Aleksieva, 1979, 1987, 1995; Stankov, 1995; Nedelchev et al., 1995, 2004; Kafedjiev, 1997; Kafedjiev et al., 1998; Panayotov, 2003; Nikolov, 2004). More detailed studies on the Copper-Red and Karakachan sheep are reported by Boykovski (2003), Boykovski et al. (2005)

(Hlebarov, 1940; Aleksieva, 1979, 1987, 1995; Stankov, 1995; Nedelchev et al., 1995, 2004; Kafedjiev, 1997; Kafedjiev et al., 1998; Panayotov, 2003; Nikolov, 2004).

Boykovski (2003), Boykovski et al. (2005) and Staykova (2005).

and Staykova (2005).

Breed preservation on the basis of scientifically justified genetic structures necessitates information and statistically justified analysis of the genetic potential and of the factors determining its phenotypic performance. The evaluation of the effect of breeding line outlines the degree of homozygosity in the population. The dynamics of the heritability coefficients of the main productive traits brings additional information about the breed-specific genetic variability or its limitation. This motivates the present study.

The objective of the study was to evaluate the effect of breeding line on some productive traits in autochthonous sheep breeds and the genetic predetermination of the studied traits.

MATERIAL AND METHODS

Subject of the study were 315 ewes of the Copper-Red Shumen (CRS) breed and 303 ewes of the Karakachan breed (KK) borne in the period 2003-2012 on the state farm Kabiuk, near Shumen. The following traits were studied: live weight at weaning (3 months of age), and at the ages 9 months, 1.5 and 2.5 years, and the fleece performance – yield and length of wool at 1.5 years. The information was extracted from the pedigree books of the breeds. The data were obtained in conformity with the standard methods and guidelines in the Instruction Codex for Breeding for Preservation of the Local (Indigenous) Sheep Breeds in Bulgaria (2003). Live weight is measured with accuracy within 0.5 kg, the precise weaning age being recorded in days, after which is adjusted to a standard of 100 days to make data comparable. The wool yield from first shearing (18 months of age) is measured with accuracy within 0.1 kg, and the length of the staple at the respective body location – within 0.5 cm.

315
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(3), 9 , 1,5 2,5
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1,5
() (2003).
0,5 kg.,
100
(18
0,1 kg,
–

0,5 cm.

(Harvey, 1990),

$$Y_{ijklm} = \mu + A_{ijklmno} + B_{pqhij} + e_{ijklm}$$

where:

μ -

$A_{ijklmno}$ -

(3 - 4 + 1) 6 (5 + 1)

B_{pqhi} -

e_{ijklm} - $N(O, e^2)$

Student (Hayter, 1984):

$$(y_i - y_j) / S \sqrt{(1/n_i + 1/n_j) / 2}$$

where $(y_i - y_j)$ is the difference between the LSM values per the respective levels of the factor; S - quadratic deviation; n_i and n_j - the number of observations (individuals) per the respective levels.

1

28,644 kg, 9

38,915 kg, 1,5 - 47,663 kg

2,5 - 51,079 kg.

8,57 12,81%.

(Staykova, 2005)

25 %

9 - 17 %, 1,5

10 % 2,5 - 3%,

9,49% 21,06%.

Nakev (1977)

Boykovski (2003)

Analysis of variance was carried out on the basis of multi-factor linear statistical model (Harvey, 1990) for each studied trait for each breed, having the following expression:

$$Y_{ijklm} = \mu + A_{ijklmno} + B_{pqhij} + e_{ijklm}$$

where:

μ is the mean value of the trait;

$A_{ijklmno}$ - fixed effect of breeding line for all ages: 4 levels for the CRS subset (3 lines + 1 of undetermined lineage) and 6 for KK (5 + 1);

B_{pqhi} - fixed effect of year of birth: 10 levels for the complete dataset;

e_{ijklm} - residual effects, $N(O, e^2)$

The differences among the levels of the studied factors are established on the basis of the degree of Student's distribution (Hayter, 1984):

RESULTS AND DISCUSSION

The data in Table 1 show that in CRS the mean live weight at weaning is 28.644 kg, at 9 months - 38.915 kg, at 1.5 yr - 47.663 kg, and at 2.5 yr - 51.079 kg. The coefficients of variability of the trait are relatively low - from 8.57 to 12.81%. The highest variability is attributed to the differences in growth rate in dependence on the dams' milk yield. In a previous study (Staykova, 2005) was established lower performance of the trait - relatively by 25 percent at weaning, by 17 percent at 9 months, and by 10 percent at 1.5 yr, variability ranging wider - from 9.49% to 21.06%. In an earlier work of Nakev (1977) weaning weight was reported to be way lower, while Boykovski (2003) published little lower weaning weight and similar values to ours at the later ages. These differences are to be attributed to the highly significant effect of year, as a complex factor reflecting the effect of the

LS- (Staykova, 2005).
 CRS-055 CRS-93,
 18
 2,5
 (< 0,05).
 CRS-64
 18
 1. LS- (LSE)

environmental components, observed for the same flock (Staykova, 2005). The lines CRS-055 and CRS-93 are presented with positive LS-estimates for live weight at all studied ages, excluding 18 months ($P < 0.05$). The animals with unknown lineage have negative deviation from the LS mean up to 2.5 yr and significant differences between the line groups at weaning and at 18 months ($P < 0.05$). The ewes from line CRS-64 are characterized by inconsistent estimates at the different ages.

Table 1. LS-estimates (LSE) of the effect of breeding line on live weight at different age

Line	e		9		1,5		2,5	
	Weaning		9 months		1,5 years		2,5 years	
	n	LSE	n	LSE	n	LSE	n	LSE
/ Copper-Red								
CRS-055	106	1,659*	101	0,373	100	0,058	60	0,781*
CRS-64	44	-0,025	38	0,537	37	0,475*	6	- 1,239*
CRS-93	179	0,716	175	0,075	174	- 0,101	136	0,229
NL	4	-2,350*	4	-0,986	4	- 0,432*	4	0,228
μ	333	28,644	318	38,915	315	47,663	206	51,079
CV		12,81		10,39		8,57		7,14
/ Karakachan								
KK-0517	112	1,565**	101	0,319	98	0,608	82	1,186*
KK-1512	68	0,605	61	0,269	61	0,177	51	0,879
KK-2509	120	0,037	109	-0,559	102	- 0,384	85	- 0,639*
KK-3996	32	-1,443**	26	-0,469	25	- 0,089	12	- 1,053
KK-5103	12	0,510	12	0,093	12	0,034	11	- 0,541
NL	5	-1,275	5	0,349	5	- 0,345	5	0,167
μ	349	26,783	314	37,391	303	44,815	246	48,603
CV%		17,51		14,73		9,76		9,14
NL –	/ undetermined lineage;							
μ –	LS-	/ overall LS mean; ** – $P < 0,01$; * – $P < 0,05$						

1) ()
 –
 26,783 kg, 9 – 37,391
 kg, 1,5 – 44,815 kg 2,5
 – 48,603 kg.
 9,14%
 17,51%

In the Karakachan breed (Table 1) the study resulted in live weight of 26.783 kg at weaning, 37.391 kg at 9 months, 44.815 kg at 1.5 yr and 48.603 kg at 2.5 yr. The variability coefficients of the trait are between 9.14 and 17.51%.

Like CRS, variability is highest for the pre-weaning age due to the maternal

Staykova (2005) - 32 %
 1,5 - 10 %
 10 %
 9 - 25 %
 2,5 -
 8,75% 20,87%.

Odjakova (1994), Kafedjiev (1997)
 Genkovski (2002)

(Staykova, 2005).

12

LS-
 KK-0517
 KK-1512
 KK-5103 (1),
 KK-3996
 25,340 kg -
 KK-0517
 11%
 (< 0,01).

2,5
 5%.

2) 3,015 kg
 13,979 cm,
 15,04% 18,98%
 (Staykova,
 2005)
 - 3,316 kg

Tonchev
 (1924) Ganchev (1926)

effect. Staykova (2005) established performance of the trait that was lower by 32 percent at weaning, by 25 percent at 9 months, and by 10 percent at 1.5 and 2.5 yr, the variability ranging wider – from 8.75% to 20.87%.

In earlier studies, Odjakova (1994), Kafedjiev (1997) and Genkovski (2002) reported even lower values, presumably due to the highly significant non-genetic effects on this trait (Staykova, 2005).

For the particular Karakachan flock, subject of the present study, there is a trend for increasing live weight at all ages for a period of 12 years. Evidently, the good management of the two flocks affords the upper limits of the genetic potential for live weight of the two breeds to be reached, also contributing for the reliability of the assessments aimed herein.

At weaning, best and positive are the LS-estimates of the animals from line KK-0517 (< 0.01, < 0.05) followed by KK-1512 and KK-5103 (Table 1), while the ewes from KK-3996 are marked with lowest live weight of 25.340 kg – by 11 % relatively lower than KK-0517 (< 0.01). But after the maternal effect is eliminated, the live weight values of the progenies of all KK lines gradually approximate – the relative differences at 2.5 yr being below 5 %. The animals with undetermined line belonging are characterized by inconsistent values without statistical significance.

The mean wool yield from first shearing in CRS (Table 2) is 3.015 kg and the mean wool length is 13.979 cm, the variability coefficients being 15.04% and 18.98% respectively. Previously accomplished our studies (Staykova, 2005) have established little higher wool yield with much higher variability and wool length with similar values of phenotypic performance and variability.

Ivanov (1957) and Nakev (1977)

Ivanov (1957) 3,745 kg
 Nakedev (1977)
 Aleksieva (1984)
 Hlebarov (1940)

published values close to our mean wool yield. The performance by Alexieva (1984) is considerably higher – mean wool yield 3,745 kg, while in the earliest works it is lower (Tonchev, 1924; Ganchev, 1926). As for the staple length, Hlebarov (1940) and Ivanov (1957) resulted in little higher values.

2. LS- (LSE)
 1,5

Table 2. LS-estimates (LSE) of the effect of breeding line on wool productivity at 1.5 years of age

Line	Greasy wool yield, kg		Staple length, cm	
	n	LSE	n	LSE
/ Copper-Red				
CRS-055	65	0,124	65	0,873
CRS-64	13	0,322	13	0,172
CRS-93	156	0,114	156	0,492
NL	4	- 0,561	4	- 1,536
μ	238	3,015	238	13,279
CV	15,04		18,98	
/ Karakachan				
KK-0517	87	- 0,035	87	0,593
KK-1512	47	0,062	47	- 0,642
KK-2509	80	- 0,092	80	- 0,221
KK-3996	11	- 0,057	11	1,092
KK-5103	12	- 0,042	12	- 1,099
NL	5	0,164	5	0,277
μ	242	2,978	242	21,644
CV%	16,55		17,71	

NL – / undetermined lineage;
 μ – LS- / overall LS mean; P> 0,05

KK-5103 (2) KK-2509
 2,978 kg
 1,5
 21,64 cm,

In the Karakachan breed, the lines KK-2509 and KK-5103 (Table 2) are characterized by negative estimates of the two traits, while the other KK breeding lines have inconsistent low LS-estimates without statistically proved differences among. These results deny any lineage-related variation of the traits of wool productivity. In 1.5-year old ewes, our study established 2.978 kg mean wool yield and 21.64 cm mean staple length, with respective variability of 16.55% and 17.71%. Previous our studies (Staykova,

16,55% 17,71%.
(Staykova, 2005)

Kafedjiev (1997)
Tyankov et al., (2003),
Hinkovski et al. (1984)
Aleksieva (1979),
Odjakova (1994) Genkovski (2002),
Kafedjiev (1997)

(3)

(Staykova, 2005).
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49,9 58,9 %

2005) resulted in similar to the present values for the two traits of wool productivity, as well as those of Kafedjiev (1997) for wool yield and of Tiankov et al. (2003) for staple length. Lower are the values established by Hinkovski et al. (1984) and Alexieva (1979), and higher than the presently studied wool performance are the estimates of Odjakova (1994) and Genkovski (2002), and also of Kafedjiev (1997) for wool length.

The results of the analysis of variance (Table 3) indicate that in the Copper-Red sheep breeding line is not a significant source of variation of live weight, in contrast to the established effect at an earlier stage of the same flock (Staykova, 2003). Evidently, for the period of 12 years occurred diminishment of the genetic effect and prevalence of the environmental factor on live weight at different ages. The coefficients of determination show that the constructed models explain 49.9 to 58.9% of the variance of the trait.

3. F-

Table 3. F-values of significance of the factor breeding line from the analyses of variance of the productivity traits

Traits	Copper-Red				Karakachan			
	df	F	P	R ²	df	F	P	R ²
, kg / Live weight, kg								
9 / Weaning	3	2,021	n.s.	0,505	5	1,871	*	0,637
1,5 / 9 months	3	0,248	n.s.	0,589	5	0,558	n.s.	0,701
2,5 / 1.5 years	3	0,169	n.s.	0,510	5	0,624	n.s.	0,613
9 / 2.5 years	3	0,612	n.s.	0,499	5	1,755	n.s.	0,535
/ Wool productivity								
Wool yield, kg	3	3,099	*	0,598	5	0,692	n.s.	0,419
Staple length, cm	3	1,048	n.s.	0,291	5	1,105	n.s.	0,447

As for the wool productivity, lineage has caused variation only in

1,5	(< 0,05). (Staykova, 2005)	-	greasy wool yield at 1.5 yr (< 0.05). In the previous study (Staykova, 2005) paternal breeding group affected significantly wool yield at 2.5 yr (< 0.001).
2,5	(< 0,001).	-	The model for wool yield resulted in sufficiently high determination coefficient of $R^2 = 0.599$, while the reliability of that for staple length is low.
$R^2 = 0,599,$		-	
	3,	-	As Table 3 indicates, live weight of Karakachan breed is affected significantly by lineage at weaning age only (< 0.05). This finding is in disagreement with the previously established highly significant effect of breeding line on live weight up to 3.5 yr (Staykova, 2005).
(< 0,05).	(Staykova,	-	
2005) –		-	
	3,5	-	The observation for reduction of the genetic effect in the recent 12 years and the presumption for dominating role of the environmental factor apply to this breed as well.
12		-	
	$R^2 = 0,535$	-	The determination coefficients for the four studied ages range from $R^2 = 0.535$ to $R^2 = 0.700$, explaining relatively high portion of the variation.
$R^2 = 0,700,$		-	
		-	
1,5	(3)	-	There is no variation in the traits wool yield and staple length reliably caused by lineage (Table 3) in this study. In a previous study (Staykova, 2005), paternal breeding group played highly significant effect on wool yield at all ages (< 0.001) and significant effect on natural staple length at 18 mo (< 0.05).
	(Staykova, 2005)	-	
		-	
(< 0,001)		-	
(< 0,05)	18	-	In their book summarizing the available information for the KK population in Bulgaria, Boykovski et al. (2005) also established significant effect of line on wool productivity.
Boykovski et al. (2005)		-	
		-	
		-	Studies on heritability of different economic traits in the nationally bred autochthonous sheep breeds are scarce in the agricultural literature. This is due to

1,5
0,402

(
($h^2 = 0,315$

h^2

18
 $h^2 = 0,014$)
($h^2 = 0,069$ $h^2 = 0,245$).

(Staykova, 2005)
 h^2

12

(Staykova, 2005),

the fact that in these breeds is practiced supporting selection with no intensive pressure towards production improvement.

The heritability coefficients for live weight in both breeds are relatively low (Table 4), except for those at 1.5 yr ($h^2 = 0.315$ for CRS and $h^2 = 0.402$ for KK). At this age, after the maternal influence is off, the genetic potential of an individual can manifested.

The data indicate also very low h^2 estimates for the traits wool yield and natural staple length in the Copper-Reds ($h^2 = 0.045$ and $h^2 = 0.014$) and low to moderately low in the Karakachan ewes ($h^2 = 0.069$ and $h^2 = 0.245$).

The results regarding heritability show the decisive impact of environment on productive performance, in confirmation to the analysis of variance. In a previous study on the two breeds, Staykova (2005) established higher heritability coefficients of live weight in Copper-Red and Karakachan.

The comparison with it implies that a definite change has occurred during the 12-year period. The supporting selection on the basis of pure line breeding is to preserve the genetic diversity. Though, decrease in the genetically determined variance is apparent in the productive traits, to an extent depending on the nature of the trait.

For the traits wool yield and natural staple length were established also higher heritability estimates in CRS (respectively $h^2 = 0.268$ and $h^2 = 0.401$) and KK ($h^2 = 0.362$ and $h^2 = 0.168$) at an earlier stage of populations' development (Staykova, 2005), in comparison to the present study.

Table 4. Heritability coefficients (h²) of the productive traits in sheep at different ages

Traits	Copper-Red			Karakachan		
	N	n	h ²	N	n	h ²
Live weight, kg / Weaning	29	333	0,031	34	349	0,211
9 / 9 months	29	318	0,059	33	314	0,259
1,5 / 1.5 years	28	315	0,315	31	303	0,402
2,5 / 2.5 years	17	206	0,065	19	246	0,168
Wool productivity						
Wool yield, kg	19	238	0,045	22	242	0,245
Staple length, cm	19	238	0,014	22	242	0,069

- The low heritability coefficients
- represent the narrowed genetic diversity, expressed by the additive genetic component of the variability, of the main productive traits. Diversifying the allelic pool by creation of new outbred breeding lines would widen the phenotypic variability of all productive traits.
- Using sires or semen from isolated regions of the country, to ensure certain genetic distances, can contribute for the population vitality.

CONCLUSIONS

Breeding line affects significantly wool yield at 1.5 yr in the Copper-Red sheep ($p < 0.05$) and the weaning live weight in the Karakachan breed ($p < 0.05$).

No significant genetically determined variance on the basis of lineage was established for live weight and staple length in the Copper-Red breed, and for the post-weaning live weights and wool yield in the Karakachan breed.

In the Karakachan breed, the heritability coefficients of the traits live weight at different ages ($h^2 = 0.211$, $h^2 = 0.259$, $h^2 = 0.402$,

<p>$h^2 = 0,168$), 18 $h^2 = 0,069$</p> <p>($h^2 = 0,031$, $h^2 = 0,059$, $h^2 = 0,315$, $h^2 = 0,065$), 18 ',</p>	<p>($h^2 = 0,245$</p> <p>($h^2 = 0,045$ $h^2 = 0,014$)</p>	<p>$h^2 = 0.259$, $h^2 = 0.402$, $h^2 = 0.168$), wool yield and staple length at 18 months ($h^2 = 0.245$ $h^2 = 0.069$) have low to moderate values.</p> <p>The genetic determination of the traits live weight at different ages ($h^2 = 0.031$, $h^2 = 0.059$, $h^2 = 0.315$, $h^2 = 0.065$), wool yield and staple length at 18 months ($h^2 = 0.045$ $h^2 = 0.014$) is low, implying reduction of the genetic variability in the Copper-Red population.</p>
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*E-mail: gercho_g@abv.bg

Fatty acid composition of milk of Tsigai and Karakachan sheep and meat of their lambs F1 crossbreeds of Awassi

Gercho Gerchev^{1*}, Nikolina Naydenova², Tsvetelina Dimitrova¹,
Gyurga Mihailova², Nikolay Markov¹

¹Research Institute of Mountain Stockbreeding and Agriculture, 5600 Troyan, Bulgaria

²Trakian University, 6000 Stara Zagora, Bulgaria

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SUMMARY

The aim of the present investigation was to study the fatty acid composition of milk fat of Tsigai and Karakachan sheep and meat from their lambs of F1 crossbreeds of Awassi. The study was conducted with two groups of 6 sheep with male lambs in Tsigai and Karakachan herds. The main milk chemical composition and lamb meat was reported (m. Longissimus, Dorzi). The fatty acid composition of milk and lamb meat was evaluated.

Saturated fatty acids in milk of Tsigai and Karakachan sheep are with short and medium chain length ranging from 68.06% to 69.72% with a myristic acid content of 9.10 and 8.58%, respectively. There is a high palmitic acid content C16: 0, which is a part of the saturated fatty acids in meat with a long

F1
6
(m.
Longissimus Dorzi)
68.06%
69.72%,
9.10
8.58%.

16:0, chain, while myristic acid content is lower (C14: 0).

(14:0).

(4.24 - 4.0%).

(4:0)

0.058), / (0.06-

(1.20-1.27%).

25.32%.

0.90 0.87, - 0.78 - 0.70.

(Σ 4:0- 11:0)

C25:0) (Σ C17iso-

2002).

(Simopoulos,

16:0

(Williams,

The total amount of poly-unsaturated fatty acids in the studied milk of both sheep breeds is relatively low and with close concentrations (4.24 - 4.0%).

The choleric type in Karakachan lambs contributes to an increased content of arachidonic acid (C4:0) in Karakachan lamb meat. PUFAs/SFA ratio (0.06-0.058) had low values of, while in the meat these ratio values were higher (1.20-1.27%).

Monounsaturated fatty acids in the meat, mainly represented by oleic acid (C18: 1), ranged from 21.92% to 25.32% in both breeds.

The atherogenic index of milk fat in both breeds is in the range of 0.90 and 0.87, while in lamb it is lower 0.78 - 0.70.

Short-chain fatty acids (C4: 0- C11: 0) are closely related in the two sheep breeds. Medium-chain acids (C C17iso-C25: 0) have an increased concentration in the milk of Karakachan sheep, and vice versa, for the long-chained ones their content is higher in Tsigai sheep.

Key words:sheep milk, milk fat, lamb, fatty acids

INTRODUCTION

Studies on the effects of different fatty acids on human health indicate that only a few are the fatty acids responsible for the negative effects on the health of consumers (Simopoulos, 2002).

Some saturated fatty acids such as lauric, myristic, and palmitic may have cholesterol effects, but only C16: 0 has a proven effect on cardiovascular disease (Williams, 2000).

2000).

(CLA),

(Mills et al., 2011).

Ulbright and Southgate (1991).

6/ 3,

(Simopoulos, 2002).

et al. (2008)

(CLA)

(Av si, Lakaune, Frieslandand, Chios), Tsiplakou et al. (2006)

F1

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The milk contains unsaturated fatty acids, including conjugated linoleic acid (CLA), the benefit of which is the reduction of total blood cholesterol, anti-carcinogen, antidiabetic and immunomodulatory effect (Mills et al., 2011). It is also important to compare the content of saturated and unsaturated fatty acids by calculating the atherogenic index proposed by Ulbright and Southgate (1991). Another way to evaluate the fatty acid composition of milk is the ratio of omega 6/omega 3, which has antagonistic physiological functions necessary for the human body (Simopoulos, 2002).

Many studies also address the various factors influencing the content of individual fatty acids in milk such as breed, nutrition, lactation stage, and so on. Signorelli et al. (2008) did not detect differences in the content of CLA and polyunsaturated fatty acids in local Italian breeds unlike monounsaturated acids. Investigating the milk fat composition of four sheep breeds (Avasi, Lakaune, Frieslandand, Chios), Tsiplakou et al. (2006) found that the breed had no effect on the fatty acid profile, whereas pasture feeding resulted in a lower saturated and higher proportion of unsaturated fatty acids.

The aim of the investigation was to study the fatty acid composition of milk fat of Tsigai and Karakachan sheep and meat from their lambs F1 crossbreeds of Awassi.

MATERIAL AND METHODS

The study was conducted with two groups of 6 sheep in Tsigai and Karakachan herds of RIMSA - Troyan. In each group, six male lambs F1 were crossed by Avasis with mothers matched by type of birth. Lambs were recorded live birth weight and every 15 days to the end of the trial. The mothers were raised

15
 300 g
 3 3%,
 46.5%,
 - 89.7%,
 - 16.49%,
 2.13%, - 7.2%,
 6.71, - 0.49%,
 - 0.21%, - 0.34%.

Milko-skan.
 3
 m. Longissimus
 Dorzi
 Food-Skan.
 5
 47.5 +/- 0.05 g.

-WAX, 30 m, ID 0.25 mm,
 Film:0.25µm.
 ()

upside down during the two months of experience, fed in the morning and evening with roughage and 300 g of concentrated fodder, and during the day of grazing.

Lambs after the seventh day were fed with a starter mix consisting of: corn, sunflower sprouts SP3 3%, wheat, wheat bran, soybean SP46.5%, chalk, additives and premixes. The nutritional value is as follows: Dry matter - 89.7%, Crude protein - 16.49%, Fat - 2.13%, Crude fiber - 7.2%, Ash - 6.71, Calcium - 1.19%, Phosphor - 0.49%, Magnesium - 0.34%.

During the mammalian period, control of the milk of the ewes was carried out. The basic chemical composition of the milk is determined by Milko-skan.

At the end of the experiment, the two groups of male lambs slaughtered 3 units for slaughter analysis. From each animal was sampled meat from m. Longissimus Dorzi for determining the qualitative indicators. The tests for the quality characteristics of the meat samples on the relevant indicators were carried out on a Food-Skan apparatus. Under the "Fragility" metric, the measurement is in the penetrometer units for 5 sec. at the weight of the puncture needle - 47.5 g +/- 0.05 g.

The fat extraction from the milk samples and from the meat was carried out in the Laboratory of the Department of Dairy at the Faculty of Agriculture of the Thracian University - Stara Zagora by the method of Rosa-Gottlieb. The methyl esters of the fatty acids are separated by means of a gas chromatograph. The fatty acid composition was determined by a gas chromatograph with a flame ionization detector and a ESTM-WAX column, 30 m, ID 0.25 mm, Film: 0.25 µm.

The atherogenic index (AI) is calculated on the basis of the content of medium chain fatty acids C12: 0, C14: 0

$$\begin{aligned}
 &12:0, \quad 14:0 \quad 16:0, \\
 &\quad \quad \quad (\quad) \\
 &\quad \quad \quad (\quad) \quad \text{Ulbricht} \\
 &\text{and Southgate (1991):} \\
 &\quad \quad \quad 12:0 + 4 \quad 14:0 + \quad 16:0 \\
 &= \frac{\quad}{\quad} +
 \end{aligned}$$

Statistica for Windows (Release, 4.3, Stat. Soft. Inc., 1994),

t-
 1 e
 (<0.05) (<0.05)
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1.

Table 1. Chemical analysis of sheep milk and lamb

/Breed	Dry matter %	Milk fat %	Protein %	Dry matter %	Fat %	Protein %	Collagen %
	X+Sx	X+Sx	X+Sx	x+Sx	x+Sx	x+Sx	x+Sx
	/milk			/meat			
/Tsigai	15.93+0.43	4.72+0.37	5.25+0.13	23.56+0.74	2.14+0.36	20.59+0.45	1.39+4.18
/Karakachan	17.83+0.57	5.91+0.53	5.89+0.25	23.93+0.45	2.48++0.47	20.75+0.62	1.41+0.37

1 -
 m. Longis. Dorzi. -
 : -
 , -
 . -

and C16: 0, and the groups of monounsaturated (MUFAs) and polyunsaturated fatty acids (PUFAs) according to Ulbricht and Southgate (1991):

$$\text{AI} = \frac{12:0 + 4 \quad 14:0 + \quad 16:0}{\text{MUFAs} + \text{PUFAs}}$$

Data are statistically processed through Statistics for Windows (Release, 4.3, Stat, Soft, Inc., 1994), and the mean values are compared to the Stuttgart-Fisher t-criteria tables.

RESULTS AND DISCUSSION

Table 1 follows the composition of sheep's milk and lamb. It was found that the milk of the Karakachan sheep is higher in dry matter (p <0.05) and in protein (p <0.05) and in a tendency for a higher percentage of fat compared to Tsigai. Although all of the indicators are in favor of the milk of Karakachan breed, the growth in the cross-lamb is lower than that of Tsigai crossbreeds.

Table 1 presents the quality characteristics of the lamb sample from m. Longis. Dorzi. With respect to indicators: dry matter, water content and collagen, significant differences between lambs are not established. Differences are recorded in the fat, protein and mineral content in favor of the meat of Karakachan crossbreeds, with no confidence in the respective groups of

2
(4:0),
(3.35 %)
(6:0)
2.40 %.

lamb.

Table 2 shows the results for the saturated fatty acid content in the milk of Tsigai and Karakachan sheep. In case of short-chain fatty acids, incl. fatty acid (C4: 0), close values were observed, with the higher content (3.35%) in Karakachan sheep. Concentration of capronic acid (C6: 0) is higher in Tsigai sheep and lower in Karakachan - 2.40%, respectively.

2. , g/100 g
Table 2. Saturated acids, g/100 g fat in the milk and in the lambs mean

Fatty acids	/Saturated fatty acids			
	/Tsigai		/Karakachan	
	x	Sx	x	Sx
	/milk			
C4:0	3.29	0.135	3.35	0.141
C6:0	2.54	0.117	2.40	0.101
7:0	0.01	0.004	0.035	0.007
C8:0	2.97	0.173	3.47	0.146
9:0	0.07	0.021	0.21	0.020
C10:0	8.36	0.261	8.13	0.214
11:0	0.06	0.011	0.49	0.027
C12:0	3.16	0.096	3.47	0.095
C13:0	0.11	0.021	0.129	0.007
C14:0	9.10	0.235	8.58	0.195
C15:0	1.40	0.353	1.33	0.098
C16:0	25.33	0.171	26.11	0.284
C17:0	1.60	0.115	0.83	0.033
C18:0	10.01	0.48	10.46	0.205
	/meat			
	F1 & F1crossbreeds Tsigai & Awassi		F1 & F1crossbreeds Tsigai & Awassi	
14:0	3.36	0.51	2.61	0.42
16:0	28.10	0.62	27.89	0.39
18:0	11.75	0.33	11.57	0.90
20:0	0.73	0.13	0.76	0.17

(7:0)
(<0.1).
(8:0) -

In enanthic (C7: 0) acid, the values of Karakachan sheep are higher, the differences with the milk of Tsigai sheep are reliable (p <0.1). Similar is the difference in caprylic acid (C8: 0) - in both breeds ranging from 2.97-3.47%, with

2.97-3.47%,
(<0.1)

(9:0)
(0.07 0.21)
(<0.001).
(10:0)

13:0

(<0.1).

(12:0)

(14:0)

Mihaylova et al. (2008)
(6.55±0.29 10.11±0.29)
(Gerchev et al., 2011) (9.09±0.64 12.29±0.77).

- 15:0,

17:0 (<0.01)

(16:0),

(18:0),

(16:0).
- 16:0 18:0,

(Mihaylova et al.,
2008), 21.02±0.49 9.24±1.19
(Gerchev et al.,
2011), 26.24±0.74

credible differences (p <0.1) in favor of milk from Karakachan breed. While in pelargonic acid (C9: 0) the quantities are minimal (0.07 and 0.21) and the differences are highly reliable (p <0.001). For capric acid (C10: 0) the results are close among breeds. The content of C11: 0 and C13: 0 is in minimum concentrations during the trial and the differences are in favor of the milk of Karakachan sheep (p <0.1).

Lauric acid (C12: 0) is an acid with antimicrobial activity, close values are observed between the tested breeds. The myristic acid content (C14: 0) in the milk of both breeds is almost the same. This acid determines the smell of milk and meat for the animal species concerned. The observed values are similar to those in the milk of Karakachan breed studied by Mihaylova et al. (2008) (6.55 ± 0.29 to 10.11 ± 0.29) and those in Srednostaroplaninska sheep breed (Gerchev et al., 2011) (9.09 ± 0.64 to 12.29 ± 0.77).

Saturated fatty acids with an odd number of carbon atoms - C15: 0, are of close proximity to both breeds, with differences in C17: 0 (p <0.01) in favor of Karakachan sheep. Palmitic acid (C16: 0) has the highest values for the four groups - sheep and lambs, with no credible differences between breeds. This acid affects the elasticity and strength of the skin.

For stearic acid (C18: 0), which determines to some extent the hardness of the fat and its melting temperature, the trend is similar to palmitic acid (C16: 0). Concentrations of saturated C16: 0 and C18: 0 fatty acids in the sheep milk of the two groups of sheep are significantly higher than those of Karakachan breed (Mihaylova et al., 2008), respectively 21.02 ± 0.49 and 9.24 ± 1.19 and lower than the milk of Srednostaroplaninska breed sheep (Gerchev et al., 2011), respectively 26.24 ± 0.74 and 13.03 ±

13.03±0.10.

m. Longis. Dorzi

(14:0)

(16:0),

27.89%

28.10 %

11.57%

11.75%.

(0.73-0.76%),

14:1,

(16:1)

(18:1)

-9

(18:1)

Tsiplakou et al., 2006)

18:1

CLA,

2,

0.10. It is likely that the feeding of the sheep in the individual herds during the experiment - feeding and grazing early spring - is likely to have an impact.

Looking at Table 2, we see that in m. Longis. Dorzi has established medium and long carbon saturated acids that determine the energy and taste qualities of the meat. Myristic acid content (C14: 0) is lower than that of milk, more pronounced in the meat of the Karakachan breed. The most common fatty acid in the meat is palmitic (C16: 0), where lipogenesis can produce acids with longer chains. Its content in meat is quite high at 27.89% for Karakachan crossbreeds to 28.10% for Tsigai. Stearic fatty acid is one of the main ingredients of meat. In lamb, it has close values - ranging from 11.57% to 11.75%. Arachidonic acid has a very low concentration in the examined lamb (0.73-0.76%), but plays a major role in the body as it participates in the construction of the skeletal muscles.

The content of unsaturated fatty acids - C10: 1 C12: 1 as well as C14: 1 in milk fat in both sheep breeds studied is low, with close values (Table 3). Concentration of palmitoleic acid (C16: 1) in the milk studied in both sheep breeds is high and close, whereas C16: 2 is lower in Karakachan breed.

The content of oleic acid (C18: 1) in milk fat is significant but with minimal differences between breeds. It is an important part of Omega-9 fatty acid group. Typical of oleic acid (C18: 1) is the high percentage at the beginning of the grazing period. In many studies of milk produced during the grazing period (Atti et al., 2006; Tsiplakou et al., 2006) a positive correlation between C18: 1 concentration in milk fat and the amount of CLA is found because it appears as a substrate in the synthesis of the latter.

3. , g/100 g ,
Table 3. Unsaturated acids, g/100 g fat, in the milk and in the lambs mean

Fatty acids	, g/100 g Unsaturated fatty acids, g/100 g fat			
	/Tsigai		/Karakachan	
	x	Sx	x	Sx
/milk				
C10:1	0.21	0.030	0.23	0.022
C10:2	0.11	0.008	0.08	0.020
C12:1	0.16	0.001	0.19	0.024
C12:2	0.10	0.053	0.23	0.006
C14:1	0.22	0.010	0.28	0.017
C14:2	0.23	0.035	0.12	0.009
C16:1	0.54	0.021	0.52	0.033
C16:2	0.23	0.027	0.15	0.029
C17:1	0.61	0.054	0.71	0.042
C18:1	25.23	0.280	24.92	0.186
C18:2	2.67	0.084	2.65	0.136
C18:3	1.57	0.138	1.35	0.89
/meat				
	F1 & F1crossbreeds Tsigai & Awassi		F1 & F1crossbreeds Tsigai & Awassi	
18:1	43.72	1.00	44.22	1.84
18:2	6.04	0.01	6.70	1.11
18:3	1.26	0.16	1.12	0.23
20:4	1.79	0.13	2.50	0.25

(18:2) - (18:3), Polyunsaturated essential fatty acids - linoleic (C18: 2) and linolenic acids (C18: 3) reported a relatively high concentration but no differences in the milk of both breeds. It should be noted that the values of these acids in milk fat depend mainly on animal nutrition because they are not synthesized in the body and their absence causes a number of biological disorders. The established concentration of these two acids in the milk of Tigaki and Karakachan sheep is similar to that of Karakachan and Staroplaninska sheep raised under the conditions of the Balkan Mountain (Mihailova et al., 2008, Gerchev et al., 2011).

(3), - Unsaturated fatty acids also participate in the meat composition (Table 3), they form long chain

Karakachan, showing good oxidative stability of the milk. MUFAs have a preventive effect on coronary and cardiovascular diseases, similarly to PUFAs, but they are more unstable for oxidation due to their higher unsaturation. PUFAs protects the membranes of the cells from the oxygen radical almost as much as the tocopherol and lower than the carotene.

With regard to the total amount of saturated fatty acids in the meat, the differences are small and in favor of Tsigai lambs, with no proven reliability. For MUFAs and PUFAs, the difference between the breeds of about one percent is unreliable for both types of fats and is beneficial to Karakachan lambs. Low values of the ratio of SFA / PUFAs are 4.75%, respectively, for the meat of Tsigai lambs and 4.8 for Karakachan ones.

Short-chain fatty acids are similar in milk values to Karakachan and Tsigai sheep. The content of long-chain fatty acids is higher in the milk of Tsigai breed, while in the milk of Karakachan sheep, the average chain lengths are predominant. Short-chain fatty acids are not reported. While middle-chain fatty acids predominate in the meat of both breeds.

The content of long-chain fatty acids in the meat of both breeds is close to and is significantly lower than that of milk. As with milk and meat, no credible differences between breeds are found.

The biologically important ratio of PUFAs/SFA ratio or so-called P / S ratio in sheep's milk is low and varies within a narrow range of 0.06 for Tsigai sheep and 0.058 for Karakachan. While in the meat, PUFAs/SFA ratio is significantly higher - 1.20 in the case of Tsigai lambs and 1.27 in Karakachan ones. These values indicate that Karakachan lambs with their choleric type determine the higher content of PUFAs.

			P/S
/		4.75%	
		4.8	
			0.06
		0.058	
/			1.20
		1.27	

-6/ -3 (4). (Tsipakou and Zervas, 2008).
 0.90 0.87
 0.78 0.70.
 (Mierlita, 2012; de Renobles et al., 2012).

One of the criteria for evaluating the preventive value of milk is the atherogenic index and omega-6/omega-3 ratio (Table 4). Food with a high index and ratio are considered harmful to human health (Tsipakou and Zervas, 2008). Milk fat is generally considered to be pro-atherogenic because of the high content of saturated fatty acids (mainly lauric, myristic and palmitic). The atherogenic index is a criterion for the level and connectivity of some fatty acids that may have atherogenic properties. In the present study, the milk index is in the range of 0.90 for Tsigai breed and 0.87 for Karakachan breed, while for meat it is lower - 0.78 and 0.70, respectively. The values obtained are lower than those of other authors of sheep's milk (Mierlita, 2012; de Renobles et al., 2012).

4.

Table 4. Groups of fatty acids in the milk from sheep

Groups of fatty acids	Tsigai		Karakachan		F1 & Meat F1 crossbreeds Tsigai & Awassi		F1 & Meat F1 crossbreeds Tsigai & Awassi	
	x	Sx	x	Sx	x	Sx	x	Sx
SFA	68.06	1.96	69.42	1.44	43,21	1,46	42,07	1,71
MUFA	27.34	0.55	26.89	0.39	43,72	0,28	44,22	1,84
PUFA	4.24	0.22	4.00	0.23	9,09	0,30	10,32	1,59
C4:0-C11:0	17.37	0.77	17.47	0.58				
C12:0-C16:1	40.60	1.06	41.66	0.76	31,46	1,13	30,50	0,81
C17iso-C25:0	41.68	0.90	40.91	0.69	12,47	0,46	12,33	1,07

1.7:1
 6.21
 8.21
 20:4

The omega-6/omega-3 ratio in milk fat does not differ significantly for the two breeds: 1.7: 1 for Tsigai and 1.96: 1 for Karakachan breeds. While for meat, this ratio is higher - 6.21 for Tsigai lamb crossbreeds and 8.21 for Karakachan lamb crossbreeds, respectively.
 - These high values are due to the higher C20: 4 content in Karakachan crossbreeds. Milk data are slightly lower than the recommended healthy ratio of unsaturated fatty acids - from 2: 1 to 4: 1 (Sretenovic et al., 2009) and close to that

(Sretenovic et al., 2009) – 2:1 4:1
 (Mierlita et al., 2012.

found by other authors of sheep's milk (Mierlita et al., 2012).

69.72%, 68.06%
 9.10 - 8.58%
 16:0,
 (14:0).
 (4.24 - 4.0%).
 (4:0)
 / (0.06-0.058%),
 (1.20-1.27%).
 (18:1),
 21.92 % 25.32%.
 0.90 0.87,
 - 0.78 – 0.70.
 (Σ 4:0- 11:0)
 (Σ C17iso-C25:0)

CONCLUSIONS

Saturated fatty acids in milk of Tsigai and Karakachan sheep are of short and medium chain length ranging from 68.06% to 69.72% with a reduced myristic acid content of 9.10 - 8.58%, respectively. Saturated fatty acids in the meat are long chain and high in palmitic acid C16: 0, but with lower myristic acid content (C14: 0).

The total amount of polyunsaturated fatty acids in the studied milk of both sheep breeds is relatively low and with close concentrations (4.24 - 4.0%). The choleric type in Karakachan lambs contributes to an increased content of arachidonic acid (C4: 0) in Karakachan lamb meat. There were low values of PUFAs/SFA ratio (0.06-0.058), while in the meat these ratio values are higher (1.20-1.27%). Monounsaturated fatty acids in the meat, mainly represented by oleic acid (C18: 1), ranged from 21.92% to 25.32% in the two breeds.

The atherogenic index of milk fat in both breeds is in the range of 0.90 and 0.87, while in lamb is lower 0.78 - 0.70.

Short-chain fatty acids (ΣC4: 0-C11: 0) are closely related in both sheep breeds. Medium-chain acids (ΣC C17iso-C25: 0) have an increased concentration in the milk of Karakachan sheep, whereas for long-chain fatty acids, their content in Tsigai is higher.

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Dynamics of chemical indicators in milk of Bulgarian White Dairy breed and its crossbreeds with Anglo-Nubian and Toggenburg breeds in the lactation period

Tsvetelina Dimitrova

Research Institute of Mountain Stockbreeding and Agriculture, 5600 Troyan, Bulgaria

E-mail: c.dimitrova@abv.bg

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SUMMARY

- The main chemical indicators of goat milk were studied of three groups of animals – Bulgarian White Dairy breed (BWD) and its crossbreeds with Anglo-Nubian (BWDxAN) and Toggenburg (BWDxTG) during the lactation for a two-year period. The animals are raised in the herd at the Experimental Base of the Research Institute of Mountain Stockbreeding and Agriculture - Troyan under the same production conditions.

The milk samples milk were taken at the beginning, the middle and the end of the lactation period from each animal individually as well as mixed samples from each goat group and were examined using a Milko-Skan FT 120 apparatus.

- The goats of BWD breed has the highest milk yield for control milking, followed by the crossbreeds of BWDxTG and BWDxAN.

- The values of the content of dry matter, dry fat-free residue, protein and

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Milko-Skan FT 120.
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milk fat are higher in the milk of crossbreeds in comparison with BWD breed.

In wider variation ranges, the content of milk fat and dry matter varied, and minor variations were observed for the dry fat-free residue and the protein in the three groups.

In all three study groups of goats, the lowest results were recorded in the middle of the lactation period for the indicators of dry matter, dry fat-free residue, protein and milk fat.

Key words: goat, goat milk, dynamics, chemical composition

INTRODUCTION

Milk and dairy products have been a major part of people's diet since the beginning of domestication of livestock. Over the last decade there has been an increased interest in dairy products of goats. The amino acid composition of goat milk is the closest to human milk - its assimilability by the human body is 94-98%. Compared to cow milk, goat milk is richer in dry matter, proteins, fats, minerals (Ca, P, Mg), vitamins B1, B3. (Tziboula-Clarke, 2003; Park, 2006; Kumar et al., 2012, 2016).

Goat milk has good digestibility, alkalinity, buffer capacity and certain therapeutic characteristics that are valuable to medicine and human nutrition (Haenlein, 2007, Park et al., 2007).

The milk composition, especially the fat and protein content, determines its cheese processing potential, corresponding to the yield and organoleptic characteristics of the products (Lawrence, 1991a, Brito et al., 2002; Guo et al., 2004).

In Bulgaria, the main ingredients of milk obtained by BWD goat breed are studied by a number of authors (Balevska

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In Bulgaria, the main ingredients of milk obtained by BWD goat breed are studied by a number of authors (Balevska

(Balevska et al., 1973; Balevska and Tyankov, 1971; Zunev et al., 1999; Mihaylova et al., 2000; Petrova et al., 2000; Slavov et al., 2000; Odjakova, 2002 .)

et al., 1973, Balevska and Tyankov, 1971, Zunev et al., 1999; Mihaylova et al., 2000; Petrova et al. ., 2000; Slavov et al., 2000; Odjakova, 2002, etc.)

The aim of the present study is to track the chemical indicators of goat milk from three groups – Bulgarian White Dairy breed (BWD) and its crossbreeds with Anglo-Nubiann (BWDxAN) and Toggenburg (BWDxTG) during the milking period for two years.

MATERIAL AND METHODS

Experiments are conducted in 2015-2016 in the Experimental Base at the Research Institute of Mountain Stockbreeding and Agriculture in Troyan. In the experiment are used animals from one herd divided into three groups – Bulgarian White Dairy breed (25 animals) and its crossbreeds with Toggenburg (BWDxTG) (14 animals) and Anglo-Nubian (BWDxAN) (16 animals). These are pure-bred crossbreeds that have passed to internal breeding, in the process of establishing the desired breeding type, through various forms of selection. The system of breeding is in cattle-shed and grazing, and the animals are raised in a natural pasture of transitional type in the period of April and in a cattle-shed during the rest of the year. During the cattle-shed period, the animals were fed by meadow and lucerne hay, treated with a feed-distributing mixer for fast cutting and distribution of the roughage. The hay is put daily on a nutritional trail in the amount of 1.8-2.0 kg per head with secured access to feed, water and salt. Throughout the year, the mother goats are fed with a concentrate of 0.6 kg per day. Concentrated feed is dispensed in the milking room before milking.

The milk samples were taken for analysis at the beginning, the middle and the end of the lactation period (April-September) for two years, from each animal individually, as well as mixed

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2015-2016 .

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1,8-2,0 kg

0,6 kg

samples from each goat group, as for that purpose the animals are being milked by hand following the necessary hygienic conditions. Samples were tested in the Milk Technology Laboratory at RIMSA - Troyan.

Protein, milk fat, dry matter, dry fat-free residue and lactose are analyzed using the apparatus Milko-Skan FT 120.

The variation-statistical processing of the data is done through Statistica and the graphic one – through Excel. The mean values of the groups in the individual studies were compared according to the Student-Fisher t-criteria tables.

RESULTS AND DISCUSSION

Milk productivity of goats depends mainly on the breed, feeding, lactation period, breeding, individuality, age of the animals, climate and many other factors.

Data on milk yield for control milking and chemical composition of the goat milk for a two-year lactation period are presented in Table 1. The highest milk yields are found in goats of BWD breed compared to BWDxAN and BWDxTG, the differences being mathematically proven ($p < 0.05$; $p < 0.01$). Our results of milk yield are higher than these of Slavov et al. (2000) for BWD breed – (1.94 l) in comparison with local goats (1.58 l) and their crossbreeds – (1.70 l) raised in the region of Stara Zagora.

Table 1. Milk yield and chemical composition of goat milk average for the years
Table 1.

/ Parameters	/ Groups		
	/ BWD (n=150)	x / BWDxTG (n=84)	x H / BWDxAN (n=96)
	x±Sx	x±Sx	x±Sx
/Control milk yield, l	2.39±0.048ab	2.04±0.043b	2.06±0.046a
/ Total solids, %	12.77±0.100a	13.68±0.123a	13.76±0.136a
/ Milk fat, %	4.55±0.077a	5.13±0.100	5.28±0.099a
/ Dry fat-free residue,%	8.46±0.041b	8.84±0.047b	8.69±0.051
/ Protein, %	3.08±0.026a	3.35±0.034a	3.41±0.040a
/ Lactose, %	4.32±0.032	4.40±0.035	4.18±0.046

/ Note: a – $p < 0.05$; b – $p < 0.01$

5.28, 3.41, 8.69)
 5.13, 3.35, 8.84)
 8.16)
 8.04),
 4.18%
 (x H) 4.40% (),
 Anifantakis and Kanadarakis (1980) –
 4.33%
 1,
 II) – 2.68 I, -
 x H – 1.93 I
 (I).
 II
 (III),
 0.55%
 , 0.20%
 x H.

- Dry matter content, milk fat, protein
 and dry fat-free residue with a tendency
 - for higher results for BWDxAN (13.76,
 5.28, 3.41, 8.69) and for BWDxTG
 (13.68, 5.13, 3.35, 8.84) groups
 compared to BWD breed with differences
 within the tolerable errors of the method
 used. Our results are higher than these of
 Mihaylova et al. (2000) for BWD breed
 (11.49, 3.33, 2.96, 8.16) and meat goats
 (11.50, 3.46, 2.99, 8.04) raised in
 collection herd of Trakia University in
 Stara Zagora.

Milk sugar in the studied milk
 samples ranged in close limits – from
 4.18% (BWDxAN) to 4.40% (BWDxTG),
 and they are similar to those found by
 Anifantakis and Kanadarakis (1980) –
 4.33% for the milk of Saanen goats.

Differences in milk composition in
 the three groups are mainly due to breed
 differences as the animals are raised
 under the same conditions.

Milk dynamics during the different
 - lactation phases is shown in Figure 1,
 - where the highest values for BWD breed
 at the midpoint of lactation (II control)
 were 2.68 I and the lowest for BWDxAN –
 1.93 I in the beginning of lactation (I
 control).

I The trend is to increase the values of I to
 - II control of the milk indicator as the
 animals have gone to pasture when the
 grass was at maximum development and
 again decreasing at the end of the
 - lactation period (the third control), which
 is related to the physiological animal
 characteristics naturally reduce their milk
 productivity, and pasture itself became
 poorer. The difference between the
 - lowest and the highest milk rates is
 0.55% for the BWD breed, 0.20% for
 BWDxTG and 0.28% for BWDxAN.

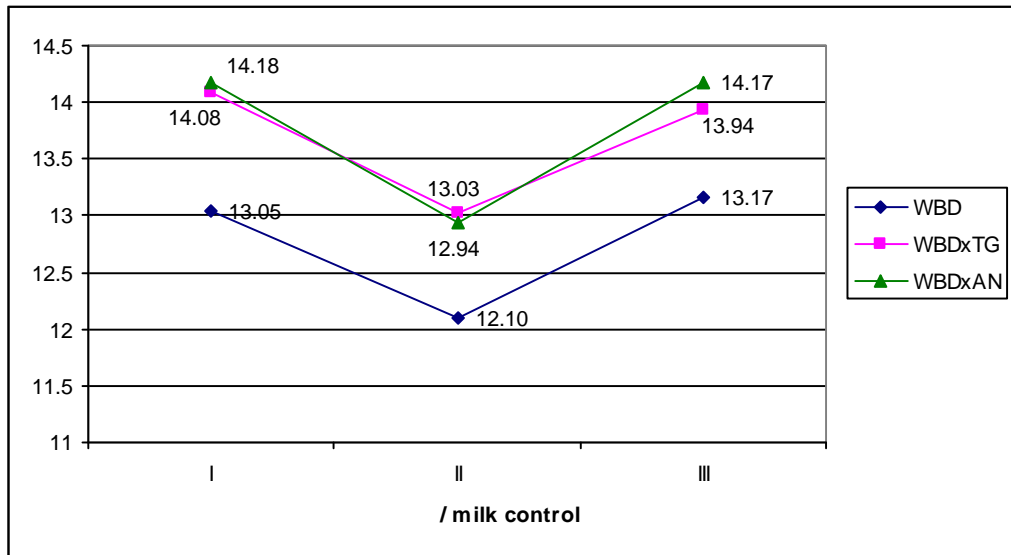


Fig. 2. Variation of the milk dry matter content, %

(3),
 4.96% x H – 5.60% III
 – 5.38% I
 - 0.87%
 , 0.67% 0.77%
 x H.
 (2002) – 3.56% Odjakova
 3,45% Terziyska
 et al. (1994) – 3.49%
 (Brozos et al., 1998; Haenlein,
 2001; 2004), (Petkova,
 1997).
 (Petkova, 1997).

The milk fat content also varies widely in the three groups (Figure 3), with the highest result reported for BWD – 4.96%; and for BWDxAN – 5.60% for the IIIrd control, while for BWDxTG – 5.38% for the Ist control. The difference between the lowest and the highest fat content is 0.87% for BWD, 0.67% for BWDxTG and 0.77% for BWDxAN. Our results are higher than those found by Odjakova (2002) – 3.56% for milk in local goat milk and 3.45% for crossbreeds raised in the Middle Rhodopes region, as well as those obtained by Terziyska et al. (1994) – 3.49% for BWD breed.

For all three groups of goats, the trend of decreasing and increasing fat in milk coincides with groups and controls, which corresponds to the season, because at the end of the lactation period the fat content is increasing (Brozos et al., 1998, Haenlein, 2001; 2004), while (Petkova, 1997). The influence of the ration during the different seasons is determined (Petkova, 1997).

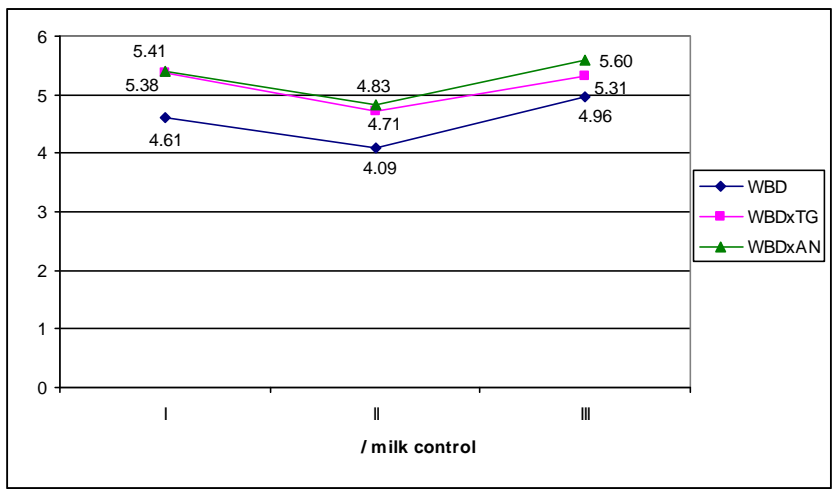


Fig. 3. Variation in the content of milk fat, %

III (8.98%) x H (8.92%), (8.61%) I 853/2004 (8.0%).

The fluctuations in dry fat-free residue are lower than the milk fat for the three tested milk (Figure 4), as the values of the IIIrd control are the highest for BWDxTG (8.98%) and BWDxAN (8.92%), and the lowest for BWD (8.61%) of the Ist control. For all three groups of goats, the milk is with dry fat-free residue higher than the requirements of Regulation 853/2004 (8.0%).

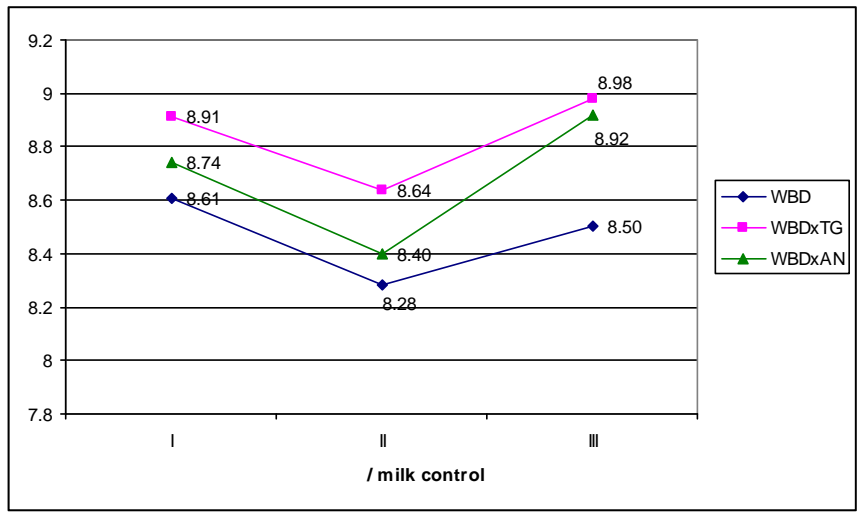
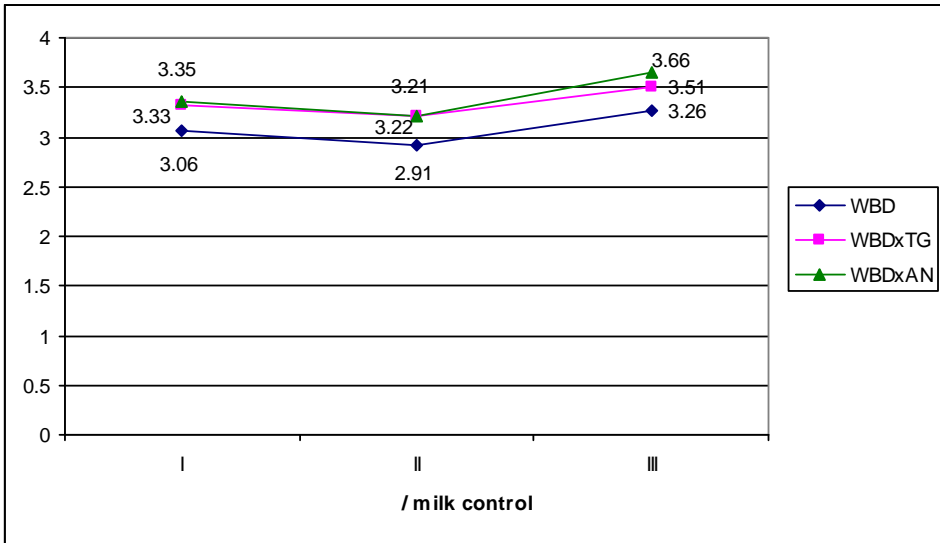


Fig. 4. Dynamics in the content of dry fat-free residue, %

0.29%
 0.35%
 0.45%
 Terziyska et al. (1994)
 - 3.11%
 Zunev (1991)
 (3.18-3.41%, 3.26%).

5. Changes in protein values are shown in Figure 5. Higher results were observed for the three groups of III control, with the difference between the lowest and highest values being 0.35% for BWD, 0.29% for BWDxTG and 0.45% for BWDxAN. Our data is similar to that of Terziyska et al. (1994) for total protein in BWD breed – 3.11% and to Zunev (1991) for the same breed (3.18-3.41%, on average 3.26%). For all three studied groups, the protein indicator tends to increase at the end of the lactation period.



5. Dynamics in the protein content, %

4.68%
 4.53%
 Kandarakis (1980)
 - 4.15-4.70%.

6. The data on lactose change in the milk controls tested are shown in Figure 6. The Ist control has the highest results in all three groups, respectively 4.68% for BWD, 4.69% for BWDxTG and 4.53% for BWDxAN, which is close to that obtained from Anifantakis and Kandarakis (1980) for lactose in the milk of Saanen goats - from 4.15-4.70%.

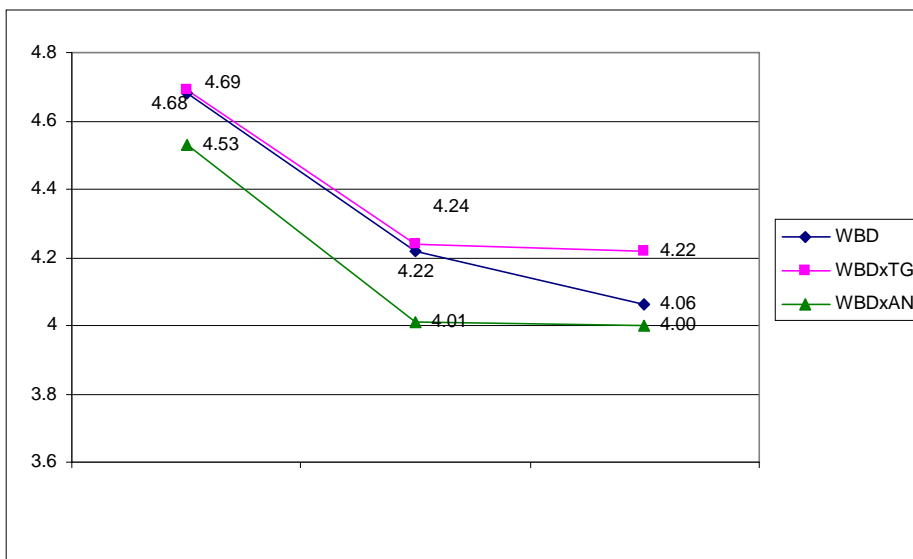


Fig. 6. Dynamics in the content of lactose, %

CONCLUSIONS

The goats of BWD breed has the highest milk yield, followed by the crossbreeds of BWDxTG and BWDxAN.

The values of the content of dry fat-free residue, protein and milk fat are higher in milk of crossbreeds in comparison with BWD breed.

The milk fat and dry matter content varied in wider variation ranges, and minor variations were observed for the dry fat-free residue and the protein in the three groups.

In all three study groups of goats, the lowest results were recorded in the middle of the lactation period for the indicators of dry matter, dry fat-free residue, protein and milk fat.

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(*Apis mellifera* L.)

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Investigation of the impact of some stimulant products on the total protein content in worker bee hemolymph (*Apis mellifera* L.)

Rositsa Shumkova^{1*}, Ivanka Zhelyazkova²

¹Experimental Station for Livestock and Agriculture - Smolyan, Agricultural Academy, Bulgaria

²Faculty of Agriculture, Trakia University, Stara Zagora, Bulgaria

*E-mail: rositsa6z@abv.bg

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SUMMARY

Results of the studies have shown that hemolymph, as the internal environment of the bee, is influenced by nutrition and can be used as a criterion for assessing the impact of applied stimulant products on feeding bee families and as a bioindicator for environmental pollution.

The study of stimulant products affecting the hemolymph composition was conducted after a spring-induction feeding of bee families in 2011 of the Experimental Apiary of the Experimental Station for Livestock and Agriculture (ESLA), Smolyan, and of a beehive of the Bee Farm Mitevi in the town of Smolyan.

For feed to the bee families, the products "Vita feed gold" (includes natural beetroot extract and molasses in distilled water),

"Vita feed gold" (

(Lazarov et al., 2016).

(Zhelyazkova and Gurgulova, 2000; Zhelyazkova et al., 2004; Balkanska, 2017).

20) -
(Slowinska et al., 2016).

Apis mellifera L.

“Vita feed gold”, „Ecophil-”, „”, „”, „”, „”, „-7”.
„Vita feed gold”

Nosema apis.

oxygen in apart.

The immunity in bees depends exclusively on the protein content of the hemolymph (Lazarov et al., 2016).

The results of studies conducted in our country show that hemolymph, as the internal environment of the bee's organism, is affected by nutrition (Zhelyazkova and Gurgulova, 2000; Zhelyazkova et al., 2004; Balkanska, 2017). In this regard, the authors assume that bee blood can be used as a criterion for assessing the impact of applied stimulant products on feeding bee families and as a bioindicator for environmental pollution with pesticides and other poisonous substances. Antioxidant protection of the bees is age-related, with adult bees (over 20 days of age) more resistant to the toxic effect of pesticides and having higher antioxidant protection (Slowinska et al., 2016)

The aim of the study was to determine the total protein content in workers' bee hemolymphs after spring stimulation with stimulant products.

MATERIAL AND METHODS

The study included bee colonies of the local *Apis mellifera* L. bee colonized in multicoloured hives - Langstroth-Ruth.

To feed the bee families, the products "Vita feed gold", "Ecophil-P", "Apidas", "Oligophos" and "Anolyte -7" were used.

„Vita feed gold” is a liquid concentrated beverage feed consisting of natural beetroot extract and molasses in distilled water. Its purpose is to stimulate the growth and development of bee families. It has a scientifically proven effective in reducing the number of spores of *Nosema apis*. It has a positive effect in the treatment of diarrhoea. It can be used

feed gold" 990 ml
(1:1).

„Ecophil- ”

„Ecophil- ”

500 ml

1:1.

-7

+1200 mV,

() = 0,005 ÷ 0,05

: 10 ml "Vita

1:1.

5 ml

5 ml

500 ml

: 2 ÷ 7,

(ORP) = +800 mV ÷

at any time of the year but the best effect is achieved if applied in spring or autumn in weak families.

To stimulate the bee families, prepare the following solution: 10 ml "Vita feed gold" to 990 ml sugar syrup (1: 1).

„Ecophil- ” is a plant protection product with acaricide and insecticide action. It is an extract of plants containing active substances - tannins, flavonoids, sesquiterpenes, carbohydrates, azulenes, terpenoid compounds, organic acids, sugars, etc. The product also includes a stabilizer that protects it from mould growth and fermentation and provides it with a shelf life of up to one year. "Ecophil-P" keeps the taste and technological performance of agricultural products and does not accumulate in them.

Apidas is a Georgian preparation based on plant extracts. It is a dark liquid. Easily digested by bees with 1: 1 sugar syrup. Apply 5 ml of a bee family once to 500 ml of sugar syrup.

Oligophos is a Georgian preparation based on amino acids. It is a dark liquid that is easily absorbed by bees with 1: 1 sugar syrup. Apply 5 ml of a bee family once to 500 ml of sugar syrup.

Anolyt-7 is a colourless liquid with a specific odour of oxidants. It is characterized by the following parameters: pH 2 ÷ 7, Oxidation Reduction Potential (ORP) = +800 mV ÷ +1200 mV, Oxidation Concentration () = 0,005 ÷ 0,05 . The active substances in the anolyte are a metastable mixture of chloroxylenol and peroxide compounds that in the course of their relaxation alter their relative concentrations. After this period, the anolyte is returned to its original state - a low mineralized aqueous solution.

08.04.2011 06.06.2011

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• -

(1 : 1) ;

• -

“Vita feed gold” 10 ml/l

(1:1);

• -

“Ecophil-P” 50 ml/l

(1:1).

• -

“ ” - 5 ml -

500 ml .

• -

” ” - 5 ml -

500 ml .

• -

” -7“ . 1:1

.

5 l ,

500 ml 2-3 .

3-

2-

The spring stimulating family meal started on 08.04.2011. and continued until 06.06.2011. The test was conducted on two apiaries: experimental beekeeping of ESLA Smolyan and an apiary of beekeeping farm Mitevi Smolyan.

There were 7 groups - two control groups (for each apiary one) and five experienced with 5 bee families equalized by the number of bees (force), the brood and the food stocks (honey and pollen).

The nursing of bee families during the spring period was carried out according to the following scheme:

- Control group - feeding with sugar solution (1: 1) without additives;
- Experimental group - feeding with the addition of "Vita feed gold" at a dose of 10 ml/l of sugar solution (1: 1);
- Experimental group - feeding with "Ecophil-P" supplement at a dose of 50 ml/l sugar solution (1: 1).
- Experimental group - feeding with "Apidas" supplement in a dose of 5 ml once to 500 ml of sugar solution.
- Experimental group - feeding with "Oligophos" supplement in a dose of 5 ml once to 500 ml of sugar solution.
- Experimental group - feeding with "Anolyte-7". Prepare a 1: 1 solution of sugar and anolyte

Each of the bee families provided a total of 5 L of a sugar solution fed into the 500 ml per hives at intervals of 2 to 3 days.

At the end of the spring experimental period after completion of feeding on each bee family, samples of young non-flight bees. The bees were anaesthetized with diethyl ether and immediately afterwards a hemolymph was obtained according to the conventional beekeeping method – suction of haemolymph with a pasteurized pipette at the boundary between 2nd and 3rd abdominal tergite. The obtained haemolymph is poured into a small tube or eppendorf. It is imperative that the

Audit Diagnostics.

vessels in which the hemolymph is collected are kept in ice.

The obtained haemolymph samples were stored in the freezer until the assays were performed.

The total protein content in honeybee samples of worker bees from the bee families included in the study was determined spectrophotometrically with a diagnostic test of Audit Diagnostics. The analyses were carried out at the National Reference Laboratory "Bee Health" at NNNDNM Sofia.

RESULTS AND DISCUSSION

The results for total protein content in samples of hemolymph samples from beehives from ESLA apiary and beehive Mitevi - Smolyan in the spring period are shown in Table. 1.

1.

(g/l)

Table 1. Total protein content (g/l) in workers' bee hemolymph

Group	Control	„Anolyt-7“	„Vita Feed Gold“	„ cophi- “	„Apidas“	Oligophos
/ Apiary ESLA, Smolyan						
Total protein	26,93±8,27	29,49±5,56	27,86±3,26	63,21±36,84	-	-
Probability of the differences	- cophi- ***; 7- cophi- ***; VFG- cophi- *** - cophil- - ***; Anolyt-7- cophi- ***; VFG- cophi- ***					
/ Apiary Mitevi, Smolyan						
Total protein	46,48±7,18	-	-	-	50,43±9,99	54,14±12,06
Probability of the differences	- pidas*; - ligophos**; pidas- ligophos*					

* 0,05 ** 0,01 *** 0,001

(1)

e

„ cophil- “ (63,21±36,84 g/l),
2,14-2,35

From the obtained results (Table 1) for ESLA apiary, it is evident that the maximum value for total protein content in haemolymph samples is determined for bees, received the stimulant product „ cophil- “ (63,21±36,84 g/l), which is with 2,14-2,35 times higher than other groups. The reported differences are of a high degree of probability (0,001). The

(0,001).
 „Vita Feed Gold”
 (29,49±5,56 g/l; 27,86±3,26 g/l)
 (26,93±8,27 g/l).

(46,48±7,18 g/l).
 (0,05)
 50,43±9,99 g/l 54,14±12,06 g/l.

(0,01) –
 „Ecophil-P” (

„Ecophil-P” (

Varroa destructor (Glinski and Jarosz, 1987; Glinski and Kauko, 1995; Kynchev et al., 1997; Zhelyazkova and Gurgulova, 2000; Gurgulova et al., 2001; Zhelyazkova et al., 2004; Shumkova, 2016; Lazarov, 2017).

study of the bees taking the preparations „Anolyt-7” and „Vita Feed Gold” has average values (29,49±5,56 g/l; 27,86±3,26 g/l) similar to those of the control group (26,93±8,27 g/l).

In the bee families of apiary Mitevi, the minimal value for total protein content in hemolymph was found for bee samples from the control group (46,48±7,18 g/l). In the experimental groups (received "Apidas" and "Oligophos"), measurable values for the characteristic protein content of the haemolymph – respectively 50,43±9,99 g/l and 54,14±12,06 g/l. The reported differences are unreliable. With low probability (0,05) are the differences between Control Groups – „ pidas“ and „ pidas“ – „ ligophos“, but with an average degree of probability (0,01) – between Control – „ ligophos“.

The analysis of the obtained results showed an increase in the total protein content in the workers' bee hemolymph, who received the stimulant preparations with their food „Ecophil-P“ (for apiary ESLA), „ pidas“ and „ ligophos“ (for apiary Mitevi).

The results of this study confirm the findings of other Bulgarian and foreign authors that the amount of total protein in worker bee hemolymph is influenced by a number of factors (breeding and feeding technology of bee families, degree of hygiene behavior, environmental pollution, bee and brood diseases, infestation with *Varroa destructor* etc.) (Glinski and Jarosz, 1987; Glinski and Kauko, 1995; Kynchev et al., 1997; Zhelyazkova and Gurgulova, 2000; Gurgulova et al., 2001; Zhelyazkova et al., 2004; Shumkova, 2016; Lazarov, 2017).

CONCLUSIONS

It is definitely an increase in the total protein content in the hemolymph of bees workers who have received with

„Ecophil-P“, „ „ „.

their food the stimulant preparations "Ecophil-P", "Apidas" and "Oligophos".

The total protein content in worker bee hemolymph can be used as a criterion for determining the nutritional value of apiculture products used in beekeeping.

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SWOT

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SWOT analysis of the Bulgarian beekeeping

Lyubomir Lyubenov

University of Ruse "Angel Kanchev", 7017 Ruse, Bulgaria
E-mail: LLyubenov@uni-ruse.bg

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SUMMARY

- The factors of the international business environment are analyzed based on a survey of the global trends on the markets of bee products, their competitors and substitutes, and the situation on global markets. The study also covers the domestic business environment with the size and structure of domestic product markets and factor and resource markets for bee products.

- Based on the abovementioned analyses, the possibilities and threats to the Bulgarian beekeeping are determined. The sectoral business environment has been studied by analyzing the development and state of Bulgarian beekeeping. The potential for development of the domestic beekeeping sector is also reported based on a survey. As a result of these studies, the strengths and weaknesses of Bulgarian beekeeping are determined. Based on the analyzed information from the international, national and sectoral business environment a SWOT analysis is made and is synthesized in a SWOT matrix of Bulgarian beekeeping.

Key words: Bulgarian beekeeping, business environment, SWOT analysis

INTRODUCTION

The main objective of the SWOT analysis is to identify the strengths and weaknesses, opportunities and threats to the Bulgarian beekeeping because they determine its present and future condition and are a prerequisite for developing different strategies. The SWOT analysis is based on an analysis of the factors of the international and the domestic business environment from which derive the opportunities and threats in the beekeeping sector, as well as an analysis of the factors of the sectoral business environment that shape the strengths and weaknesses of the national beekeeping sector.

Given the presence of significant difficulties faced by the Bulgarian beekeeping related to the liquid and profitable realization of the bee products produced on the international and national markets (Lyubenov, 2016), it is necessary to develop marketing, innovative and other strategies for these markets that will allow it to develop its potential. The basis for the development of different strategies is the SWOT analysis. Its implementation will study the international, national and sectoral business environment of Bulgarian beekeeping.

In order to determine the opportunities and threats, the international and national business environments have been analysed by studying the trends of markets for bee products and their competitors and substitutes, the size, structure and conjuncture of international and domestic markets. To determine the strengths and weaknesses of Bulgarian beekeeping, the state of the sector and its development potential are analysed. The results of SWOT analysis of Bulgarian beekeeping are synthesized in a SWOT matrix.

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MATERIAL AND METHODS

Lyubenov, 2018)
(Lyubenov, 2016)

- Data from international and national institutions as well as publications (Lyubenov, 2017a; Lyubenov, 2018) and research by the author (Lyubenov, 2016) on the development of markets for bee and other agricultural products, both nationally and internationally, are used as information base of materials. The research methods used include the complex combination of induction, deduction, grouping, comparison, analysis, synthesis, and others.

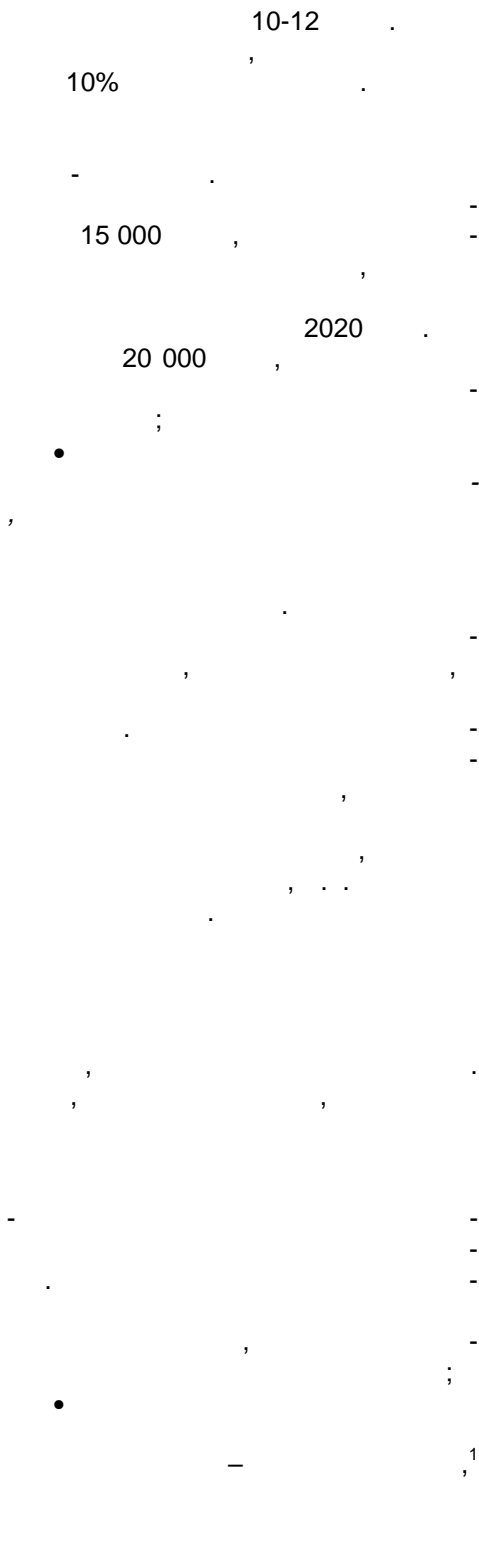
RESULTS AND DISCUSSION

(Lyubenov, 2018),

- Based on a survey on international and domestic markets for bee products (Lyubenov, 2018), the global trends, substitutes and competitors, the size and structure of international and national product markets and factor and resource markets for bee products will be synthesized:

- *The global trends in the markets for bee products* show that simultaneously with the ongoing processes of globalization, which permeates agriculture slower and more difficult, there are also opposing processes of regionalization and localization which lead to an increase in the demand for local agricultural raw materials, and respectively for bee products. A characteristic feature of agrarian markets, which also fully applies to the markets for bee products, is their high volatility, which is expressed mainly by the variability of their prices.

- This volatility will increase as a result of the current climate changes, the population growth, and the depletion of fossil fuels. Modern European, Bulgarian and other consumers prefer healthy food and would like to consume fresh,



tons of honey per year can cover about 10% of this deficit. Due to a significant deficit in the European production, the organic bee honey is one of the most sought after. Today, the European market for organic bee honey exceeds 15 thousand tons and is one of the most developed global markets. It is expected that by 2020 the annual consumption in Europe will exceed 20 thousand tons, making it a promising target market for Bulgarian beekeepers;

- *Bulgarian beekeepers should also pay attention to other markets, as European markets are extremely competitive* precisely because of high demand and good profits for traders. The European organic honey markets are the most logical choice because they are accessible, have a demand deficit and good prospects for profit. Bulgaria is one of the largest producers of certified organic honey in the EU but when beekeepers sell it mainly to wholesalers at prices of conventional honey, i.e. as a raw material, they do not earn well. On the EU markets Bulgarian honey is offered mainly as a raw material and enters into a strong price competition with global leaders such as China and the countries in South America, which is not in our favour. Japan, Saudi Arabia, the United Arab Emirates and even North America are suitable destinations for Bulgarian bee products due to their higher export prices and profits vis-à-vis domestic and European ones. The United States is a particularly good destination for Bulgarian producers of queen bees and swarms, due to the significant losses of bee colonies in the USA;

- *The Bulgarian markets for bee products* are differentiated into three main segments - organizational,² consumer and markets of factors and means of production of bee products. The

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64

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2/3

wholesalers realize the bee honey bought from the domestic organizational markets amounting to more than 54 million BGN/year on international markets. As a result their export revenues are over 56 million BGN/year, and their export earnings exceed the size of the domestic organizational markets. The domestic organizational markets for honey and other bee products that amount to 64 million BGN/year are not organized and therefore cannot reduce the risk, improve competitiveness and profits, and the opportunities to obtain higher prices for beekeeping farms because they are not involved in the creation of added value. The Bulgarian beekeepers have an economic interest in realizing on international markets the produced bee products because they will obtain better prices and profits, but they must also meet certain quantitative and qualitative requirements which requires production and marketing co-operation among them through appropriate marketing strategies;

- The domestic consumer markets of bee honey exceed 44 million BGN/year. They provide opportunities for Bulgarian beekeeping farms to obtain higher prices but require additional investment and competencies to create bee products with high added value - packaged, organic, branded, etc. The consumer markets for bee products cover three main segments - traditional and modern commerce, and the public catering sector. The traditional trade segment occupies over 2/3 of consumer markets for honey. The competition in the segment of modern trade is the most acute, and the segment of public catering is the least developed. The national beekeeping sector is a generator for the development of many other markets of production factors, such as labour, financial and production markets – pollination, centrifuges, beehives, queen bees, veterinary medicines, forages, etc. with a total value of more than 107 million BGN/year. The

107 . / .

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108 . / .

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5%.

15%,

1 . ,

60%

40%,

- proportionality between factors and means of production at the entry of the beekeeping sector of more than 107 million BGN/year, and the organizational and consumer markets at its output of about 108 million BGN/year is established. The economic effect of pollination (about 1 billion BGN/year) is commensurate with the market potential of the domestic markets for bee products.

State of the Bulgarian beekeeping sector

- The agrarian sector occupies an important place in the national economy, providing food security and a contribution to the GDP of about 5%. The relative share of the agrarian sector in the total export of the country in the recent years fluctuates about 15%, with the sector annually generating a positive trade balance of about 1 billion EUR, as opposed to industry and services with a negative trade balance. Over 60% of agrarian exports are directed to EU countries, which is driven by the more favourable economic conditions in trade on the common European market. As for crop production, grain and technical crops account for more than 40%, and for livestock breeding- meats, cheeses and bee honey.

- Bulgaria has long-standing traditions in the production of honey and bee products. The prerequisite for this on the Balkan Peninsula is the varied and rich vegetation for producing honey and creating excellent conditions for beekeeping. Our country is one of the leaders in Europe by vegetation species diversity. The favourable natural, climatic and ecological conditions are a prerequisite for the high quality of honey and bee products. Beekeeping provides alternative employment for the underprivileged rural population, and may be the source not only of additional but

also of substantial permanent income.

1. (National Beekeeping Program NBP 2017-2019, Ministry of Agriculture and Food (MAF), Agrarian Report, 2016) – 1% 5,5% 29,94%

Over the past decade, several pronounced and sustainable trends in the domestic beekeeping sector can be seen – Table 1. The first trend is aimed at increasing the bee colonies, and the other one is in the direction of reducing the number of beekeeping farms. The average honey yields per bee colony and the producer's prices show a slight upward trend. There is a consolidation and permanent increase of the professional orientation of the Bulgarian beekeeping farms. There is a significant decrease in the small farms, and the number of large professional farms with more than 150 bee families is increasing (National Beekeeping Program NBP 2017-2019, Ministry of Agriculture and Food (MAF), Agrarian Report, 2016) - from less than 1% at the beginning, to 5.5% as farms, but accounting for 29.94% as the number of hives at the end of the period being analyzed.

1. **Table 1. Number of hives and farms, average and total yields and prices of honey**

	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
Hives, thousands.	710	652	625	613	548	529	542	588	748	754
Farms, thousands	34,5	31,1	29,2	27,5	21,9	19,3	17,2	16,1	17,9	18
Yield, kg/hive	8,6	17,6	16,2	19	20	19,6	21,1	18,1	19,2	20
Honey, thousand tons	6,14	11,4	9,53	10,6	9,6	9,2	10,1	9,3	11,4	10,2
Wholesale prices, BGN/kg	3,5	4,9	4,8	4,8	5,2	4,9	5,2	4,9	5,4	5

MAF Agrostatistics Department, National Statistical Institute NSI, Eurostat

2008-2010 7,43
2011-2013 19,59
2014-2016

The subsidizing of the domestic beekeeping sector has grown significantly since the introduction of three-year National Beekeeping Programs. The first one covers the period 2008-2010 with a financing plan of 7.43 million BGN, the second one for the period 2011-2013 with a financial plan of 19.59 million BGN, the third covering the period 2014-2016 with a financial plan of 19.5 million BGN and

19,5 2017-2019
15,9
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. 2008-2010
5
70% (Agrarian Report, 2011).
2011-2013 90%.
. 2014-2016
13,3
2014 75%
. 2015 82%
(NBP 2017-2019).
. ,
. 2014-2020
. ,
.
2011 2014
. 5-8%
. ,
21%. 2014
(http://bioselena.com, 22.02.2017).
2016
21%
(www.capital.bg, 01.03.2016).
. , 2014

- the fourth one for the current period 2017-2019 with a financial plan of BGN 15.9 million. For the three completed National Beekeeping Programs, the envisaged total financial resources exceed 46 million BGN, where half of it is European funding. In spite of the dire need for financial resources in the sector, however, these funds are often reduced, and the absorption of these funds for the past programs is incomplete.

- For the first National Beekeeping Program 2008-2010, the real financial support amounted to a little more than 5 million BGN, and less than 70% of these funds were absorbed (Agrarian Report, 2011). The second National Beekeeping Program, covering the period 2011-2013, achieves absorption of funds of around 90%. For the third period of the Program 2014 - 2016, the reported amount of the financial support was adjusted to the amount of 13.3 million BGN, of which 75% is absorbed in 2014 and 82% in 2015 (NBP 2017-2019). The Rural Development Program (RDP) also offers a possibility of subsidizing the beekeeping sector, and for the current program period 2014-2020 there is some advantage for the development of organic farming, livestock breeding and smaller farms, where also belong most of the beekeeping farms.

- The strong growth of organic beekeeping in Bulgaria begins in 2011 and by 2014 the number of bee colonies grew almost four times. From 5-8% at the end of the past decade, today their share in the sector is more than 21%. Since 2014 Bulgaria is one of the world leaders in the number of certified bee colonies (http://bioselena.com, 22.02.2017). In 2016, 21% of the bee colonies in Bulgaria are certified (www.capital.bg, 01.03.2016). According to industry expert estimates, 85,000 bee colonies were developed using organic methods in 2014, and in 2016 they exceeded

85
 2016 . , 200
 .
 , ,
 21%,
 .
 179 106 .,
 2015 .,
 146 692 .,
 96 478 ., 96 367 .,
 81 583 ., 51 978
 ,, 48 470 .,
 47 118 . (FIBL, 2016; http://inteliagro.bg,
 13.04.2016).
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 2014 .
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200,000, thus placing Bulgaria back into a leadership position in the EU. Considering the total number of bee colonies in Bulgaria of about one million, it is confirmed that organic bee colonies already account for over 21%, considering that some of them are still in transition.

Bulgaria ranks first in the world with 179,106 organic bee colonies in 2015, followed by Italy with 146,692, France with 96,478, Brazil with 96,367, Romania with 81 583, Zambia with 51 978, Spain with 48 470, Portugal with 47 118, (FIBL, 2016; http://inteliagro.bg, 13.04.2016). An interesting paradox is that Eurostat and the Department of Agrostatistics at the Ministry of Agriculture and Food (MAF) from our accession to the EU until 2014 report higher average yields of honey in organic beekeeping compared to conventional beekeeping, while the production of organic honey remains with a much lower share of that of the national beekeeping sector - about 15%. This is probably due to the much weaker sensitivity to quality of the domestic markets for bee products because the organic honey is bought out by wholesalers at prices of conventional honey.

The emphasis on organic production in the Common Agrarian Policy of the EU has led to substantial changes in the domestic beekeeping sector. The support is mainly provided under the Rural Development Program (RDP). The assistance during the first programming period was less than during the second period. For example, the rate under measure 214 "Agri-environment payments" was 18.4 EUR, regardless of the fact whether the farm is certified or in transition. After 2014, the support for organic production is differentiated into a separate Measure 11, the payments for organic beekeeping have been differentiated and increased by the

33 30
 22 22
 21
 20 18,4
 (FAO, 2013). 16,2

Spain - 22,000 tons, Germany - 21,000
 tos, Belgium - 20,000 tons, Hungary -
 18.4 thousand tons, Brazil - 16.2
 thousand tons (FAO, 2013). Since joining
 the EU, Bulgaria has established itself as
 a traditional exporter of bee honey.

2.

Table 2. Export and import of bee honey

	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
Export, thousand tons	3,81	3,36	6,12	8,54	6,85	9,31	12,64	10,13	9,8	8
Export, million BGN	13,4	16,4	29,2	44	37	48	67	61	65	49
Import, thousand tons	0,24	0,85	0,2	0,23	0,29	0,65	1,86	1,15	1,4	1,5
Import, million BGN	0,84	3	0,82	0,83	1,1	2,4	5,8	3,7	4,8	6,3

MAF Agrostatistics Department, NSI, Eurostat

- About half of our exports are to
 - Germany, followed by major consumers
 - of Bulgarian honey like Greece, Poland,
 - France, Great Britain, Italy, Belgium,
 - Austria, etc. Over the last six years, a
 - steady import of honey has been formed,
 - and it started to grown, the main reasons
 - being not only its re-export but also the
 - price sensitivity of Bulgarian consumers,
 - giving priority to Ukrainian, Russian and
 - Chinese honey, the last one selling for
 - 1.1 EUR/kg. The main weakness of
 - Bulgarian export of honey is its realization
 - by merchants in barrels as a raw
 - material, which carries the added value
 - far along the supply chain - trader,
 - importer, processor, distributor and
 - retailer abroad. The domestic beekeeping
 - sector and the Bulgarian producers of
 - bee products do not actually benefit from
 - the export of their produce.

- The EU countries are among the
 - world's largest importers of honey and
 - bee products, with the EU being the
 - second largest global market for organic
 - products, following the USA. There is a
 - potential to penetrate the markets of
 - Great Britain and Switzerland, both of
 - which are among the largest importers of

honey and having the most developed consumption of organic food. The proximity to another large and solvent importer - Saudi Arabia, as well as the evolving consumption of such premium products in South-East Asia shall also be considered. At the same time, the geographical distance of some of the major exporters of bee honey is also a competitive advantage and provides good opportunities for our beekeeping sector.

The Bulgarian producers of bee honey and bee products are predominantly small. The majority of our beekeeping farms do not have the resources to invest in processing, own brand and innovative bee products. This is a limiting factor for obtaining a higher price on domestic and international markets for bee products. It is difficult to penetrate markets such as Germany and Austria, where there are well established, large and recognized local brands, some of which are owned by the retail chains and organic food shops. From this point of view, the markets in the Middle East and South-East Asia may be more accessible. Good opportunities are also offered by the markets in the USA, which are the largest buyer of queen bees and bee swarms.

2017)

100

(Capital,

Not a single representative of the domestic beekeeping sector or processors and traders integrated with it are not present in the K 100 ranking of the largest companies in Bulgaria (Capital, 2017), as opposed to the sectors for production of grain, pigs and poultry, and the processors and traders associated with them. Although today's markets for bee products are much smaller than the markets of the leaders listed in the ranking, they have great potential - organic, manufacturing and marketing - which are yet to be developed, given the needed solution to the global problem of pollination of entomophiles to ensure food security and the global trend towards consumption of

160 . / .
 150 . / .,
 1%
 77%
 16%

(Lyubenov, 2017b),

SWOT

(strengths – S)
 W)
 O)
 :
 •
 S₄)
 (S₁, S₂, S₃,
 (1, 2, 4, 5)
 – 3.
 (S₁, S₂, S₃, S₄, S₆)

healthier products, which will increase the consumption of bee products and reduce the consumption of their substitutes – sugar and other sweeteners.

An alternative to labour-intensive and low-yielding honey production is pollen, parchment, apilarynyl, royal jelly and bee venom. The economic potential of bee products such as bee venom, royal jelly, parchment, etc. is enormous but requires a strategic orientation towards the right markets. Based on a research on the potential of Bulgarian beekeeping (Lyubenov, 2017b), we will only supplement, update and highlight the most important data. For example, the bee venom has the greatest market potential of bee products, and is worth 160 million BGN/year, and royal jelly worth 150 million BGN/year, and today they have not yet absorbed even 1% of their potential. Bee honey has absorbed about 77% of its potential, and the pollination market has a potential perspective which is better than the one of the domestic organizational markets for honey and bee pollen. Factor and resource markets have absorbed about 16% of their potential.

SWOT Analysis of Bulgarian Beekeeping

We will analyze the four main types of interaction between strengths - S and weaknesses - W of Bulgarian beekeeping farms, as well as the opportunities (O) and threats (T) of the business environment:

- *Strengths and Opportunities* – the production of significant quantities of conventional and organic honey of high quality at a relatively low cost (S₁, S₂, S₃, S₄) and growing demand (O₁, O₂, O₄, O₅) allow the realization of competitive advantages of the Bulgarian beekeeping – see Table 3. It has strengths (S₁, S₂, S₃, S₄, S₆) to satisfy the growing demand for honey products other than bee honey such as propolis, pollen, parchment, royal jelly and bee venom, as well as services

3. (3, 6) –

(S₅),

3. (1, 2, 3, 8) (S₁, S₂, S₃, S₄, S₅, S₆) –

(4)

(5).

(6)

(7)

such as pollination and api-tourism (O₃, O₆) – see Table 3.

The strengths of Bulgarian beekeeping farms allow them to take advantage of the opportunities, but not to the full. The considerable biological, production and market potential of Bulgarian beekeeping (S₅), which is not developed, will allow it to take advantage of all the opportunities provided the existence of marketing strategies for its absorption to the fullest;

• *Strengths and Threats* – the Bulgarian beekeeping farms can successfully counter some of the threats (T₁, T₂, T₃, T₈) based on their strengths (S₁, S₂, S₃, S₄, S₅, S₆). – see Table 3. The successful counteraction to the more competitive non-agricultural enterprises (T₄) requires the beekeepers to cooperate with each other. The counteraction to the mechanism of the non-organized markets of bee products (T₅) requires the establishing of own markets or the reorientation towards organized markets. The development of organic beekeeping is slowed down by the higher costs in comparison to conventional beekeeping, and the fact that wholesale prices of organic bee products are equal to conventional bee products (T₅). They can partially counter the global substitutes of honey, which is a major product of the Bulgarian beekeeping (T₆) with their competitive advantages or through diversification with other bee products and services. They can use their branch organizations to improve it the ineffective legislative base (T₇). The strengths do not provide full protection for beekeeping farms from threats, thus co-operation and diversification are needed;

3. SWOT

Table 3. SWOT Matrix of Bulgarian Beekeeping

/ Strengths:	/ Opportunities:
<ul style="list-style-type: none"> • S₁ production of significant quantities of high quality bee products with comparative competitive advantages due to favourable natural conditions and low cost; • S₂ rich experience, traditions, motivation and deepening professional specialization in the production of bee products, as the sector is in the top 15 of the world's largest exporters of honey; • S₃ EU leader in the number of biologically certified bee colonies with over 21% of the total number, and our honey is also sought to improve the quality of foreign bee honey; • S₄ provided access to subsidies, financial credits, scientific and consultancy network; • S₅ significant biological, production and marketing potential for honey and bee products such as propolis, royal jelly, pollen, parchment, bee venom, etc., which have no substitutes; • S₆ multifunctional role of beekeeping - foods, drinks, pharmaceuticals, cosmetics, tourism, etc. 	<ul style="list-style-type: none"> • O₁ growing and sustained demand on international conventional and organic markets delivering substantial export revenues commensurate with, and even greater than, domestic markets; • O₂ as a member state we can satisfy about 10% of the EU deficit of honey, Suitable markets are Japan, Saudi Arabia, the United Arab Emirates and North America; • O₃ the role of bee-keeping will steadily increase on a global and domestic level as well as the demand for bee products beyond the honey category - propolis, pollen, parchment, royal jelly, bee venom; • O₄ consumer markets have more than twice higher prices of bee products than organizational markets, but require additional investment; • O₅ bee honey is a healthier alternative to other sweeteners; • O₆ the domestic beekeeping sector is a generator for the development of many other factor and resource markets.
/ Weaknesses:	/ Threats:
<ul style="list-style-type: none"> • W₁ mainly low-value products are produced - honey, pollen, etc. as a raw material; • W₂ insufficient market awareness and poor 	<ul style="list-style-type: none"> • T₁ climate change and reduction of vegetation appropriate for honey production, escalation of diseases and poisoning of bees; • T₂

<p>diversification of beekeeping farms - mainly honey is produced;</p> <ul style="list-style-type: none"> • W₃ <p>;</p> <ul style="list-style-type: none"> • W₃ the majority of beekeeping farms cannot meet the quantitative requirements of trade chains and for export; • W₄ <p>;</p> <ul style="list-style-type: none"> • W₄ the majority of beekeeping farms use technologies with predominantly manual labour and low productivity; • W₅ <p>;</p> <ul style="list-style-type: none"> • W₅ growing cost of bee products and services in Bulgaria; • W₆ <p>;</p> <ul style="list-style-type: none"> • W₆ difficulty in accessing markets because of the small-scale nature of beekeeping farms, poor co-operation amongst them, and poor integration with processors and traders; • W₇ <p>;</p> <ul style="list-style-type: none"> • W₇ low potential utilization due to poor staffing, technology and markets; • W₈ <ul style="list-style-type: none"> • W₈ very low relative share of mobile beekeeping within the total beekeeping sector. 	<p>;</p> <ul style="list-style-type: none"> • T₂ slower penetration of globalization, coupled with increased volatility on the markets for bee products and the presence of a gray sector; • T₃ <p>;</p> <ul style="list-style-type: none"> • T₃ deepening demographic crisis with labour shortages in rural areas and the sector; • T₄ <p>-</p> <ul style="list-style-type: none"> • T₄ non-agricultural enterprises at the entrance and exit of beekeeping farms are more competitive; • T₅ <p>;</p> <ul style="list-style-type: none"> • T₅ the mechanism of domestic organizational markets of wholesalers cannot reduce the risk, improve the price and the profit of beekeeping farms; • T₆ <p>-</p> <ul style="list-style-type: none"> • T₆ presence of very strong and global competitors and substitutes for honey - the main product of Bulgarian beekeeping; • T₇ (26 .) <p>;</p> <ul style="list-style-type: none"> • T₇ the legislative base (Ordinance 26, etc.) limits the direct realization of bee products on the consumer markets; • T₈ <p>;</p> <ul style="list-style-type: none"> • T₈ a decline in bee product prices, increased imports and growing prices of production resources.
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- (W₁),
- (W₂)
- (W₃)
- (4).
- (W₆)
- (1, 2, 3, 5, 6)
- 3.

• *Weaknesses and Opportunities* – the production of raw materials such as honey and pollen (W₁), as well as the failure to meet the quantitative requirements of the retail chains (W₃) do not allow for realization at higher prices on consumer markets (O₄). Poor diversification and market orientation of beekeeping farms (W₂) and difficult access to markets due to low cooperation and integration (W₆) do not allow the full use of most of the opportunities (O₁, O₂, O₃, O₅, O₆) – see Table. 3. The low degree of utilization of the potential due to poor staffing, technology and markets

(W₇),
(W₈)
(W₄),
(W₁)
(W₂)
(W₇)
(W₄),
(W₅)
(W₃, W₆, W₇) –

(W₇), the low relative share of mobile beekeeping (W₈) and predominantly manual labour and low productivity technologies (W₄) are serious reasons that significantly limit all favourable opportunities. The weaknesses of the Bulgarian beekeeping farms significantly impede the complete realization of the opportunities;

• Weaknesses and Threats – the production of mainly raw materials such as honey and pollen (W₁) and their realization on non-organized markets dominated by wholesalers (T₅) does not improve the profit and the risk of beekeeping farms. The poor diversification and market orientation of beekeeping farms (W₂) do not allow them to mitigate the effects of climate change (T₁) and volatility on markets for bee products (T₂). The low degree of utilization of their potential due to poor staff availability, technology and markets (W₇) and predominantly manual labour and low productivity technologies (W₄) are due to the labour shortage in the sector (T₃) and the constraints of the regulatory base for direct realization (T₇). The increase in the cost of the bee products (W₅) is also due to the increase in the prices of the production resource markets (T₈). In order to overcome the greater competitiveness of non-agricultural enterprises and substitutes (T₄, T₆), the beekeeping farms have to overcome their weaknesses (W₃, W₆, W₇) – see Table 3, by cooperating and integrating. The weaknesses of Bulgarian beekeeping farms are preventing them from adapting to threats.

The development of marketing and other strategies is based on the interrelation between the strengths of Bulgarian beekeeping farms and the opportunities of the business environment, and this relationship is a priority for their future development. It is also important to analyse how combining the strengths of Bulgarian beekeeping

SWOT

3.

farms with the opportunities of the business environment can be used to overcome their weaknesses and threats. This analysis will allow determining the strategic orientation of Bulgarian beekeeping farms by using their strengths to realize opportunities, overcome weaknesses and mitigate threats. A SWOT matrix of the Bulgarian beekeeping is compiled (see Table 3) based on the analysed and synthesized information from the international, national and sectorial business environment.

For harnessing *opportunities*, beekeeping farms shall offer not only honey, which has many competitive analogues and is rich in sugars, whose consumption is declining globally for health reasons. The demand for propolis, pollen, parchment, royal jelly and bee venom will grow in the future because they are extremely healthy and have no substitutes, unlike bee honey, whose demand will grow much slower on domestic and international markets. Beekeeping farms must also offer api-pollination and api-tourism services, given their growing demand. They offer opportunities for the so-called reverse integration into the markets for means of production and for a direct integration into the markets for bee products and services. There are relatively good opportunities to realize revenues from the export of conventional and organic honey and bee products to markets in the EU, Saudi Arabia, the United Arab Emirates, and Japan.

15

The Bulgarian beekeeping farms shall use their *strengths* such as high quality of produced bee products at relatively low cost. The sector is among the top 15 largest exporters of bee honey in the world, as well as the EU leader in the number of biologically certified bee colonies. The Bulgarian beekeeping has considerable biological and production capacity and respectively- market potential, which allow it to offer significant

quantity and quality of honey and bee products without substitutes - propolis, pollen, parchment, royal jelly and bee venom. Beekeeping is supported financially by the state through subsidies, scientific and consulting activities. Bee products can be realized on many markets - foods, drinks, cosmetics, pharmaceuticals, tourism, etc.

In order to overcome the *weaknesses*, beekeeping farms have to implement technologies for biological, precision, mobile beekeeping, etc., as well as for the production of products with high added value. Technologies are the basis for improving productivity, quality, cost and profitability. Beekeeping farms can be diversified based on their large biological, production and market potential. They have to orient themselves to bee products and other complex products and services with higher added value – bio-certification, branding, api-tourism, etc. Beekeeping farms must cooperate to be competitive with non-agricultural enterprises and to integrate with processors and traders to improve market access and better control and profit in distribution channels.

In order to mitigate the *threats*, the Bulgarian beekeeping farms can diversify their activities in order to reduce the irregularities in the incoming cash flows generated by the volatility of markets for bee products. They must offer not only bee honey, but also other products without competitive analogues and with higher added value - propolis, pollen, parchment, royal jelly and bee venom. Beekeeping farms need to cooperate in order to improve their opportunities for innovation and investment in tangible and intangible assets. The legislative basis for api-pollination, the access to consumer markets and the protection of bee population through selection, prevention, treatment, poisoning control, etc. should be improved. To improve the access to

- markets for bee products beekeeping farms shall establish their own consumer and organizational markets - mostly organized.

CONCLUSIONS

The developed SWOT analysis allows to synthesize the *main success factors* for the Bulgarian beekeeping, starting from the pursuit of the favourable opportunities through the strengths, in overcoming the threats and the elimination of the weaknesses:

- *Diversification* – based on the great potential and multi- functionality, pollination, api-tourism, etc.; mitigating the volatility of markets for bee products and improving profitability;

- *Cooperation* – improving the competitiveness against non-agricultural enterprises and meeting export requirements of international markets and large retailers; improving productivity, quality, cost and profitability;

- *Integration* – to the markets for means of production and to those for bee products for diversification and improvement of market access, including through its own markets for bee products and means of production; creating bio-certified, branded, and other bee products with high added value.

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Research on genes responsible for eggshell structure and quality

Vanya Mladenova*, D sislava Abadjieva

*Department of Immunoneuroendocrinology, Institute of biology and immunology of
reproduction, Bulgarian Academy of Sciences, 73 Tzarigradsko shosse Blvd.,
1113 Sofia, Bulgaria*

**E-mail: vanya_mladenova@abv.bg*

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SUMMARY

Hens' eggshell is an important structure due to the fact that it forms an embryonic chamber for a developing embryo and controls the gas exchange. On the other hand, the shell is a mechanical protection of the egg content as a valuable food product. Cracking of the eggshell in the marketplace is causing serious concern and economic loss.

For these reasons, the hardness of the eggshell is important in poultry production and, through in-depth research upon its structure, solutions are sought. Eggshell consists of 97% calcium carbonate that should be provided to the hen with the main diet. The process of eggshell mineralization occurs in uterus for about 20 hours, consisting of three stages, initial stage (5 to 10 hours post-ovulation), rapid deposition stage (10 to 20 hours post-ovulation), and final stage (21 up to 24 hours post-ovulation).

97%

(5 10

(10

(21

in other parameters such as eggshell thickness and eggshell weight. This type of "strong and weak" eggs and the corresponding hens can be the optimal models for the investigation of molecular mechanism underlying eggshell quality (Sun et al., 2013).

(Sun et al., 2013).

(Hodges, 1974).

(Johnson, 2000).

(Solomon, 2010).

(Nys et al., 1991; Gautron et al., 1997; Nys et al., 1999; Dominguez-Vera et al., 2000).

(Hincke et al., 2010; Dominguez-Vera et al., 2000).

(Dominguez-Vera et al., 2000; Nys et al., 1999).

(Board and Halls, 1973).

in other parameters such as eggshell thickness and eggshell weight. This type of "strong and weak" eggs and the corresponding hens can be the optimal models for the investigation of molecular mechanism underlying eggshell quality (Sun et al., 2013).

The purpose of this review is to identify molecular markers, genes responsible for the process of mineralization of the egg shell, with opportunity to make recommendations for the industry.

The structure of the eggshell

The shell has an average thickness of approximately 0.3 mm and can be divided into three different layers that can be distinguished, the mammillary layer, the palisade layer and a vertical crystal layer closely connected to the cuticle (Hodges, 1974). The crystallized palisade layer is composed mainly of crystalline calcium carbonate arranged in columns perpendicular to the surface (Johnson, 2000). Each palisade column grows from one mammillary knob and during calcification these columns fuse forming the bulk of the "true shell" (Solomon, 2010). The shell gland cells secrete the compounds of a milieu which is saturated in calcium carbonate - the precursors of eggshell matrix (Nys et al., 1991; Gautron et al., 1997; Nys et al., 1999; Dominguez-Vera et al., 2000).

Organic matrix proteins help control the mechanical properties of the eggshell (Hincke et al., 2010; Dominguez-Vera et al., 2000). The shape, size and orientation of the calcite crystals are due to the interaction of calcium carbonate and the organic matrix (Dominguez-Vera et al., 2000; Nys et al., 1999). Finally a cuticle covers the eggshell which minimizes penetration of microorganism (Board and Halls, 1973).

(Na⁺, K⁺, Cl⁻),
(Wistedt, 2013).
Na⁺, Cl⁻, Ca²⁺, K⁺
Na⁺
et al., 1999; Arad et al., 1989).
Na⁺, Cl⁻, K⁺, Ca²⁺
(Nys et al., 1999).
Wistedt, 2013).
(Solomon, 2010).
TRPV6
(Jonchere et al., 2012),
(Yang et al., 2013).
HCO³⁻
HCO³⁻

Shell gland secretion

When the egg enters the shell gland plumping occurs and the albumen is diluted by adding ions (Na⁺, K⁺ and Cl⁻), water and glucose. The exact time when and where shell formation starts is somewhat controversial (Wistedt, 2013).

With more focus on the minerals of the eggshell and the formation of calcium carbonate, there is an increase in extracellular water transfer, including sodium and chloride, but the uterine fluid also contains calcium, potassium and bicarbonate ions (Nys et al., 1999; Arad et al., 1989). The content of Na⁺, Cl⁻ and Ca²⁺ is high at the beginning of shell calcification and decreases progressively, while K⁺ shows an inverse change (Nys et al., 1999). Chloride ions diffuse passively back to the blood and Na⁺ is exchanged for Ca²⁺ by the mucosal cells (Arad et al., 1989). The transport of Na⁺, Cl⁻, K⁺, Ca²⁺ and HCO³⁻ occurs against their electrochemical gradients and involves ion exchangers and ion channels (Nys et al., 1999). The regulation of calcium secretion is strictly linked to the arrival of an egg and synchronized to ovulation (Nys et al., 1999). Evidence suggests that the surface epithelium provides the bulk of Ca²⁺ needed for shell formation (Etches, 1996; Wistedt, 2013) and the ciliated cells contain the highest concentration of calcium (Holm et al., 2003). Ultrastructural studies show that these cells produce secretory granules and the production of granules reaches its greatest intensity during the early stages of shell formation (Solomon, 2010). Recent findings implicate the calcium ion channel TRPV6 in shell formation (Jonchere et al., 2012) and immunohistochemistry revealed that the protein is located in the surface epithelial cells, but the location to a specific type of surface epithelial cell was not evident (Yang et al., 2013). However, it has been suggested that the tubular gland cells may have a dual function and also provide the HCO³⁻ needed for shell

(Nys et al., 1999).

(17 -estradiol) (ER).

ER, Ca²⁺ (Deroo and Korach, 2006). ER (ER , ER), - ESR1 ESR2, (Morani et al., 2008, Deroo and Korach, 2006). (Ball et al., 1999), ER (Hansen et al., 2003; Isola, 1990). ER (Hrabia et al., 2008; Mattsson et al., 2008).

formation. The production of HCO₃⁻ originates mainly from the hydration of CO₂ by CA, which is present in the tubular gland cells (Nys et al., 1999).

Estrogen receptors

The biological actions of estrogen (17 -estradiol) are mediated via estrogen receptors (ER). A classical estrogen action pathway is when estrogen diffuses into the cell and binds to the receptor located in the nucleus to regulate gene transcription, resulting in a physiological response. This response occurs over the course of hours. Estrogen can act more quickly, within seconds or minutes, via a non-genomic mechanism through ER located in or adjacent to the plasma membrane, with cellular responses such as increased levels of Ca²⁺ (Deroo and Korach, 2006). The ER exists in two main forms, referred to as alpha and beta (ER , ER), encoded by separate genes - ESR1 and ESR2, and found on two different chromosomal locations (Morani et al., 2008; Deroo and Korach, 2006). Both receptors have been localized in laying hens (Ball et al., 1999), and in reproductive organs nuclear ER has been localized in the shell gland (Hansen et al., 2003; Isola, 1990). Expression of ER seems to be predominant compared to ER in reproductive organs during sex differentiation in quail and in ovaries of adult domestic hens (Hrabia et al., 2008; Mattsson et al., 2008).

Calcium from the feed

A sufficient amount of calcium in the feed is a necessity for both shell formation and skeletal health and resorption of calcium takes place in duodenum. any herbs have been the focus of attention in recent years as natural food supplements containing biologically active substances with multilateral action (Kistanova, 2005; Grigorova, 2014), such as phytoestrogens. They are estrogenic

<p>ERb.</p> <p>et al., 2006; Kuiper et al., 1998).</p>	<p>ERa</p> <p>ER</p> <p>(Dusza</p> <p>Kuiper et al., 1998).</p>	<p>compounds in plants able to evoke biological responses by activating ER and ER (Dusza et al., 2006; Kuiper et al., 1998). Phytoestrogens are high affinity ligands especially for ER , but the doses that are biologically active differ between species (Dusza et al., 2006; Kuiper et al., 1998). Reproductive disturbances such as impaired ovarian function have been found in ewes grazing forages with clover (Adams, 1995). However, health benefits are reported in humans after consumption of phytoestrogen, with a high intake of dietary phytoestrogens, may show a reduced risk of breast and prostate cancer (Adlercreutz et al., 1991) or against osteoporosis (Potter et al., 1998). The latter was also found by Gjorgovska et al. (2016) in laying hens, indicating that isoflavones are effective supplements for improving body weight and bone calcium content even during the late laying period.</p>
<p>(Adams, 1995).</p> <p>(Adlercreutz et al., 1991)</p> <p>(Potter et al., 1998).</p> <p>Gjorgovska</p> <p>et al. (2016)</p>	<p>-</p> <p>-</p> <p>-</p> <p>-</p> <p>-</p>	<p>of soybean daidzein, along with various concentrations of endogenous estrogen in animals, can result in completely different effects. For example, a supplement of daidzein improves the laying performance during post peak laying of Shaoxing duck (Zhao et al., 2005) and in ISA layers the amount of cracked eggs decreases and eggshell thickness and egg production increases (Ni et al., 2007).</p>
<p>(Zhao et al., 2005),</p> <p>(Ni et al., 2007).</p>	<p>-</p> <p>-</p> <p>-</p> <p>-</p> <p>-</p>	<p>Different isoflavone concentrations of soybean daidzein, along with various concentrations of endogenous estrogen in animals, can result in completely different effects. For example, a supplement of daidzein improves the laying performance during post peak laying of Shaoxing duck (Zhao et al., 2005) and in ISA layers the amount of cracked eggs decreases and eggshell thickness and egg production increases (Ni et al., 2007).</p>
<p>(SPP1)</p> <p>(CALB1)</p> <p>Ca²⁺</p> <p>al., 2012).</p> <p>(Pines et al., 1995),</p>	<p>-</p> <p>-</p> <p>-</p> <p>-</p> <p>-</p>	<p><i>Genes</i></p> <p>The genes of calbindin (CALB1) and osteopontin (SPP1) have been extensively explored for their roles in eggshell calcification in recent decades. Calbindin as a active Ca²⁺ transcellular transporter exerts significant effects in delivering active calcium in the uterus (Jonchere et al., 2012). Osteopontin is a main eggshell matrix proteins (Pines et al., 1995), regulating the eggshell calcification process by inhibiting calcium carbonate precipitation in a</p>

(Hincke et al., 2008; Pines, 2007).
 Bar et al. (1992)
 CALB1
 Yang et al., (2013).
 Jeong et al., (2012)
 CALB1 SPP1
 3 20
 DMP4 (dentin matrix protein-4)
 BMP2 (bone morphogenetic protein 2)
 (Jonchere et al., 2010). DMP4
 -4,
 (Hao et al., 2007)
 (Jonchere et al., 2010).
 BMP2,
 TGF
 (Duprez et al., 1996),
 (Sekiya et al., 2005).
 CALB1, SPP1
 DMP4

phosphorylation-dependent manner (Hincke et al., 2008; Pines, 2007). Bar et al. (1992) pointed out that the expression of CALB1 fluctuates markedly during the daily egg cycle, in close temporal association with egg shell calcification, which was consistent with Yang et al. (2013).

Similarly, Jeong et al. (2012) demonstrated the expression of CALB1 and SPP1 increased significantly in the shell gland between 3h and 20h postovulation, revealing temporal changes in gene expression in different stages of the laying cycle. No research however has been carried out to illustrate the association of these two genes with eggshell mechanical property.

The other two genes, DMP4 (dentin matrix protein-4), BMP2 (bone morphogenetic protein 2) were also observed for their over expression during eggshell calcification, and they have been speculated for their potential roles in determining eggshell strength (Jonchere et al., 2010). The gene of DMP4 encodes dentin matrix protein-4 which is a calcium-binding protein that plays a role in dentin mineralization (Hao et al., 2007) and was newly identified as uterine protein (Jonchere et al., 2010). The protein encoded by BMP2 belongs to the transforming growth factor-beta (TGFB) superfamily (Duprez et al., 1996), acting as a disulfide-linked homodimer and induces bone and cartilage formation (Sekiya et al., 2005). However, there aren't data for these two genes effects' on eggshell strength.

In summary, the current review provided a light to investigate the association of candidate genes with eggshell mechanical property. We suggested that genes CALB1, SPP1 and DMP4 in mRNA level, may play a positive and negative role respectively in the formation of strong eggshell, of course,

this need further evidence to support

20.12.2017

16/4

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