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Operational evaluation of goat milking installation

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SUMMARY

- The goat milking installation DIK-24PF was developed. The distinctive feature of that installation is the application of the principle of arranged fixation for the design of the fixation system. Experimental evaluation of the operational indicators of the milking installation was made. In order to improve the reliability of obtained results, the experimental study was carried out in two different stages of the lactation period: at high level of milk yield and at low level of milk yield of the goats. The values of the following operational indicators were found during working with DIK-24PF milking installation:
- productivity of labour per hour of net working time, W_1 ;
- ✓ $W_1 = 72,98$ / ((✓ $W_1 = 72.98$ goats/man-hour (at a high level of productivity);
- ✓ $W_1 = 102,45$ / ((✓ $W_1 = 102.45$ goats/man-hour (at a low level of productivity).
- productivity of labour per hour of operational working time, W_{02} ;
- ✓ $W_{02} = 59,66$ / ((✓ $W_{02} = 59.66$ goats/man-hour (at a high level of productivity);
- ✓ $W_{02} = 79,42$ / ((✓ $W_{02} = 79.42$ goats/man-hour (at a

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 : 77,52 %
 - 81,74 %;
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 - 12,91÷15,02 %;
 - 5,35÷7,36 %.
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low level of production).
 The experimental results also show
 - that during milking with DIK-24PF the
 - relative value of the technological
 operations is changing per one milking
 cycle among the following limits,
 depending of the stage of lactation and
 the current level of milk yield of the goats:
 - actual milking: from 77.52 % to 81.74 %;
 - preparatory technological operations –
 12.91÷15.02 %;
 - Closing technological operations –
 5.35÷7.36 %.
 DIK-24PF milking installation also helps to
 improve:
 - welfare of goats in the milking process;
 - the working conditions of the operators-
 milkmen;
 - Zoo-veterinary treatment conditions and
 the productivity control of goats.
 The results of the operational
 evaluation of DIK-24PF milking installation
 confirm validity of the working hypothesis
 for the positive impact of the principle of
 arranged fixation on the quality of
 operational process of the linear milking
 installations, side-by-side type.
Key words: goats, goat milking;
 goat milking installation; operational
 evaluation; output of a milking installation

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INTRODUCTION
 In Agricultural Engineering Journal
 - (ISSN 0037-1718), issue No 6/2011, was
 - published an article 'Study on some
 - operational indicators of milking
 installation for goats DIK-24'. The article
 - presents the results of the operational
 - evaluation of the milking installation. The
 - following operational indicators were
 - assessed: 'Productivity of labour per hour
 - of net working time, W_1 ', productivity of
 - labour per hour of operational working
 - time, W_{02} ', duration and relative share of
 - technological operations per one milking

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cycle in working with DIK-24. It was found that within one milking cycle, the auxiliary technological operations occupied a relatively high share, reaching up to 34.06%.

Milking installation DIK-24 is with 24 stalls, linear, one-row, side by side type. It is equipped with a fixing system, which applies the random fixing principle.

The results of an a priori experimental study on the impact of the fixation principle on workflow quality in line milking installations (Sabkov et al., 2017) show that, compared to the principle of random fixation, the principle of arranged fixation helps to improve the quality of the workflow of the milking installation.

In order to overcome these shortcomings, a sophisticated construction of DIK-24 was created – ‘Milking installation for goats DIK-24PF’. The main distinctive feature of this construction is the advanced fixing system created on the principle of arranged fixation.

The aim of this study is to evaluate the operational indicators of the milking installation for goats DIK-24PF.

MATERIAL AND METHODS

The milking installation DIK-24PF is intended for group milking of goats in a milking parlour. It is equipped with a feeder for concentrated fodder for feeding the goats during the milking process. It is served by two operators-milkmen, who are working with 6 milking clusters.

Figure 1 shows a combined general view of the milking installation.



a) view from the side of feeding-trough for concentrated forage

b) view from the side of milking area

Fig. 1. Combined general view of DIK-24PF goat milking installation

The study was conducted in the experimental goat farm in the Research Institute of Mountain Stockbreeding and Agriculture - Troyan. The basic parameters of the goat herd are presented in Table 1.

Table 1. Key parameters of the goat flock

	/ Parameter	Measure	Value
1.	Size of the flock (mother-goats)	Number	160
: / Breed composition:			
2.	/ Bulgarian White Dairy	%	54,4
-	/ Anglo-Nubian		15,6
-	/ Toggenburg		30,0
/ Fertility:			
3.	/ Bulgarian White Dairy	%	171
-	/ Anglo-Nubian		162
-	/ Toggenburg		155
- / Live weight of mother-goats:			
4.	/ Bulgarian White Dairy	kg	54.5
-	/ Anglo-Nubian		53.3
-	/ Toggenburg		51.3
/ Lactation milk yield:			
5.	/ Bulgarian White Dairy	l	677
-	/ Anglo-Nubian		611
-	/ Toggenburg		514

The following operational indicators

- T_c ;
 - p_i ;
 - t_i ;
 - W_{1i} ;
 - W_{02} .
 (Kardashevskii et al., 1979; 3527-72. 99).
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of the milking installation were studied:
 - the average duration of one milking cycle, T_c ;
 - the relative share of preparatory technological operations, p_i ;
 - the relative share of the final technological operations, t_i ;
 - productivity of labour per hour of net working time, W_{1i} ;
 - productivity of labour per hour of operational working time, W_{02} .
 In the study of the technological operations the methods of the control shifts and the chronometric observations were used (Kardashevskii et al., 1979, 3527-72.99). The duration of the operations was measured using electronic chronometers with an accuracy of 0.01 s.
 The names of the chronometric technological operations and their symbolic designations are presented in Table 2.

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Table 2. Technological operations during milking with DIK-24PF

Name of the technological operation		Symbol
<i>I.</i> <i>Technological operations during the actual milking</i>		T_1
1.	/ Machine milking	$T_{1;1}$
2.	/ Machine stripping	$T_{1;2}$
3.	Waiting for the end of milking the group	$T_{1;3}$
<i>II.</i> <i>Preparatory technological operations</i>		T_2
1.	Loading the feeding-trough with concentrated fodder	$T_{2;1}$
2.	Routing the goats onto the milking platform	$T_{2;2}$
3.	Γ / Attaching the 1 st teat cup	$T_{2;3}$
<i>Terminative technological operations</i>		T_3
1.	Detaching the last teat cup	$T_{3;1}$
2.	Waiting for the release of the group	$T_{3;2}$
3.	Getting the group out of the milking parlour	$T_{3;3}$
1.	1. Determination of the indicators	

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$$T_3, \dots$$

$$c = \sum_{i=1}^3 i$$

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$$1 = \sum_{i=1}^3 1;i, s.$$

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- 1;2 -
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"Duration of a one milking cycle",
 "Relative share of the preparatory technological operations" and "Relative share of the final technological operations".

The milking cycle in working with DIK-24PF includes all the technological operations (see Table 2) performed on the single loading of the milking platform with animals. This includes all operations related to the milking group's preparation, milking and removal from the milking parlor.

1.1 Duration of one milking cycle

The duration of a one milking cycle T_c is measured with time from the moment marking the beginning of the 'Loading of the feeding-trough with concentrated fodder' to the moment that takes into account the end of the process 'Taking the group out of the milking parlour'.

It is expressed by the sum of the net time T_1 , the time for the preparatory T_2 and the time for the final technology operations T_3 , i.e.:

(1)

The net milking time for milking with the installation, T_1 is the time immediately after the 1st cup has been placed, just before the last milking cup has been removed.

T_1 is the sum of all the times during which the milking process is carried out or

(2)

In this equation with $1;i$ is denoted the time for:

- 1;1 - machine milking, s;
- 1;2 - machine stripping, s.
- 1;3 - waiting for the end of milking of the group.

1.2 Relative share of preparatory technological operations

The auxiliary time T_2 is the sum of all the times during which the preparatory

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$$T_2 = \sum_{i=1}^3 T_{2,i} \cdot S_i \quad (3)$$
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$$p = T_2 / T_c, \% \quad (4)$$
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$$T_3$$
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technological operations necessary to implement the milking process are carried out. The preparatory technological operations group includes the operations that precede and are necessary for the beginning of the actual milking: loading the feeding-trough with concentrated fodder, laying and stacking the goats on the milking platform, fixing them for milking and placing the 1st teat cup.

The duration of T_2 is determined by the moment marking the beginning of 'Loading of the feeding-trough with concentrated fodder' until the end of 'Attaching the 1st teat cup'.

where $T_{2,1}$ is the loading time of the feeding-trough with concentrated fodder;
 - $T_{2,2}$ – the time for stacking and self-fixing of goats on the milking platform;
 - $T_{2,3}$ – time for setting the 1st milking apparatus.

The relative share of the preparatory technological operations is expressed by the percentage ratio of the auxiliary time T_2 and the time for one milking cycle T_c , i.e.:

1.3 Relative share of final technological operations

The auxiliary time T_3 is the time for performing the final technological operations. This group includes operations carried out immediately after completion of the actual milking: removal of the last teat cup, unlocking of the fixing system, release of the group of animals that are milked, removal from the milking parlor and preparation of the milking installation for introduction of the next milking group.

The duration of the final technological operations is limited by the moment marking the beginning of 'Removing the last teat cup' up to the

$$T_3 = \sum_{i=1}^3 T_{3,i}$$

moment that takes into account the end of the process "Take the group out of the milking platform and locking the fixing system" and is measured with the auxiliary time T_3 :

(5)

Here with $T_{3,i}$ is indicated the time for:
 $T_{3,1}$ – removing the last milking apparatus;
 $T_{3,2}$ – waiting for release of the group;
 $T_{3,3}$ – taking the group of goats out of the milking parlour.

The relative share of the final technological operations is determined by the expression:

(6)

2. Determination of the indicators 'Productivity of labour per hour of net working time, W_1 ' and 'Productivity of labour per hour of operational working time, W_{02} '

Labour productivity per hour of net working time, W_1 is expressed by the number of animals milked from 1 operator-milkman per hour of actual milking, i.e.:

$$W_1 = \frac{3600 \cdot Q}{T_1 \cdot N} \quad / \quad (\text{goats/man-hour})$$

(7)

where N is the number of operators-milkmen;
 Q - number of animals in a group for milking.
 The number of animals in the group, Q , depends on the structure of the milking installation and is determined by the number of milking sites on the milking platform. For DIK-24PF: $Q = 24$.

Labour productivity per hour of operational working time, W_{02} is expressed by the number of animals milked by 1 operator-milkman per hour of operational time for milking and is presented by the expression:

$$W_{02} = \frac{3600 \cdot Q}{T_{02} \cdot N} \quad / \quad (\text{goats/man-hour})$$

(8)

$T_{02} = T_c$

(Bozhanov and Vuchkov, 1983; Mitkov and Minkov, 1989).

In this expression, the operational time for working time of the installation T_{02} is the sum of the actual milking times, the preparatory and final technological operations, i.e. $T_{02} = T_c$.

The preparation of the experimental study and the processing of the obtained statistical evaluations of the parameters of the technological operations were performed on the basis of known methodologies (Bozhanov and Vuchkov, 1983; Mitkov and Minkov, 1989).

RESULTS AND DISCUSSION

The results of the conducted study on the milking technology with DIK-24PF are shown in Table 3.

Table 3. Numerical characteristics of the technological operations during milking with DIK-24PF

Name of the operation	Symbol	At high milk yield			At low milk yield		
		\bar{X} , s	S, s	V, %	\bar{X} , s	S, s	V, %
1 Loading the feeding-trough with concentrated fodder	$T_{2;1}$	56.51	2.85	5.04	50.42	2.46	4.88
2 / Arranging the goats onto the milking platform	$T_{2;2}$	32.57	2.67	8.20	27.13	1.98	7.30
3 Attaching the 1 st teat cup	$T_{2;3}$	4.38	0.49	11.19	4.17	0.37	8.87
4 Machine milking (1 st goat)	$T_{1;1}$	176.54	47.05	26.65	124.67	37.64	30.19
5 Machine stripping (1 st goat)	$T_{1;2}$	17.40	6.29	36.15	14.27	5.61	39.31
6 / Waiting for the end of milking the group	$T_{1;3}$	397.99	57.32	14.40	282.72	52.95	18.73
7 Detaching the last teat cup	$T_{3;1}$	2.78	0.28	10.07	2.71	0.27	9.96
8 / Waiting for the release of the group	$T_{3;2}$	4.34	0.94	21.66	4.32	0.92	21.30
9 / Getting the group out of the milking parlour	$T_{3;3}$	31.62	2.07	6.55	33.53	2.48	7.40

The duration of the operations is represented by the point estimates of the numerical characteristics of the studied

operations.

In order to increase the reliability of the results, the exploitation study was conducted in two different stages of the lactation period: at high and low level of productivity of the animals (respectively in June and October).

Using the results of Table 3 and the expressions from (1) to (8) are determined the values of the assessed operational indicators (Table 4).

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Table 4. Results of the operational evaluation of goat milking installation DIK-24PF

Evaluative indicators	Symbol	Measure	Results for:	
			high milk yield	low milk yield
1 Duration of one milking cycle	T_c	s	724.16	543.94
2 / Relative value of the preparatory technological operations	p	%	12.91	15.02
3 / Relative value of the terminative technological operations	t	%	5.35	7.36
4 / Productivity per hour of net working time	W_1	goats/ man-hour	72.98	102.45
5 / Productivity per hour of operational working time	W_{02}	goats/ man-hour	59.66	79.42

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Figures 2 and 3 graphically represent the results for the relative share of technological operations within 1 milking cycle, with high and low levels of animal productivity. The analysis of the results shows that the lactation stage and the current productivity of the animals have an influence over the duration of the technological operations during the 'Milking' process.



Fig.2. Percentage share of time per 1 milking cycle in milking with DIK-24PF (at a high level of productivity)

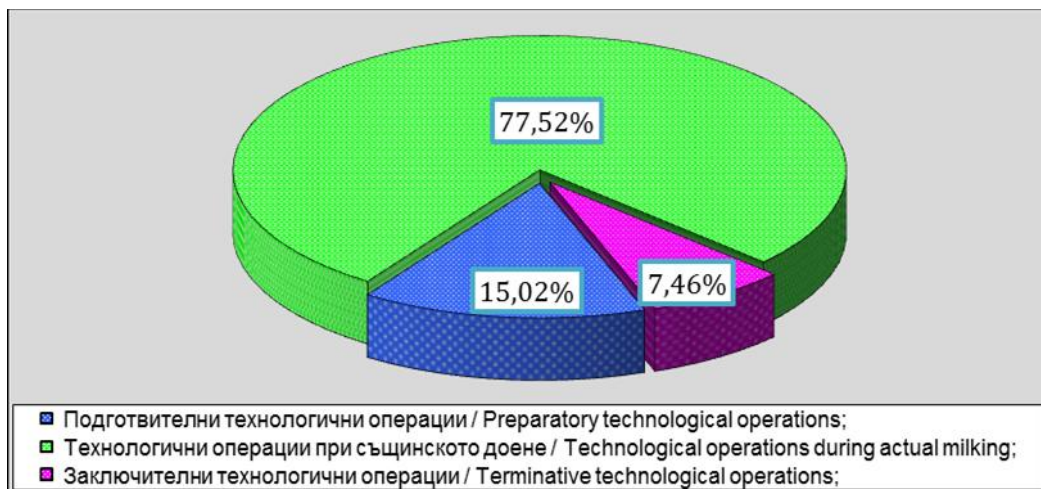


Fig. 3. Percentage share of time per 1 milking cycle in milking with DIK-24PF (at a low level of productivity)

81,74 %.

77,52 %

In absolute terms, the impact of ongoing productivity on the duration of technological operations in actual milking is the strongest. The relative share of these operations within a one milking cycle ranges from 77.52% to 81.74%. Moreover, the increase in the relative share of actual milking is directly proportional to the increase in the level of productivity.

	12,91	15,02 %	
	5,63÷6,16 %		
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The influence of productivity on the duration of preparatory technological operations is less. Depending on the lactation stage, their relative share ranges from 12.91 to 15.02%. What is special here is that the increase in the relative share of preparatory technological operations is inversely proportional to the change in the level of productivity.

The least pronounced is the influence of current productivity on the final technological operations. Their relative share varies within the range of 5.63 ÷ 6.16%. The inversely proportional dependence is also in effect in this case.

In the final evaluation of the effect of the creation of DIK-24PF is interesting the comparison with the results of the operational evaluation of DIK-24 base milking installation in terms of operational indicators, influenced by the improvement of the new installation. The temporal distance of both experimental studies and some methodological differences in their implementation imply some conditionality in the objectivity of the direct comparability of both constructions. However, and with a certain degree of conditionality, in general these results give grounds for some comparisons.

Table 5 presents the results of the comparison of the operational evaluations of both installations.

The results in Table 5 show that, in general, DIK-24PF milking installation has helped to improve all tested operational indicators of the base milking installation DIK-24. Both the total time of one milking cycle and the relative share of the auxiliary technological operations performed within one milking cycle are reduced.

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Table 5. Comparative evaluation of goat milking installations DIK-24 and DIK-24PF

Indicators	Measure	-24 DIK-24		-24 DIK-24PF compared to DIK-24, %	
		high milk yield	low milk yield	high milk yield	low milk yield
1 Duration of one milking cycle, T_c	s	845.47	638.76	85.65	86.57
2 / Relative value of the preparatory technological operations, ρ	%	20.04	5.61	64.42	58.44
3 / Relative value of the terminative technological operations, τ	%	25.70	8.26	95.37	90.31
4 / Output per hour of operational working time, W_{02}	man-hour	51.10	68.76	116.75	133.12

The impact on the timing of the preparatory technological operations is most pronounced. The relative share of these operations (ρ) decreases, respectively, by 35.58% (at a high level of productivity) and by 41.56%, with a low level of productivity.

The general positive effect of DIK-24PF application is reflected in productivity increase per hour of operational working time (W_{02}):

- at a high level of productivity - by 16.75%;
- at a low level of productivity - by 33.12%.

The comparative expert evaluation of the working process of the base and experimental milking installations carried out in the experimental study shows that compared to the base DIK-24, the milking installation DIK-24PF contributes for:

- improving the welfare of goats in the milking process;
- improving the working conditions of the operator-milkmen;
- improving the conditions for zoo-veterinarian treatment and control of productivity of animals.

The results of the operational evaluation of DIK-24PF milking

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The general positive effect of DIK-24PF application is reflected in productivity increase per hour of operational working time (W_{02}):

- at a high level of productivity - by 16.75%;
- at a low level of productivity - by 33.12%.

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- improving the welfare of goats in the milking process;
- improving the working conditions of the operator-milkmen;
- improving the conditions for zoo-veterinarian treatment and control of productivity of animals.

The results of the operational evaluation of DIK-24PF milking

(Sabkov et al., 2017)

installation confirm the general conclusion of a priori experimental study (Sabkov et al., 2017) of the positive impact of the fixing principle on the quality of the working process of the line milking installations for goats.

CONCLUSIONS

The results of the operational evaluation of the milking installations for goats, DIK-24PF, allow the following summaries to be made:

1. Milking installation for goats DIK-24PF is applicable for group milking of goats in a milking parlour and allows to achieve the following operational indicators for productivity:

1.1. Productivity of labour per hour of net working time:

- $W_1 = 72.98$ goats/man-hour (at a high level of productivity);

- $W_1 = 102.45$ goats/man-hour (at a low level of productivity).

1.2. Productivity of labour per hour of operational working time:

- $W_{02} = 59.66$ goats/man-hour (at a high level of productivity);

- $W_{02} = 79.42$ goats/man-hour (at a high level of production).

2. The duration of the technological operations of the milking process varies within one milking cycle, depending on the lactation stage (current productivity) of the animals.

The relative share of technological operations in actual milking ranges between 77.52% and 81.74%. Moreover, the increase in the relative share of these operations is directly proportional to the increase in productivity.

The relative share of the preparatory technological operations changed in the range of 12.91 ÷ 15.02% and the final technological operations within the range of 5.35 ÷ 7.36%. As with both preparatory and final technological operations, the change in their relative share is inversely proportional to the change in current productivity.

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- $W_1 = 72,98$ / ; (

- $W_1 = 102,45$ / ; (

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77,52 % 81,74 %.

12,91÷15,02 %,

5,35÷7,36 %.

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- | 3. The results of the operational evaluation of the milking installation DIK-24PF confirm the hypothesis of the positive impact of the fixation principle on the quality of the working process of the linear milking installations for goats.

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Evaluation of adaptive capacity of single and twins kids of BWD breed on the basis of realization speed of behavioural reactions in the early neonatal period

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SUMMARY

48 | The behaviour of 48 kids, 16 single and 16 pairs twins of Bulgarian White Dairy (BWD) goat was studied in the first hour after their birth. The development of their behaviour was evaluated on the basis of the following indicators: time to the first attempt to rise, time to the first rising, total time standing on their feet, time to the first attempt for sucking, time to the first successful sucking and total time for sucking, observed within the first hour after birth. It was found that the single and twins born BWD kids made their first attempt to rise within the first ten minutes after birth and successfully stand upright within half an hour after birth.

The single and twins born BWD kids made the first attempt for sucking and successful sucking within first hour after birth.

Key words: kids, BWD, behaviour, sucking

INTRODUCTION

Observations in the kid behaviour, immediately after their birth provides information for its adaptive capacity and their development at this earliest stage of their life. The manifestation of behaviour of new-born after birth is influenced by a number of factors one of which is number of new-borns (Dwyer, 2003).

According to Novac and Poindron (2006), new-borns have limited energy supplies and need quick access to colostrum immediately after birth to maintain their body temperature within optimal limits and to survive. The same authors note that the twins have a slower manifestation of neonatal behaviour in comparison with the single. The breed and the number of newborns also affect the ability of the mother and neonate to "recognize" in the herd and build a stable relationship during the critical period after birth (Awotwi et al., 2000).

Nowak (1989) reported that single-born lambs created faster relationship with their mothers compared to those born as twins. They also recognize their mother more quickly and spend more time with her.

According to Martinez et al. (2009) the survival of the kids during the neonatal period depends on the maternal characteristics of the goat as well as on the activity of the new-born, facilitating access to the udder and timely sucking.

The aim of present study is to assess the adaptive capacity of single and twins born kids of BWD breed on the basis of speed of realization of behavioural reactions during the first hour after birth.

MATERIAL AND METHODS

The study was conducted in the goat farm of the Experimental Base at Research Institute on Mountain Stockbreeding and Agriculture in the town of Troyan. The behavior of 48 kids, 16 single and 16 pairs twins kids was followed of Bulgarian White Dairy goat (BWD) breed within the first hour after their birth. Kidding was in February and March.

During the winter period animals were kept in a barn and fed with a ration containing 1.6 kg hay, 0.6 kg silage and 0.6 kg concentrated fodder per head. There was a free access to water and salt. In spring months (May-November), goats were grazing.

Chronometers were used, in the course of observation of the animals, to give an account of the time of all variables. Kids were observed by two researchers and the observation lasted for one hour after birth.

The following behavioural reactions were assessed, registered within the first hour after birth:

- Time to the first attempt to rise – duration of the period from birth (pushing out) to the first attempt of the kid to rise;
- Time to the first rising – duration of the period from birth to the first successful attempt for rising (the kid remains standing on its 4 limbs at least for 5 seconds);
- Total time standing – the time during which the kid remains standing on its feet within the first hour of the postnatal life;
- Time to the first attempt for sucking - time from birth to the first attempt for sucking;
- Time to the first successful sucking – time from birth to beginning of sucking;
- Total time for sucking – time spent in sucking in the course of the first hour after birth.

Data are presented as mean value (\bar{x}) and error of the mean ($S\bar{x}$). Results from all tasks were processed with

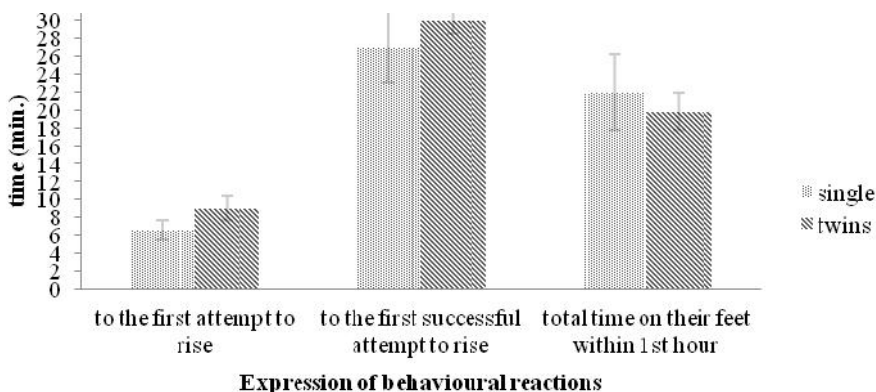
(Microsoft Excel, 2003), ANOVA

Windows statistical tools package program Windows (Microsoft Excel, 2003), and the reliability was calculated by ANOVA method through single-factor analysis.

RESULTS AND DISCUSSION

6,63±1,09 min (1)
 27±3,95 min
 (9±1.38 min
 30,06±1,62 min).
 22±4,22 min
 19,79±2,05 min
 81%

The single kids made their first attempt to rise averagely on 6,63±1,09 min (Figure 1) and successfully rose on their limbs on 27±3,95 min which was shorter than time till the first attempt to rise and the successful standing up for the twins – respectively 9±1.38 min and 30,06±1,62 min. The single kids stood longer time on their feet within the first hour after birth (22±4,22 min) in comparison with twins (19,79±2,05 min). All single kids made attempt to rise and 81% of them had succeeded. All twins of researched group made attempt to rise and had succeeded during the controlled period.



. 1.

Fig. 1. The effect of type of birth on time till the rising of the kid within the first hour after birth

Ramirez et al. (1998)

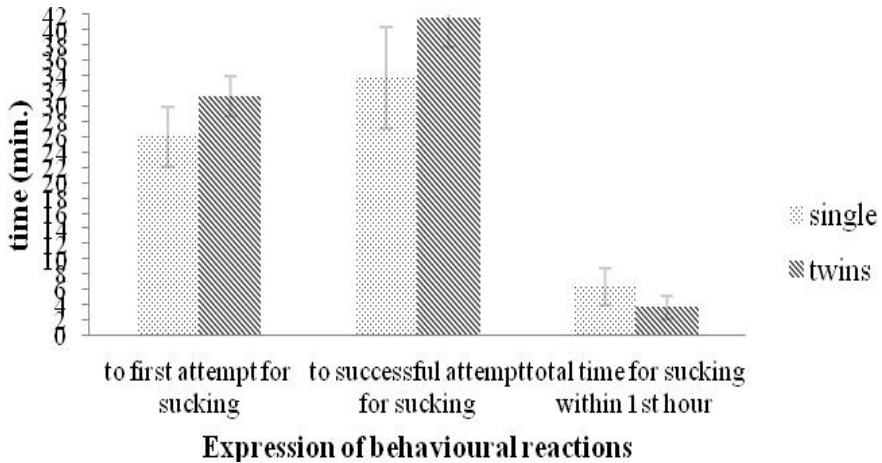
Ramírez et al. (1998) report similar to our results. They suppose that the earlier rise of single-born kids is a consequence of the higher level of maternal care as the mother's attention does not deviate to the next birth.

(Awotwi et al., 1999).

(2)	26±3,91 min	
		33,80±6,67 min	
			(31,33±2,65 min
min			41,50±3,66 min).
6,40±2,38 min,		3,65±1,52	
min. 56%		56%	
	31%		25%

The time for which new-born rises and sucks after birth can be used as an indicator of its vitality. The earlier start of sucking of new-borns gives a better opportunity to take up more colostrum and respectively a greater amount of antibodies from the mother (Awotwi et al., 1999).

The single kids (BWD) made their first attempt to suck (Figure 2) on 26±3,91 min and they sucked successfully averagely on 33,80±6,67 min after their birth, as it was earlier in comparison with twins, on 31,33±2,65 min and 41,50±3,66 min respectively. The single born kids sucked 6,40±2,38 min, and the twins 3.65 ± 1.52 min for the period of our research. 56% of single kids and 56% of twins had done first attempt to suck such as 31% of single kids and 25% of twins had sucked.



. 2.

Fig. 2. The effect of birth type on time till the kid starts sucking within the first hour after birth

Awotwi et al. (1999)

	14.3±6.6 min
	15.7±6.2 min

Awotwi et al. (1999) reported that the single kids start sucking more quickly than twins, respectively 14.3±6.6 min for singles and 15.7±6.2 for the first one of the twins. The results they have

Murciano-Granadina (Martinez et al., 2009)

- 34,76±10,26 min

- 22,45±10,26 min,

- 37,32±6,73 min

- 25,69±10,51 min.

(O'Connor et al., 1992).

registered are similar with what we have established, but for a shorter time.

Unlike our investigation, in the study on goats of Murciano-Granadina breed, Martinez et al. (2009) found, that single kids made their first attempt to suck later than twins (34.76±10.26 min in comparison with 22.45±10.26 min), and the successful sucking was also implemented later in comparison with twins (37.32±6.73 min in comparison with 25.69±10.51 min).

These results differ significantly from those obtained in our experiment, which confirms the existence of differences among breeds in the development of neonatal behaviour.

One of the reasons for earlier and more continuous sucking for single kids is that they are more active and make more attempts for sucking in comparison with twin kids (O'Connor et al., 1992).

CONCLUSIONS

It was found that the single and twins born BWD kids made their first attempt to rise within the first ten minutes after birth and successfully stand upright within half an hour after birth.

The single and twins born BWD kids made the first attempt for sucking and successful sucking within first hour after birth.

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Exterior parameters of Koprivshitsa autochthonous sheep breed on the way to consolidation of the breed type

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SUMMARY

Petrov (1930),
Spirov (1936), Savov Totev (1954),
Balevska et al. (1970), Tsochev
Bonchevska (2015). 50

Koprivshitsa sheep breed has managed to establish and prove in the specific region of Sredna Gora during its relatively long period of formation, combined with the native selection. The article is a comparative analysis of the exterior indicators of specimens from the contemporary population of Koprivshitsa sheep and Koprivshitsa sheep described by Petrov (1930), Spirov (1936), Savov and Totev (1954), Balevska et al. (1970), Tsochev and Bonchevska (2014). 50 exterior measurements were taken of the contemporary Koprivshitsa sheep, which have completed their growth in farms of farmers in the region of the town of Koprivshitsa. The study showed that the past and contemporary populations of Koprivshitsa sheep, which were described, have similar parameters, demonstrating the extremely well preserved breed type. There is a permanent increase in the size of the sheep.

Key words: sheep, exterior, breed type, population, measurements

INTRODUCTION

The assessment of sheep by the exterior is of great importance for the tribal work in sheep breeding. Animal measurement is an accurate and objective method.

Hinkovski et al. (1984)

Hinkovski et al. (1984) wrote that the exterior is one of the main controlled signs together with the type and constitution of the animals. For this purpose, the approved standards for external measurements are used, where the average values of the measurements of different points of the body are specified, which serve to ensure the objectivity of the expert assessment.

Nozhchev (1983)

According to Nozhchev (1983), the external measurements indicate the body size of given animals or group of herds of a known breed. External measurements can also be used to track the development of different parts of the animal's body over a long period of time.

Filatov Kochtigov (2012)

Filatov and Kochtigov (2012) assume that the assessment of the development and productivity of livestock gives great importance to the external forms of the animal's body, its exterior.

The same authors argue that it is the exterior that is one of the main indicators of the selection and is a reflection of the animal's productivity and its constitution.

Skorih (2010)

Skorih (2010) suggests that the assessment of the exterior and certain economic benefits is essential in tribal and commodity herds.

It is possible to assess the breed characteristics and productive qualities of livestock.

Sedefchev et al. (2011)

Sedefchev et al. (2011) made a comparative analysis of the exterior parameters of a contemporary, typical population of Karakachan sheep, in-situ preserved in Pirin and sheep described by Hlebarov (1942), Milic (1954), Baric (1952).

in situ,

Hlebarov

(1942), Milic (1954), Baric (1952).
90

1930-1970
Petrov (1930), Spirov (1936) Savov
otev (1954), Balevska et al. (1970)

Tsochev Boncevska (2015).

The dimensions of 90 sheep were taken.

- There were four specialised studies for Koprivshitsa sheep breed conducted for the period 1930-1970 conducted by Petrov (1930), Spirov (1936) Savov otev (1954), Balevska et al. (1970) and a recent study by Tsochev and Boncevska (2015).

- The aim of present study is to make a comparative analysis of the exterior parameters of the contemporary and historical population of Koprivshitsa sheep, related to the consolidation of the breed type.

MATERIAL AND METHODS

- The subject of the survey is Koprivshitsenska sheep breded in the farms of two farmers from the characteristic areal of distribution of the breed, the area of the town of Koprivshitsa in 2017. The degree of similarity of the exterior parameters will be determined by comparing current data from data measurements of breed researchers published in the XX and XXI centuries. The first four studies were conducted when the breed was widespread in Sredna Gora region, while the study of Tsochev and Bonchevska in 2015. The authors give similar descriptions of the breed they studied: a relatively small mountain sheep, a small head with a straight profile line, a heavy-set long body, a tight, healthy bone system and a long, thin tail reaching below the hock joint. The chest is deep and narrow. There are sheep with mostly red pigmented wool. The number of white sheep is smaller than the number of pigmented sheep. About 60% of the sheep are pigmented. The fleece is mostly closed. The body and stomach are wool-covered. The wool is uniform, semi-thin. Male and female animals are hornless, but they are also specimens with slightly pronounced horns.

- In the present study were made

50 outdoor measurements of 50 animals with parameters typical of the Koprivshitsa Sheep, 25 animals per herd. Wither height, body length, width and depth of chest were measured by the instrument of Lidten. The length of the metacarpus and the range are measured with a measuring tape. Average values of each indicator were calculated. The "Index of extensions", "Chest index", "Index of body mass" and "Bone development index" have been calculated for the animals of the past and the contemporary individuals of the breed. Live weight is obtained by weighing on scales with a suitable platform. The data obtained are compared with the mean values published by scientists for the breed in the 20th and 21st centuries.

The results are summarized and presented in tables and diagrams.

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The results are summarized and presented in tables and diagrams.

RESULTS AND DISCUSSION

A comparison of exterior characteristics was made of contemporary, typical Koprivshitsa sheep with data for the period of 1930-1970 and data from 2015 in order to make an objective assessment to what extent the type of studied sheep coincided with the type of breed from the beginning of XX century (Table 1).

2015 1930-1970 (1).

1.

Table 1. Body measurement of different authors

Authors	/ Body measurement							
	h /Wither height m	/Body length cm	e/Chest width cm	/Chest depth cm	ta/Tail length cm	/Metacarpus girth cm	/Live weight kg	
Petrov (1930)	51,83	59,28	18,28	26,66	26,58	-	33,9	
Spirov (1936)	52,5	61,6	16,9	26,8	36,5	-	34,75	
Savov and Totev (1954)	57,0	60,0	16,8	27,0	34,1	6,5	35,36	
Balevska et al. (1970)	57,32	62,5	17,1	25,7	34,1	7,1	47	
Tsochev and Bonchevska (2015)	70,9	81,1	24,7	34,4	-	9,1	51,2	
Markova and Stoycheva (2017)	70,3	77,6	24,6	30,1	55,1	9,0	51,5	

Petrov (1930), Spirov (1936), Savbov (1954) and Balevska et al. (1970). Tsochev and Bonchevska (2015).

(1)

Petrov (1930) 18,47 m, Spirov (1936) 17,8 cm, Savov and Totev (1954) 13,3 m, Balevska et al. (1970) 12,98 m, Tsochev and Bonchevska (2015) – 0,6 cm

It is evident from the presented results that Koprivshtitsa sheep, which are bred today, show some similarities but also significant differences with the sheep studied by Petrov (1930), Spirov (1936), Savbov and Totev (1954) and Balevska et al. (1970). There are minor differences in the measurements between Tsochev and Bonchevska (2015). The differences in indicators are attributed to improvement in feeding and breeding conditions of contemporary sheep, as well as to the random sequential genetic drift.

In relation to the height of the withers (Figure 1), contemporary sheep have the highest values, as the difference with the sheep measured by Petrov (1930) is 18.47 cm, by Spirov (1936) is 17.8 cm, by Savov and Totev (1954) is 13.3 cm, from Balevska et al. (1970) is 12.98 cm and Tsochev and Bonchevska (2015) have a minimum difference of 0.6 cm in their favor.

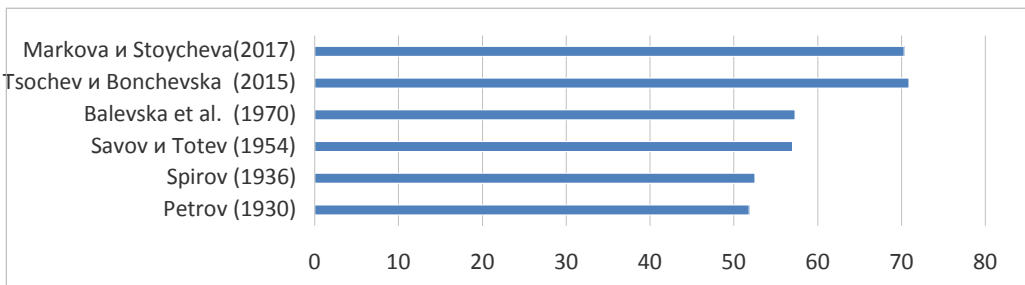


Fig. 1. Height of wither

(2)

Petrov (1930) 18,02 m, Spirov (1936) 16,0 m, Savov and Totev (1954) 17,6 m, Balevska et al. (1970) 15.1 m, Tsochev and Bonchevska (2015) 3,5 cm

The differences in the second characteristic, length of the body (Figure 2) repeat the trend: they are 18.02 cm with Petrov (1930), with Spirov (1936) being 16.0 cm, with Savov and Totev (1954) cm, with Balevska et al. (1970) - 15.1 cm and with Tsochev and Bonchevska (2015) a 3.5 cm difference in their favor.

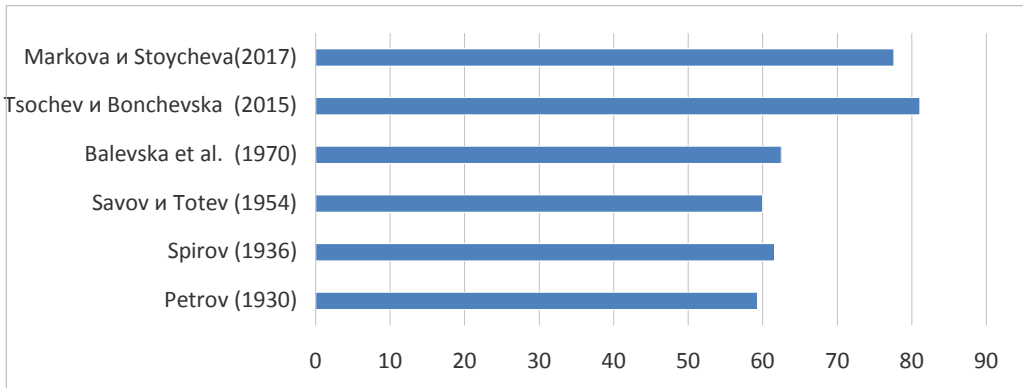


Fig. 2. Body length

(3), Petrov (1930) 6.32 cm, Spirov (1936) 7,7 m, Savov Totev (1954) 7,8 m, Balevska et al. (1970) 7,8 m, Tsochev Bonchevska (2015) 0,2 m.

The width of the chest shows the difference in the values as follows (Figure 3), they are 6.32 cm with Petrov (1930), Spirov (1936) – 7.7 cm, Savov and Totev (1954) Balevska et al. (1970) – 7.8 cm and Tsochev and Bonchevska (2015) – 0.2 cm.

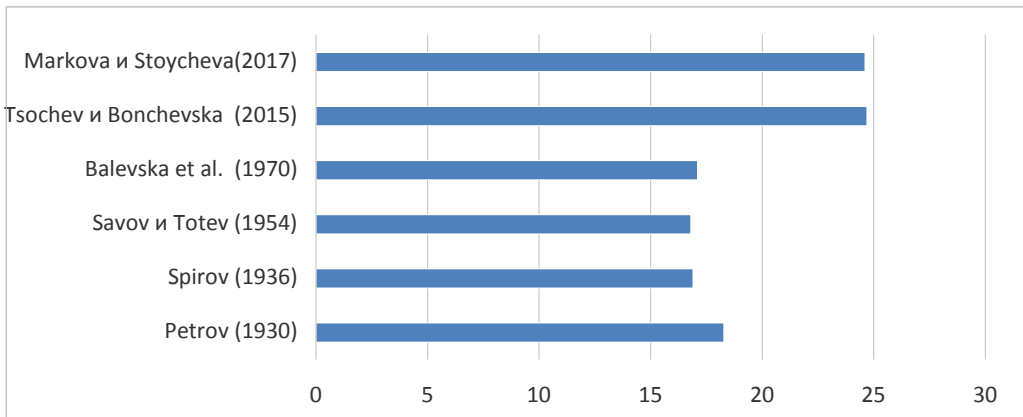
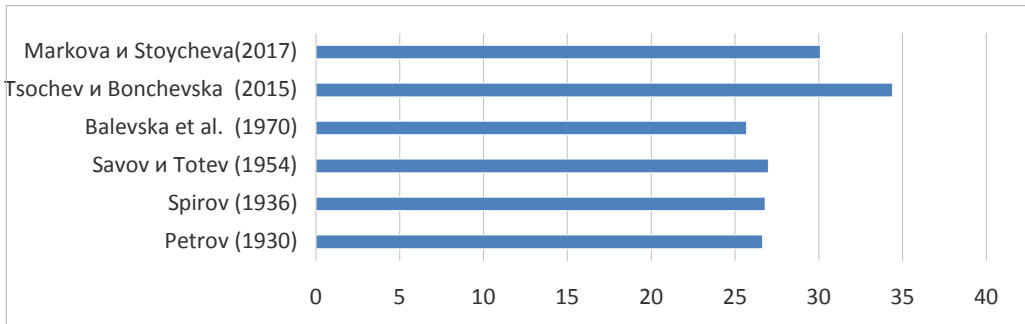


Fig. 3. Width of chest

(4) Petrov (1939) 3,44 m, Savov (1936) 3,3 m, Savov Totev(1954) 3,1 m, Balevska et al. (1970) 4,4 cm Tsochev Bonchevska (2015) 3,3 m

At the depth of the chest (Figure 4) the differences are minimal. Compared with Petrov (1939) they are 3.44 cm, with Savov (1936) – 3.3 cm, with Savov and Totev (1954) – 3.1 cm, with Balevska et al. (1970) – 4.4 cm and Tsochev and Bonchevska (2015) – 3.3 cm again for their benefit.

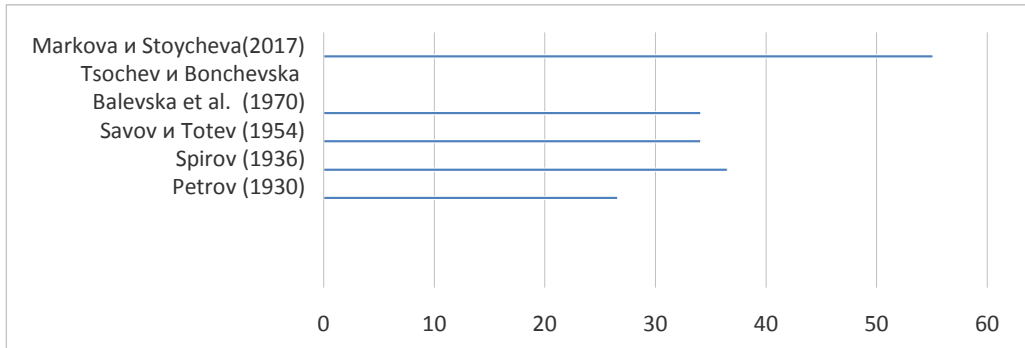


. 4.

Fig. 4. Depth of chest

(5) - -
 Petrov (1930) 26,58 m.
 Savov Totev (1954) Balevska et al.
 (1970) -
 34,1cm. Spirov
 (1936) 36,5 m,
 Markova Stoycheva (2017)
 55,1 m. Tsochev Bonchevska

The characteristic of tail length (Figure 5) shows the lowest values in Petrov (1930) of 26.58 cm, as Savov and Totev (1954) and Balevska et al. (1970) give the same value for a tail length of 34.1cm. Spirov (1936) marks a length of 36.5 cm and Markova and Stoycheva (2017) measure 55.1 cm. Tsochev and Bonchevska did not measure the tail length.



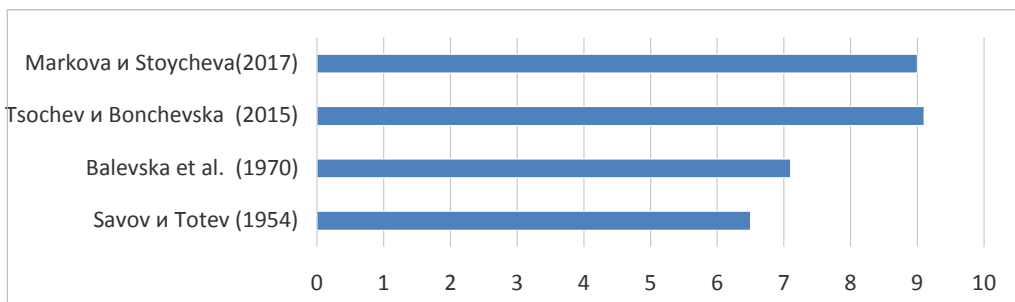
. 5.

Fig. 5. Tail length

() (6) -
 , 9,1
 m Tsochev Bonchevska (2015)
 9,0 m Markova Stoycheva (2017).
 Savov Totev (1954) Balevska et al.
 (1970)
 6,5 7,1 m. Petrov (1930)
 Spirov (1936)

The contemporary sheep has higher values in the characteristic of metacarpus girth (Figure 6), as Tsochev and Bonchevska (2015) measured 9.1 cm, 9.0 cm – Markova and Stoycheva (2017). Savov and Totev (1954) and Balevska et al. (1970) gave the following values for the metacarpus girth – 6.5 and 7.1 cm. Petrov (1930) and Spirov (1936) did not measure the metacarpus. The

metacarpus girth could be used in the selection process as a marker to prognosticate the fattening and slaughtering indicators.

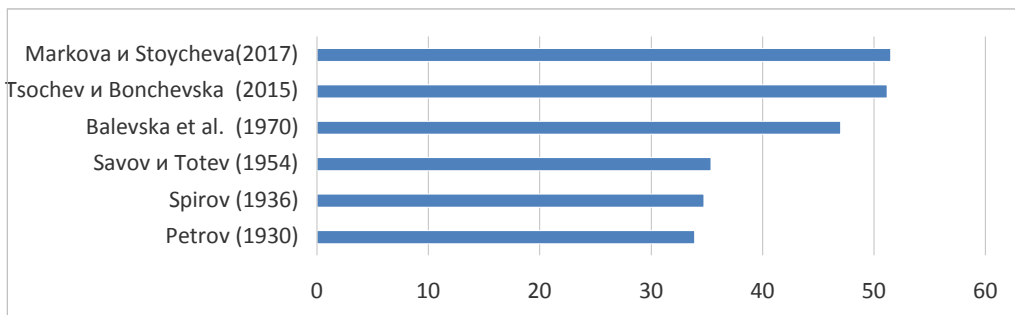


. 6.

Fig. 6 Metacarpus girth

(7)
 - Petrov (1930), 33,9 g.
 (1970) 47 g.
 е -
 Tsochev и Bonchevska (2015)
 Markova и Stoycheva (2017),
 51,2 kg 51,5 g.

Live weight (Figure 7) was the lowest in the sheep studied by Petrov (1930), 33.9 kg. Balevska et al. (1970) showed values of 47 kg. Tsochev and Bonchevska (2015) and Markova Stoycheva (2017) measured higher live weight, respectively 51.2 kg and 51.5 kg.



. 7.

Fig. 7. Live weight

- | As the absolute indices of the individual measurements do not give a complete picture of the constitution of sheep, we have calculated some body indexes that characterize the anatomically interconnected parts of the body (Table 2).
 - |
 - |
 - |
 (2).

2.

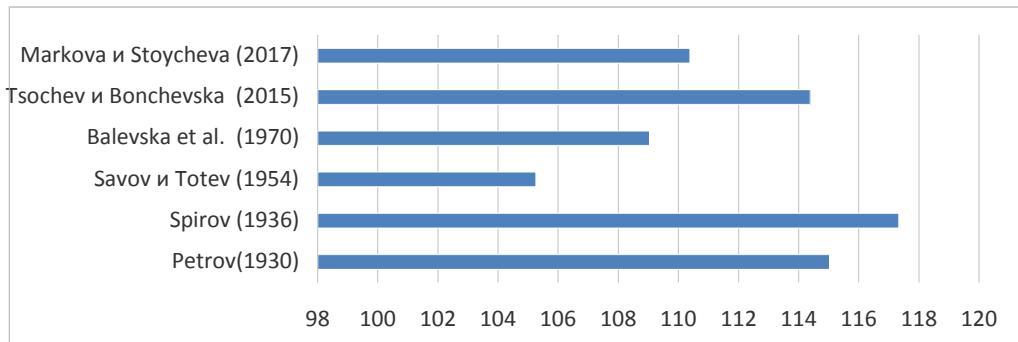
Table 2. Indices

Authors	/ Indices of constitution			
	For stretching	Chest	For body mass	/ For bone development
Petrov (1930)	115.03	68.57	47.98	0
Spirov (1936)	117.33	63.06	48.95	0
Savov and Totev (1954)	105.26	62.22	52.63	11.40
Balevska et al. (1970)	109.04	66.54	55.16	12.39
Tsochev and Bonchevska (2015)	114.39	71.80	51.48	12.83
Markova and Stoycheva (2017)	110.38	81.73	57.18	12.80

(8) ,

Spirov (1936) – 117,33, - ,
Savov Totev (1954),
Balevska et al. (1970).

Stretching index (Figure 8) show that that the sheep we are studying occupy a middle position between the most stretched, measured by Spirov (1936) – 117.33, and the shortest, measured by Savov and Totev (1954), as approaching the result of Balevska et al. (1970).

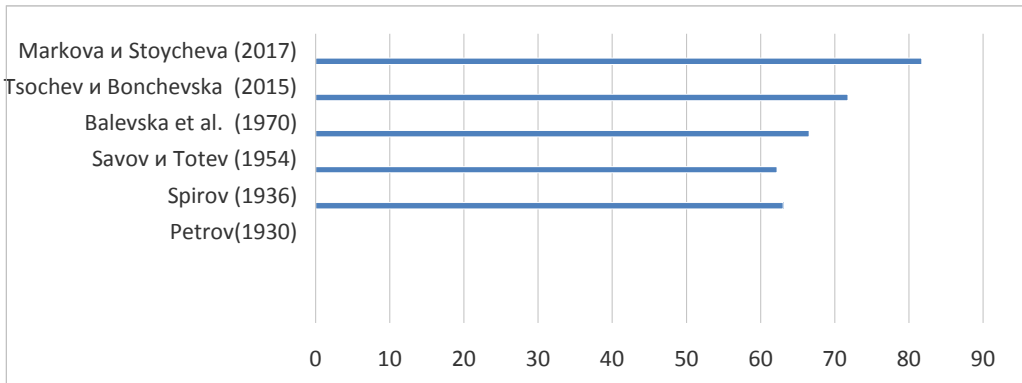


. 8.

Fig. 8. Stretching index

(9) - Savov Totev (1954) Spirov (1936). Petrov (1930) Balevska et al. (1970). - Markova Stoycheva (2017) Tsochev Bonchevska (2015) – 81,73 71,80, 9,93.

In terms of the chest index (Figure 9), Savov and Totev (1954) and Spirov (1936) measured the lowest indicators. They are followed by Petrov (1930) and Balevska et al. (1970). Markova and Stoycheva (2017) and Tsochev and Bonchevska (2015) showed the highest values in the measurements - 81.73 and 71.80, as the difference between them is 9.93.

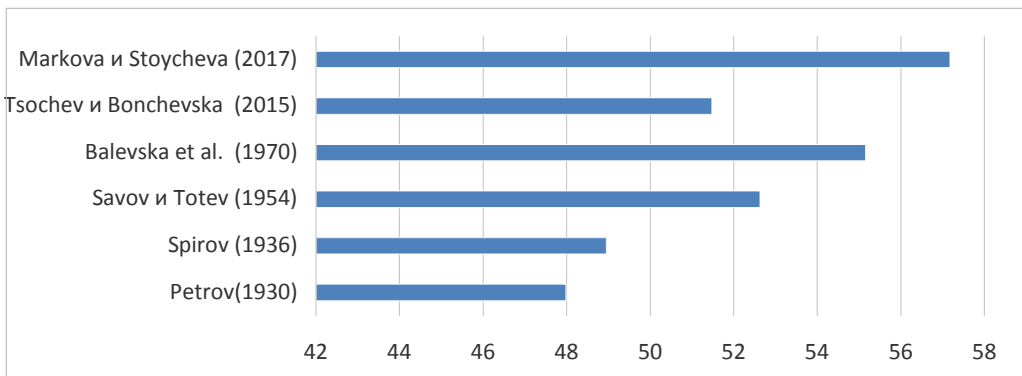


. 9.

Fig. 9. Chest index

Study	Chest index
Markova и Stoycheva (2017)	81.16
Tsochev и Bonchevska (2015)	71.48
Balevska et al. (1970)	65.16
Savov и Totev (1954)	62.98
Spirov (1936)	62.98
Petrov (1930)	47.98

The index of body mass (Figure 10) of the contemporary representatives of Koprivshitsa sheep breed takes the leading position with the values of 57.18 measured by Markova and Stoycheva (2017) and 51.48 measured by Tsochev and Bonchevska (2015). The lowest indexes are measured by Petrov (1930) – 47.98, and Balevska et al. (1970) measured the highest ones – 55.16.

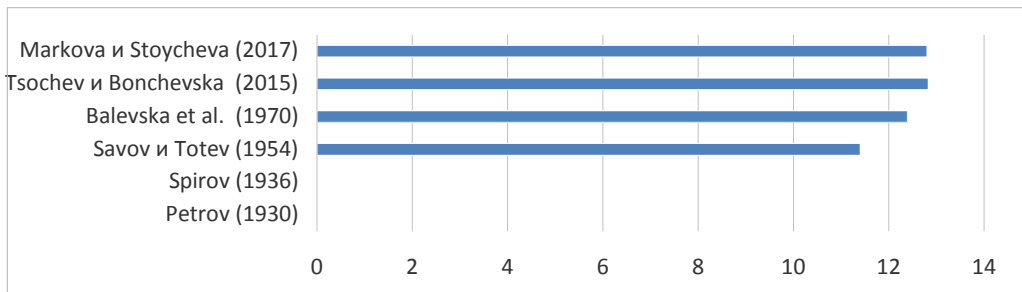


.10.

Fig. 10. The index of body mass

Study	Index of body mass
Markova и Stoycheva (2017)	57.18
Tsochev и Bonchevska (2015)	51.48
Balevska et al. (1970)	55.16
Savov и Totev (1954)	52.98
Spirov (1936)	49.98
Petrov (1930)	47.98

Bone development index (Figure 11) cannot be calculated for the sheep studied by Petrov (1930) and Spirov (1936). The lowest index values were obtained in the study of Savov and Totev (1954) –11.40, and the measurements of Tsochev and Bonchevska (2015) showed the highest values – 12.83.



11.
Fig. 11. Bone development index

70,3 m,
 77,6 m,
 30,1 m,
 55,1 m,
 51,5 kg.
 9,0 m

CONCLUSIONS

The contemporary representatives of Koprivshitsa sheep breed show height at the withers of 70.3 cm, body length of 77.6 cm, chest depth - 30.1 cm, tail length - 55.1 cm, metacarpus girth - 9.0 cm and body weight - 51.5 kg. The comparison of sheep from Koprivshitsa autochthonous breed showed that the exterior parameters are comparable to sheep that were bred in the region of Sredna Gora in the past.

The increase in sheep size is due to improved alimentary conditions, climate change and farmers' desire to increase the yields of the production. The assessment of the sheep's exterior characteristics, depending on their historical development, by measuring the body during different time periods and calculating the indexes of constitution, gives us an idea of how the consolidation of the native type has gone. By origin and type, Koprivshitsa sheep breed can be assigned to the type of autochthonous pigmented sheep.

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MnSO₄

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Feeding of the bee families with MnSO₄

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SUMMARY

(*Apis mellifera* L.)

MnSO₄

4 mg/l

(p<0.01).

- For their normal living, honey bees (*Apis mellifera* L.) require proteins, carbohydrates, lipids, vitamins and minerals. They receive all of these components from the nectar and the bee pollen. In recent years, the trend to use a variety of food supplements for bees has been ascertained in the beekeeping practices.

A special place is given to the combination of vitamins and minerals. In this respect, the aim of the study is the influence of feeding of the bee families with MnSO₄ on the chemical composition of non-flying worker bees' bodies and some bee products components.

- The results show that the addition of 4 mg/l MnSO₄ in the sugar syrup of the bee families has an influence on the average weight of non-flying worker bees (p<0.01).

- Also, statistically significant differences were found in the protein content and electrical conductivity of the royal jelly and the diastase activity and the electrical conductivity of the honey.

It can be assumed that the feeding of the

MnSO₄

MnSO₄,

(*Apis mellifera* L.)

(Nenchev and Zhelyazkova, 2010), (Ivanova, 2005), (Zhelyazkova and Nenchev, 1995, 2001), (Simkus et al., 2007; Colibar et al., 2011), (Shumkova, 2016), (Hristakov, 2012), (Stoilov et al., 2000; Sahinler et al., 2005).

Goloskov (1977).
Co, Mn

I₂

bee families with sugar syrup with MnSO₄ as a supplement has a positive influence on the hypopharyngeal glands of the worker bees.

Key words: bee honey, royal jelly, propolis, bee wax, MnSO₄, lysozyme, total protein, haemolymph

INTRODUCTION

For their normal living, honey bees (*Apis mellifera* L.) require proteins, carbohydrates, lipids, vitamins and minerals. They receive all of these components they from the nectar and the bee pollen. At the same time, in order to increase the average productivity, activation of the bee queen laying activity, more brood breeding and development of the hypopharyngeal glands, it is increasingly necessary various supplements in the beekeeping to be used. According to their chemical nature these supplements are proteins and amino acids (Nenchev and Zhelyazkova, 2010), carbohydrates and their substitutes (Ivanova, 2005), vitamins (Zhelyazkova and Nenchev, 1995, 2001), trace elements (Simkus et al. al., 2011), various stimulating products (Shumkova, 2016), plant extracts (Hristakov, 2012) and a combination of vitamins and minerals (Stoilov et al., 2000; Sahinler et al. 2005). However, the effect of different application of macro and trace elements to the bees' life processes is still a poorly studied problem.

One of the first studies in this area is on Goloskov (1977). He studies the effect of Co, Mn and I₂ during wintering of the bees after receiving sugar syrup in the autumn feeding. The results clearly show a reduction in the amount of food intake and decrease in the fecal mass of bees in the winter, respectively. Practically, this leads to better wintering of the bees. It can be assumed that these three elements favorably affect the digestive

Zhelyazkova (1999, 2005)
 Mn (2 mg/l) Co (6 mg/l)

MnSO₄

MnSO₄

- and secretory bee systems and they do not cause diarrhea. In Bulgaria Zhelyazkova (1999, 2005) found that the addition of Co (6 mg/l) and Mn (2 mg/l) salts in the bee food had a positive impact on the development and productivity of the bee families and vitamin C metabolism in the bee organism.

- The review of the available literature shows that there is no comprehensive and systematic study for the effect of MnSO₄ on the chemical composition of the bee bodies and the bee products obtained. In this regard, the aim of the study is the influence of feeding of the bee families with MnSO₄ on the chemical composition of non-flying worker bees' bodies and some bee products components.

MATERIAL AND METHODS

The study was conducted during May – August 2015 in the experimental apiary of the Institute of Animal Science, Kostinbrod. Before the experiment the bee families were balanced in regards to strength. In the experiment for royal jelly production a total of 6 bee families were observed – 3 experimental (fed with sugar syrup, sugar: water 1:1 and 4 mg/l MnSO₄ as a supplement) and 3 control (fed only with sugar syrup). The royal jelly is obtained by the artificial wax cups method (Grout, 1992). The sugar syrup is administered in a dose of 300 ml, 3 times per week in the bee feeders of the families.

Studies on the non-flying worker bees

- Average body weight of non-flying worker bees (g) – 100 honey bees were collected from each bee family from different honeycombs. The weight of each bee is measured on an analytical balance.

- Chemical composition of non-flying worker bee bodies – water content and mineral composition – weight

2015
 -
 6 - 3
 (1:1 4 mg/l MnSO₄)
 (Grout, 1992).
 300 ml, 3
 •
 (g) –
 100
 •
 –

(OAC International, 2002).

-20

Audit Diagnostics
Motavkina et al. (1979).

pH

2576693-84.

1%
(Bogdanov et al., 1997).

(HPLC)

Sesta (2006).

analysis; proteins – Keldahl method, lipids – Soxhlet method (AOAC International, 2002). The bee samples were collected at the end of the experimental period. The bees are anesthetized with diethyl ether and stored in a refrigerator at -20°C.

- Total protein in the bee haemolymph – spectrophotometric determination by the Audit Diagnostics Kit and the lysozyme content was determined by the method of Motavkina et al. (1979). The sample analysis was carried out at the National Diagnostic Science and Research Veterinary Medical Institute, Sofia.

Studies on the bee products

- Average number of queen cells – the smallest number of queen cells in each bee family is determined. From all bee families royal jelly is collected from the same number of queen cells (the smallest number of queen cells). The average queen cells number is obtained as the arithmetic average of all processes for royal jelly production.

- Average amount of royal jelly in a queen cell – it represents the total amount of royal jelly divided by the queen cell numbers. The average quantity is obtained as the arithmetic average of all samples obtained.

- Physicochemical properties of royal jelly – the methods for water content, proteins, pH and total acidity are described in ON 2576693-84.

- The electrical conductivity is determined on 1% royal jelly solution (Bogdanov et al., 1997). The fructose, glucose and sucrose content are determined by High performance liquid chromatography (HPLC) by Sesta (2006).

- Physicochemical properties of multifloral honey – water content, electrical conductivity, diastase activity

(Bogdanov et al., 1997).

Mn –

HNO₃.

Mn

Perkin Elmer Analyst 400

Windows. SPSS, 21

±

and hydroxymethylfurfural (HMF) are determined according to the Harmonised methods of the European Honey Commission (Bogdanov et al., 1997).

• Content of Mn – royal jelly, multiflora honey, propolis and bee wax samples are digested with concentrated HNO₃. The Mn content was analyzed with a flame atomic absorption spectrometer Perkin Elmer Analyst 400 with an air/acetylene flame.

The optimal instrumental parameters are determined according to the manufacturer's instructions. The analytical signal of Mn is periodically checked with a known concentration standard.

The statistical analysis was done using SPSS, version 21 for Windows. The results are presented as mean ± standard deviation.

RESULTS AND DISCUSSION

In Table 1 are present the results of chemical composition of non-flying worker bees. The analysis of the data shows that the protein, lipid and mineral content remains constant after feeding of the bee families with MnSO₄.

MnSO₄.

1.

, %

Table 1. Chemical composition of the body of non-flying worker bees, %

Parameter	Control group $\bar{X} \pm SD$	Experimental group $\bar{X} \pm SD$
/ Water content	67.27±0.61	67.96±1.85
/ Dry matter	32.73±0.61	32.04±1.85
/ Protein	53.63±0.66	46.49±2.41
/ Lipids	3.47±0.15	4.35±0.28
/ Ash	4.10±0.71	4.24±0.30

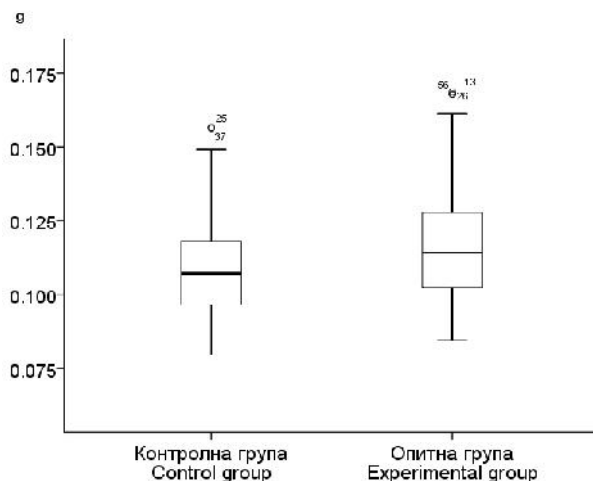
MnSO₄

Statistically significant differences were found in the average weight of non-flying worker bees fed sugar syrup with MnSO₄ (p<0.01). The mean weight and

($p < 0.01$).
 0.109 ± 0.017 g,
 0.116 ± 0.020 g.

standard deviation in the control group was 0.109 ± 0.017 g and in the experimental group was 0.116 ± 0.020 g. Figure 1 shows the minimum and maximum values of the tested indicator for the two groups.

1

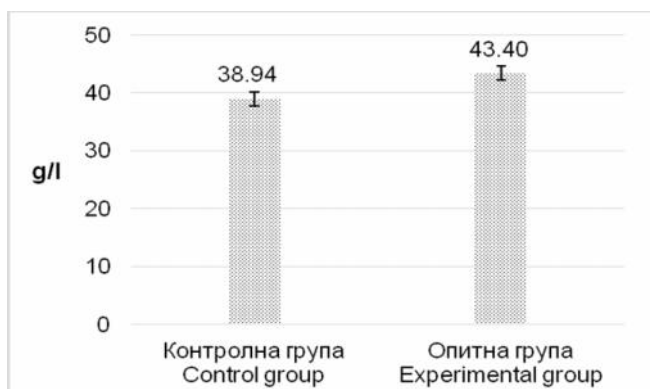


1. Box plot
Fig. 1. Box plot diagram of the average weight of non-flying worker bees, g

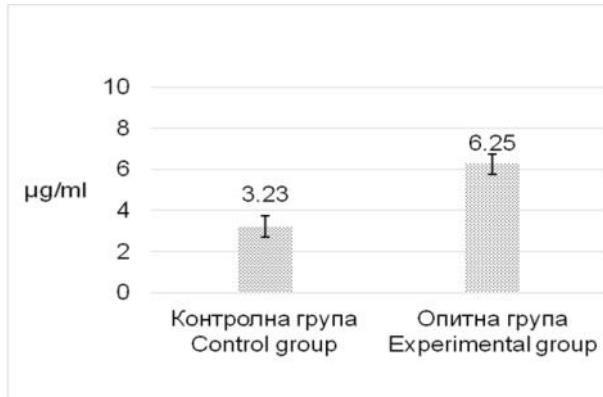
$MnSO_4$

To establish the effect of $MnSO_4$ in the sugar syrup, the total protein and lysozyme in the haemolymph of non-flying worker bees were examined. The obtained haemolymph samples are assembly samples of a large number of bees. The results for the amount of total protein and lysozyme in bee haemolymph are presented on Figures 2 and 3.

2 3.



2.
Fig. 2. Total protein content in haemolymph



3.
Fig. 3. Lysozyme content in haemolymph

4.5 g/l.
 $MnSO_4$

4 mg/l $MnSO_4$

$MnSO_4$

$MnSO_4$

The difference in the amount of total protein between the experimental and the control group is about 4.5 g/l. It can be said that the addition of $MnSO_4$ had a positive effects on the synthesis and accumulation of common protein in the bee haemolymph. In this respect, a dose of 4 mg/l $MnSO_4$ can be used in the spring feeding of the bee families and in autumn preparation for the wintering of the bees. It can be assumed that it had an immunostimulatory effect on the bees.

The differences in the lysozyme content of the experimental and control group are significant. The amount of lysozyme in the haemolymph of bee families fed with $MnSO_4$ is twice high as the control group.

In conclusion, the addition of $MnSO_4$ into the sugar syrup increased the total protein concentration and the lysozyme content in the haemolymph of the non-flying worker bees in the experimental group of bee families.

It is well known that the total protein and lysozyme values in the haemolymph can be used to detect the immunostimulatory effect of various biologically active substances (Gurgulova et al., 2001).

(Gurgulova et al., 2001).

MnSO₄.

2

MnSO₄

Until now, in the available literature, there is no data on the content of these two haemolymph components in the bee families fed with MnSO₄.

Table 2 shows the results for the average quantity of royal jelly in a queen cell. No significant differences in the average amount of royal jelly produced in the control and the experimental group.

This means that the MnSO₄ as a supplement in the sugar syrup does not lead to a larger quantity of royal jelly compared to the experimental group.

2. Table 2. Number of queen cells and quantity of royal jelly, q(X±SD)

Parameter	Control group	Experimental group
Average number of queen cells	115	115
Average quantity of royal jelly in a queen cell, g	0.112±0.028	0.126±0.035

MnSO₄ (p<0.05), (p<0.01), (1%) 166 237 μS/cm (Balkanska, 2014).

The next researches are connected to some components of the composition of the bee products obtained. It is interesting to note that MnSO₄ added to the sugar syrup influences the composition and physicochemical characteristics of the royal jelly. Statistically significant differences were found also in the protein content (p<0.05).

In addition, statistically higher values for the electrical conductivity were found in samples obtained from the experimental group (p<0.01), (Table 3). Electrical conductivity is an indicator that depends mainly on mineral content, organic acids, amino acids and proteins in the product. In royal jelly solutions (1%) it can range from 166 to 237 μS/cm (Balkanska, 2014).

3. Physicochemical properties of royal jelly, q(X±SD)

Parameter	Control group, n=6	Experimental group, n=6
Water content, %	61.82±1.01	61.97±1.05
Proteins, %	17.51±0.81*	16.32±1.09*
Fructose, %	4.99±0.91	5.53±0.40
Glucose, %	3.32±0.60	3.68±0.45
Sucrose, %	2.47±0.47	2.08±0.28
pH	4.09±0.32	4.14±0.24
Total acidity, ml 0.1 NaOH/g	3.73±0.29	3.91±0.21
Electrical conductivity, µS/cm	199.67±17.68**	173.86±10.45**

**p<0.01; *p<0.05

From the studied parameters in the honey samples – water content, electrical conductivity, hydroxymethylfurfural, only diastase activity and electrical conductivity showed statistically higher values in the samples obtained from the experimental group (p<0.05), (Table 4).

4. Physicochemical properties of multifloral bee honey, q(X±SD)

Parameter	Control group, n=5	Experimental group, n=5
Water content, %	16.32±0.23	15.90±0.30
Electrical conductivity, µS/cm	417.20±13.48*	439.40±9.37*
Diastase activity, Gothe units	22.41±1.69*	25.35±1.53*
Hydroxymethylfurfural (HMF), mg/kg	3.11±0.94	2.66±0.39

*p<0.05

Processing of nectar into honey and in royal jelly synthesis involve the same glands, which perform various functions during the different stages of the worker bees' development.

From the results obtained, it can be assumed that the bee families fed with MnSO₄ influence on the hypopharyngeal glands of worker bees. As a result they

- are better developed and produce royal jelly with higher protein content.

- These results are also confirmed by the fact that bee honey from the experimental bee families has a higher diastase activity than the control group.

- The following studies are about determining the Mn content in different bee products (Table 5).

Mn
(5).

5. Mn , (µg/g)
Table 5. Content of Mn in bee products, (µg/g)

Bee product	Control group ($\bar{X} \pm SD$)	Experimental group ($\bar{X} \pm SD$)
/ Royal jelly, n=6	0.51±0.08	0.56±0.06
/Multifloral bee honey, n=5	2.14±0.24	2.25±0.37
/ Propolis, n=7	37.27±3.40	36.54±2.28
/ Bee wax, n=6	1.87±0.25	1.92±0.17

MnSO₄

Mn

Mn

Mn

The addition of MnSO₄ on the experimental group did not affect significantly the Mn content of the studied bee products. The differences in the Mn content of royal jelly, honey, propolis and bee wax from the experimental and control groups are not statistically significant. As a major reason for the non-accumulation of Mn in royal jelly and honey can be explain with the bio-barrier function of the bee organism.

CONCLUSIONS

4 mg/l

MnSO₄

(p<0.01).

The addition of 4 mg/l MnSO₄ in sugar syrup has a positive effect on the average weight of non-flying worker bees (p<0.01).

Statistically significant differences in the protein content and electrical conductivity of the royal jelly samples and diastase activity and electrical conductivity of the honey in the experimental group bee families were found.

MnSO₄

The feeding of the bee families with sugar syrup with MnSO₄ may have a positive effect on the hypopharyngeal glands of the worker bees.

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Effect of the vitamin E enriched feed additive and superovulation on the parameters of the reproductive tract in mice

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SUMMARY

Vitamin E is one of the strongest antioxidant and necessary dietary supplement for the reproductive process. It helps reduce lipid peroxidation and prevents the adverse effects of free radicals. Implementation of nutrition enriched with vitamin E could help solve the problems associated with low or impaired reproductive function. The aim of this study was to trace the changes of some morphometric parameters of the reproductive tract in superovulated mice, received a dietary supplement, enriched with vitamin E. The experiment was conducted with 30 female laboratory mice, were divided into three groups (n=10), equalized in age and weight – control group, I-st experimental group with induced superovulation and II-nd experimental group, in which the superovulation effect was combined with an individual intake of the additive Provit E10% Super, including alpha-tocopherol acetate, for 30 days. After completion of the experiment the blood samples for the

Provital E10% Super

analysis of vitamin E content were collected. The reproductive tract was removed and the length and weight of uterine horns and uterus were measured. The results showed that the blood of animals from the experimental group II had two times higher level of alpha-tocopherol acetate than blood from the control group. The body weight and morphometric parameters of reproductive tract decreased in the experimental group II compared to the control group. Any effect of the superovulation on the morphometric parameters of reproductive tract in mice was not established. In conclusion, the study proved that alpha-tocopherol acetate from the feed additive Provital E10% Super absorbed very well and accumulated in the blood of the treated mice. The changes of the morphometric parameters of reproductive tract corresponded with the body weight changes, but were not affected by the induced superovulation.

Key words: bioactive feed additive, superovulation, mice, vitamin E

INTRODUCTION

In recent years the use of biologically active additives (BAD) becomes more and more attractive, because they are non-toxic food source and can improve the human and animal health. The natural origin of BADs is associated with their immune stimulating and immune modulating properties (Chervenkov et al., 2014; Kistanova et al., 2015). The bioactive substances of the BADs play a modulatory role for the important metabolic hormones, such as insulin, leptin and ghrelin (Tena-Sempere et al., 2013; Scaramuzzi et al., 2015). Many plants are a valuable source of vitamins. Vitamin E is the one of the vital fat-soluble vitamin for all mammals. The natural forms of vitamin E are synthesized in plants and consist a group of compounds such tocopherols and tocotrienols, which express varying biological activity.

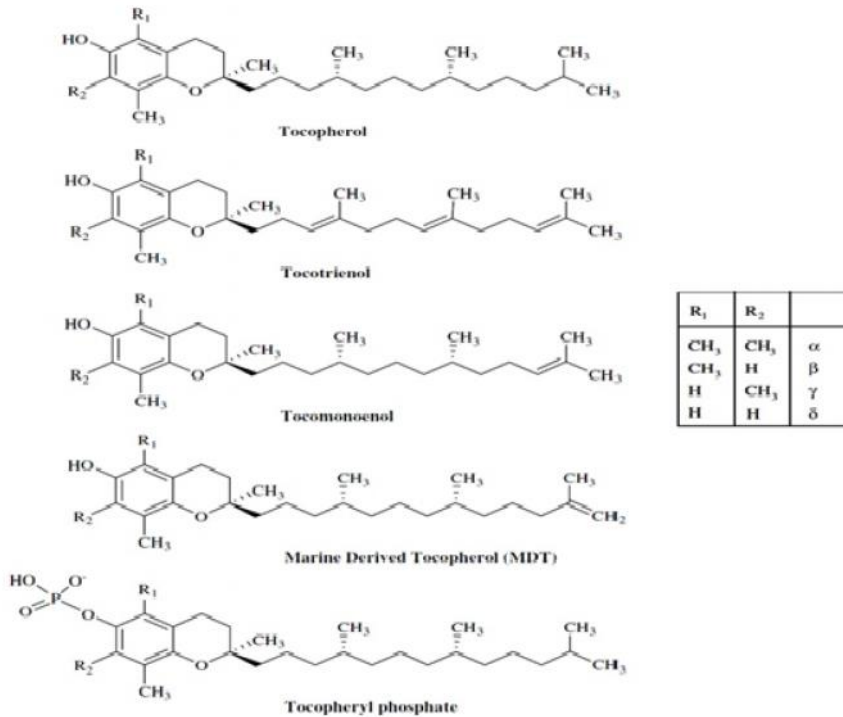
It depends on structural relationship between compounds included the

(Chervenkov et al., 2014; Kistanova et al., 2015).

(Tena-Sempere et al., 2013; Scaramuzzi et al., 2015).

(Zingg, 2007).

- hydroquinone rings and the isoprenoid chain and on a placement of the methyl group (Zingg, 2007).



1. (Zingg, 2007)
Fig. 1. Structure of Vitamin E (Zingg, 2007)

(NIH, 2007), (Zingg and Azzi, 2004; Sen et al., 2007; Zingg, 2007). (Segerson et al., 1980), (Cicek et al., 2012) (Guney et al., 2007).

Abundance of vitamin E was found in the egg yolk, liver, green and leafy plants (NIH, 2007), olive and sunflower oils (Zingg and Azzi, 2004; Sen et al., 2007; Zingg, 2007). As a strong antioxidant, vitamin E participates in the regulation of the reproductive processes: it improves the fertility of oocytes in sheep (Segerson et al., 1980.), lead to thickening of the endometrium in women with unexplained infertility (Cicek et al., 2012) and can prevent follicular degeneration and atresia in the ovaries (Guney et al., 2007).

Data regarding the effects of the dietary supplemented vitamin E on the size of the reproductive tract are scarce due to lack of the research in this field.

The aim of this study was to examine the influence of the enriched with vitamin E feed additive and superovulation on the morphometric parameters of the reproductive tract in mice.

MATERIAL AND METHODS

The experience was conducted in the vivarium of the IBIR-BAS, with 30 female Swiss white mice line BALB/C at mature age. The animals were divided into three groups (n=10), aligned in weight: control group, first experimental group – treated by the standard protocol for superovulation (Table 1), and second experimental group, in which the effect of superovulation was combined with an individual intake of dietary supplement Provit E10% Super (Profeed-Animals, Poland) in dose 1,5µg/g for 30 days.

Provit E 10% Super is a feed supplement, containing an extract of the plant artichoke and enriched with vitamin E.

white, BALB/c Swiss
(n=10), I
(1), II
Provit E10% Super
(Profeed-Animals,) 1,5
µg/g
30 . Provit E10% Super
, a
o

1.

Table 1. Superovulation protocol for female mice

0	PMSG- 6.00 I.U. (100 µL)
day 0	Injection of PMSG-6.00 I.U. (100 µ L)
2	hCG -6.00 I.U. (100 µL)
day 2	Injection of HCG -6.00 I.U. (100 µ L)
3	- 13 14 hCG
day 3	ollecting of the oocytes -13 to 14 hours after hCG

libitum.

temporal.

(: 2009-4-12/40).

d The animals received standard feed for mice and water *ad libitum*. The health status of the animals was checked daily. At the end of the experiment blood samples were collected from the *superficial temporal* vein. The vitamin E determination in the blood serum and in the feed additive was performed by gas chromatography method with a subsequent mass spectroscopic analysis. Mice were humanely killed according to the requirements of the Ethics Commission (report :2009-4-12). Reproductive tracts were collected and cleaned from the fat. The following parameters were measured: length and

STATISTI A (Stat Soft version 6.0), P 0.05.

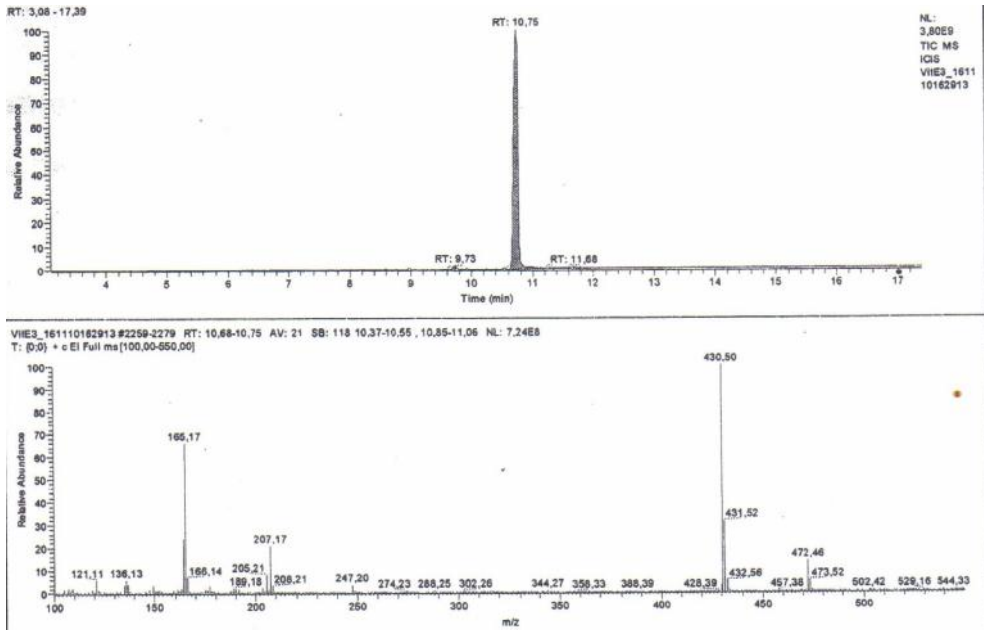
weight of the uterus, length of the uterine horns and the weight of the entire tract.

The results were developed by statistical package STATISTI A (Stat Soft version 6.0). Differences were considered significant with P 0.05.

RESULTS AND DISCUSSION

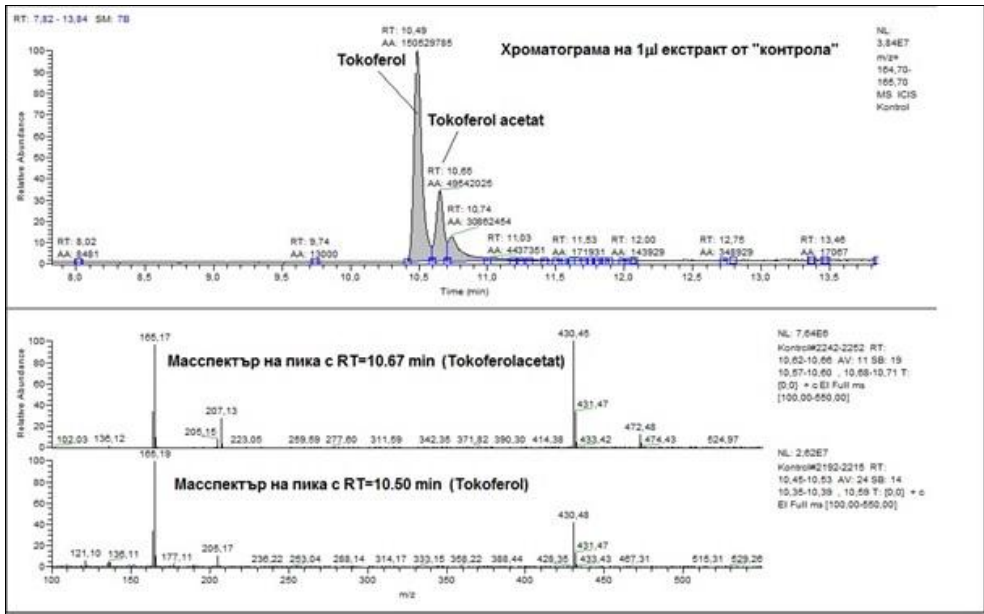
In the present study the levels of vitamin E in blood serum and its effect on the morphometric parameters of the reproductive tract in mice were investigated. Gas chromatography with a subsequent mass spectroscopic analysis is a reliable method, which can detect the qualitative and quantitative parameters of the tested substances and significantly reflects the changes of their metabolic levels (Roessner et al., 2000). Applying this method to analysis of our feed additive was shown that the main form of vitamin E in Provit E10 is an ester -tocopheryl acetate (Figure 1).

(Roessner et al., 2000).



1. () 10.75 min, e

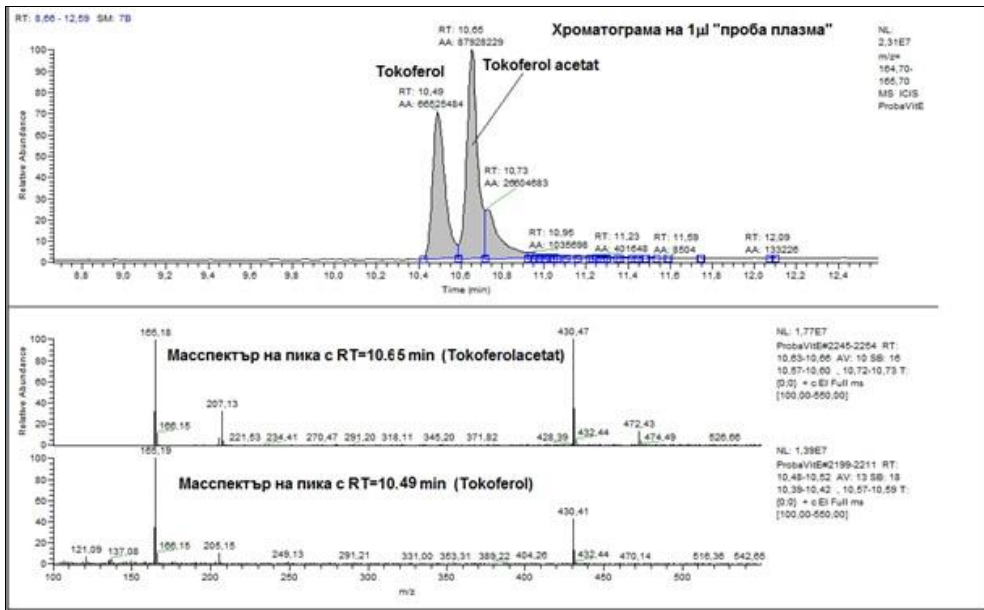
Fig. 1. Chromatogram (above) and MAs spectrogram of the supplemented feed additive. Peak with a retention time on 10.75 min is typical for the ester form of vitamin E – tocopherol acetate



. 2.

e
(n=6)

Fig. 2. Chromatogram and MAs spectrogram of the composite serum sample from the animals of the control and I experimental groups (both were not supplemented with feed additive) (n=6)



. 3.

(n=5)

Fig. 3. Chromatogram and MAs spectrogram of the composite serum sample from the animals of the II experimental group (received feed additive) (n=5)

(2). , (3), , (3.4 µg/ml 1.9 µg/ml) . 2 21 . 70 IU/kg (Umesiobi, 2009). 1000 IU , (3,43 µg/ml) - (Horn et al., 2010). , 4. , II (<0,05), 33,4±3,59 g (24,7±1,70 g).

- In control group of animals, vitamin E was mainly found in the form of tocopherol and insignificant quantities of the tocopheryl acetate (Figures 2). In the experimental group, has seen an increase of the vitamin E concentration in form of -tocopherol acetate (Figures 3), and a decrease of the tocopherol quantity.

- Thus demonstrates a good absorption of the vitamin E from the feed additive (3.4 µg/ml vs. 1.9 µg/ml in control). Umesiodi (2009) reported significantly increase of the -tocopherol serum concentrations in piglets on day 2 and 21 after addition of the vitamin E. Also author observed a sexual differences between females and males in absorption of the vitamin E in dose 70 IU/kg (Umesiobi, 2009).The addition of 1000 IU of the vitamin E to the cow diet significantly affected the serum -tocopherol concentration (3.43 µg/ml) compared to the control group. The estrus of same animals were synchronized with the aim to provide an artificial insemination, and the correlations between the hormonal treatment and levels of the -tocopherol was not found (Horn et al., 2014). Our experience confirmed this assumption, since it does not establish significant differences between control and I experimental groups regarding to the total weight of the reproductive tract and weight of the uterus after superovulation (Figure 4). At the same time we observed a significantly decrease of the reproductive tract weight in mice from the II experimental group compared to the control (<0,05). These data corresponded with significantly differences in body weight between these groups (24,7±1,70 g vs. 33,4±3,59g in control).

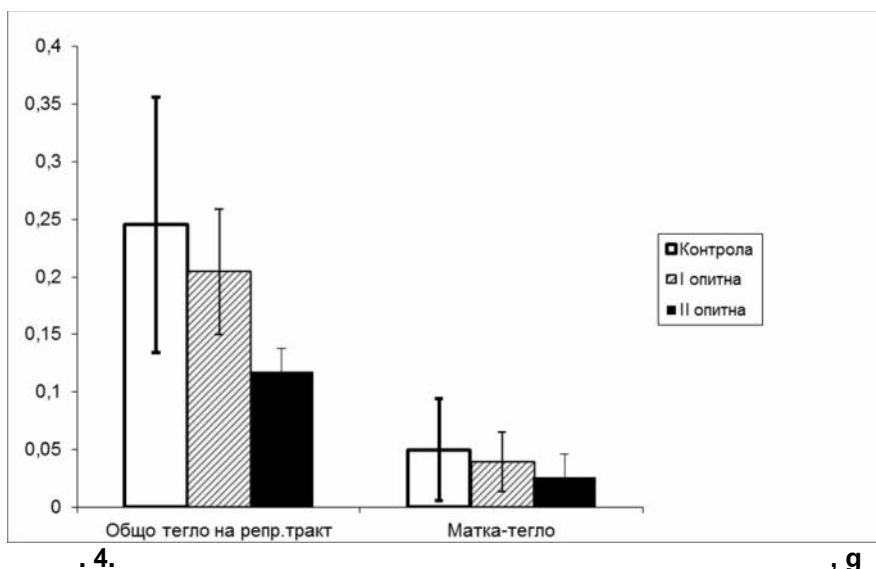


Fig. 4. Total weight of the reproductive tract and uterus, g

22 IU

(Mahan et al., 2000).

Balakrishnan (2013),

125 mg/kg -tocopherol.

(2)

E10% Super –

(Sritiawthai et al., 2013).

Similarly, the intake of 22 IU vitamin E by pigs, does not affect the live weight during of pregnancy or breastfeeding (Mahan et al., 2000). Despite that these authors did not establish the changes in serum level of the -tocopheryl acetate in these animals; they mentioned the positive effect of vitaminE addition on the prevention of the uterine problems, typical for young pigs. Contrary to these results, Balakrishnan et al. (2013) established a significant difference between initial and final weight in rats received oral dose 125 mg/kg -tocopherol (Balakrishnan et al., 2013).

Uterine morphometric analysis in our study included measure of the length of right and left horns and cervical length (Table 2). The results show a clear trend to decreasing the linear parameters of uterus horns in the II experimental group compared to the control group. Data can be explained by the composition of the supplement Provit E10% Super containing a plant artichoke, which has a properties to reduce body weight, improve hepatic function, lipid profile and blood sugar (Sritiawthai et al., 2013). In addition, Soliman et al (2014) reported a decrease in body weight when treating

- reflect the reproductive tissue content of tocopherol in animals, but could reflect the available biological forms of the vitamins in the body.

CONCLUSIONS

In conclusion, this work provides a new understanding of the effects of the dietary vitamin E on the morphometric parameters of the reproductive tract in mice, missing in the available literature. This study proves that tocopherol acetate from food additive Provit E10% Super was well absorbed by the body and accumulated in the blood of the experimental group of animals. The morphometric parameters of the reproductive tract closely correspond to the changes in the live body weight than to the level of the α -tocopherol in the blood and not affected by the induced superovulation.

Super

Provit E10%

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