Evaluation and determination of selenium deficiency in the food chain of sheep and cow reared in the endemic mountain regions of Middle Rhodope

Borislav Blazhev¹, Ljubomir Angelow²*

¹Central Laboratory for Chemical Testing and Control, 120 Nikola Mushanov Blvd., 1330 Sofia, Bulgaria
²Institute of Cryobiology and Food Technologies, 53 Cherni vrah Blvd., 1407 Sofia, Bulgaria

SUMMARY

Selenium deficiency of soils and natural pasture vegetation, as well as an irregular supply of trace-levels of selenium to ruminants due to the seasonal dynamics during the pasture period, a low bio-accumulation rates of selenium in feedstuffs have been established.

The present study for selenium concentrations in the food chain is reviewed in details and the transfer from soil to foodstuffs of selenium are reported.

The investigation had been focused on the insufficient selenium supply and related consequences on the Se-content in raw milk and white brine cheese of sheep and cow spread in the endemic areas. The low selenium concentrations in the plant species during the lactation

*E-mail: luboangelov@abv.bg
(April-June) reflected negatively on the Se-concentration in the ewe’s and cow’s milk. Recommendations for selenium suplementations are proposed.

**Key words:** selenium, deficiency, food chain, ewe’s milk, Middle Rhodope Mountain

**INTRODUCTION**

From the nutrition point of view the selenium availability in foodstuffs remains of great concern because a lot of factors affect its transfer along of the ecological chain. Selenium deficiency affects the expression and function of selenoproteins and has been involved in the degeneration of organs and tissues, leading to the manifestation of Keshan and Kashin-Beck diseases.

The selenium content of grains and vegetables generally depends on the selenium content of the soil, as well as on its geochemical characteristics (Johnson et al., 2010; Mehdi et al., 2013). The potential supply of selenium in soil can be influenced by factors which influence selenium speciation and solubility of selenium compounds (Kabata-Pendas and Pendas, 1992).

In general in acid, clay soils and soils with high organic matter the Se-content dominate in form of selenides and selenium sulfides which are slightly mobile and therefore hardly available to plants.
In alkaline soils selenates are present. They are easily soluble and highly mobile, respectively available to plants.

The solubility of selenium in most soils is rather low, therefore many agricultural areas produce forage with low selenium content.

The uptake of selenium by plants depends at first on soil pH, redox potential and water content.

Plants may be classified as selenium accumulators or non-accumulators, depending on their ability to assimilate and accumulate selenium (Terry et al., 2000; Broadley et al., 2006).

During the grazing period ruminants are subjected to variations in the selenium supply through meadow vegetation.

Thus any disturbances may occur leading to impaired animal health and production traits – low milk production, insufficient content of selenium in milk and dairy products (Angelow et al., 2004; Makaveeva et al., 2004).

Data on the forms of selenium in animal foods are limited, and the selenium content of foods from animal sources varies according to the diet of the animals (Mehdi et al., 2013). When inorganic selenium is given to animals, selenocysteine is the
main seleno-compound formed. When animals consume selenium-containing foods of plant origin, protein-containing selenomethionine will also be formed from the incorporation of plant-derived selenomethionine in place of methionine (Rayman et al., 2008).

Although water may contain selenium, predominantly as selenate, its content is typically low and does not significantly contribute to selenium intake (WHO, 2011).


In fact the bioaccumulation of selenium along the food chain is influenced of interrelated action of such factors as geological formations, soil acidity, soil organic matter content, vegetation stage of plants and species-specific uptake (Johnsson 1992; Zablocky 1990; Thornton et al. 1985).

In view of mentioned considerations data on each particular agriculture area is needed to modify the selenium
supply for animal and human nutrition.

The aim of present study was to compare the selenium offer of pastures situated at different altitudes in some mountainous regions in South Bulgaria and to give the estimation on the selenium offer of sheep, raised in the investigated endemic areas.

MATERIAL AND METHODS

The subject of investigation was a region near Smilyan village in the Middle Rhodope Mountain. Average samples of pasture grass from 10 (5 + 5) standard plots (2x2 m) situated at two levels of altitude were collected in 2 replications (Fig. 1). For assessment the effect of vegetation stage this procedure was performed once monthly during the grazing period (from May to July). In the experiment research on the dynamics of selenium in the raw milk and cheese was involved the most popular breeds in the investigated area – Karakachan sheep, Rhodope Zygay and Aborigen (Middle Rhodope breed).

Data was compared with the typical Se-content in cow’s milk received in the area. Studied are derived from white brine cheese and yellow cheese produced in the region with a homemade recipe.
The selenium content was determined using Varian AAS-HG and AGILENT ICP-MS analysis. All results were expressed as mean ± standard deviation and compared through standard t-test procedure.
The development of nitrogen-fixing bacteria is greatly reduced, it was expected that in the acidic soil nitrogen content is substantially below the required good soil that the availability. Typical of the region botanical species and lack of basic and secondary macro and micro nutrients determined botanical diversity.

### Table 1. Botanical composition of the pastures in the investigated region

<table>
<thead>
<tr>
<th>№</th>
<th>Botanical composition</th>
<th>% content</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Trifolium incarnatum</em> / пурпурна детелина</td>
<td>30.21%</td>
</tr>
<tr>
<td>2</td>
<td><em>Lolium perenne</em> L. / райграс</td>
<td>5.45%</td>
</tr>
<tr>
<td>3</td>
<td><em>Bromus mollis</em> L. / мек овсиг</td>
<td>5.55%</td>
</tr>
<tr>
<td>4</td>
<td><em>Festuca fallax</em> Thuil. / червен вл с тк</td>
<td>0.60%</td>
</tr>
<tr>
<td>5</td>
<td><em>Trifolium repens</em> L. / бял детелин</td>
<td>3.26%</td>
</tr>
<tr>
<td>6</td>
<td><em>Poa pratensis</em> L. / ливадна метлица</td>
<td>9.73%</td>
</tr>
<tr>
<td>7</td>
<td><em>Pteridium aquilinium</em> / орлов пр т</td>
<td>1.68%</td>
</tr>
<tr>
<td>8</td>
<td><em>Vicia villosa</em> Roth / див фий</td>
<td>2.28%</td>
</tr>
<tr>
<td>9</td>
<td><em>Nardus stricta</em> / картъл</td>
<td>13.18%</td>
</tr>
<tr>
<td>10</td>
<td><strong>Other plant species / други видове</strong></td>
<td>28.05%</td>
</tr>
<tr>
<td></td>
<td><strong>total / общо</strong></td>
<td><strong>100%</strong></td>
</tr>
</tbody>
</table>
Фиг. 2. Съдържание на селен в различни растителни видове

Fig. 2. Selenium content in different plant species

There is a pronounced deficit in all species close and below the critical 30 μg/kg DM.

The study of meadow grass containing all typical of the region indicator plants shows that there is no species which can compensate the low supply of selenium, so that the average level of the element is about 10-15% of the necessary needs of the animal organism (Fig. 2).

Selenium transfer during whole period is stable and depends mainly by geochemical characteristics of soil (Table 2). There are no bioaccumulation species among botanical species in the area that would have affected selenium concentrations in the food chain "soil-plant".
Fig. 3. Seasonal dynamics of selenium in meadow grass depending on the levels of altitude

At both levels of altitude (800 and 1100m) selenium concentrations were far below normal average concentrations (0.25mg/kg), and seasonal dynamics depends mainly from geological and climatic conditions during the investigated period (Fig. 3).

Table 2. Transfer factors for selenium for different altitude from two consecutive years (2013/2014)

<table>
<thead>
<tr>
<th>Area/Sample/Year/Altitude</th>
<th>Selenium, µg/kg</th>
<th>Transfer factor / Коефициент на трансфер</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smylian/soils/2013 (n=3)</td>
<td>1100m</td>
<td>0.15</td>
</tr>
<tr>
<td>Smylian/plants/2013 (n=3)</td>
<td>1100m</td>
<td>0.15</td>
</tr>
<tr>
<td>Smylian/soil/2013 (n=3)</td>
<td>800m</td>
<td>0.15</td>
</tr>
<tr>
<td>Smylian/plants/2013 (n=3)</td>
<td>800m</td>
<td>0.15</td>
</tr>
<tr>
<td>Smylian/soil/2014 (n=5)</td>
<td>800-1100m</td>
<td>0.15</td>
</tr>
<tr>
<td>Smylian/plants/2014 (n=5)</td>
<td>800-1100m</td>
<td>0.15</td>
</tr>
</tbody>
</table>
Critical deficit levels around 20-30 μg/l were observed in all milk samples. With the progress of lactation concentrations continue to decrease.

The lack of enough selenium in all studied breeds and trends are identical or similar to those in cow’s milk (Fig. 4). Significant deficiency of selenium in grass composition and their mutual synergistic relationships with iodine have a significant impact on the readings of selenium in sheep milk.

Better provision in cow’s milk is due to additional supplementation with concentrated feed contained balanced levels of selenium, to maintain a high milk yield.
Fig. 5. Selenium concentrations variation in white brine cheese from different sheep breed’s milk and cow’s milk during the investigated period (May-June).

A concentration of selenium in cow and sheep products ranges from 40-100 μg/kg. Concentrations of selenium are stable and consistent in all products of the different breeds (Fig. 5).

Deficit caused by the lack of selenium in the milk are retained in the products obtained during the entire investigated pasture period. Transfer factors in the food chain “plant-milk” and “milk-milk products” are in the range 1-2 and remain constant during investigated periods and conditions.  

CONCLUSIONS

The study on the selenium supply through pasture vegetation to ewes reared in the Middle Rhodope area revealed its irregular pattern.
It was established by seasonal variations in the Se content of meadow vegetation in the range of low selenium transfer from soil to pastures at 800 and 1100 m of altitude.

This effect was primarily determined from the spread geological structures (gneiss, syenite) and low pH of soils. The insufficient selenium availability becoming more pronounced with the advance of vegetation amounted by the end of grazing period only from 15 to 20 % of minimal nutritional needs of sheep. It was the main reason for sheep and lamb reared in the region to develop a chronic selenium deficiency.

REFERENCES


Possibilities to increase reproductive performance in sheep of Thracian merino breed by applying various hormonal methods

Nedka Dimova

Agricultural Institute, 6000 Stara Zagora, Bulgaria

The aim of the present study is to investigate possibilities for increasing the number of the lambs born by ewes from the Thracian Merino breed by applying various methods of hormonal stimulation.

The first experiment was carried out with 271 ewes treated with intravaginal sponges Sincro-part and injection of 500 UI PMSG, and 370 untreated ewes.

In the second experiment, the first group of 90 ewes were placed implants Melovine, a second group of untreated animals were 277 animals.

The following parameters were monitored: number of artificially inseminated animals, number of ewes that gave birth to lambs, number of abortions, number of lambs born, fertility and prolificacy.

Fertility of treated with sponges ewes is slightly higher than that of the untreated with 0,16% the first year and lower the second and third year, respectively 10,21% and 10,48%.

Progesterone sponges increase
Prolificacy is increased by an average of 7.36% using the intravaginal sponges and to 12.18% when using implants compared with untreated animals.

Treated with intravaginal sponges sheep are 34.19% higher prolificacy, but with 12.31% lower fertility compared to treatment with melatonin implants.

**Key words:** ewes, fertility, prolificacy, melatonin, intravaginal sponges

**INTRODUCTION**

The main factor affecting the economic effectiveness in sheep breeding, regardless of the production aspect, is reproduction performance. In order to achieve satisfactory financial results, effective reproductive management is needed. A major element of reproductive management in sheep breeding is estrus synchronization.

In sheep breeding, two methods are used for estrous synchronization for timed artificial insemination and fertility boosting: natural (non-hormonal) and hormonal.

Hormonal treatment allows planning the lambing time considering the possibilities for profitable realization of the lambs.
There are known three types of hormonal methods for estrus synchronization, by methods based on the action of progesterone or its synthetic analogues, such as progestogens; by regression of the corpus luteum with prostaglandin F2α or its synthetic analogues; by use of melatonin for induction of oestrus in sheep (Metodiev et al., 2010).


In our country in recent years Raltchev et al. (2011), Metodiev and Raicheva (2011), Slavova et al. (2012) and Slavova et al. (2013) investigated the effect of intravaginal sponges in Ile de France and Thracian Merino breeds and Bonev (2012) tested implants "Melovine" in Ile de France and various dairy ewes crosses.

The aim of the present study is to investigate possibilities for increasing the number of the lambs born by mother ewes from the Thracian Merino breed by applying various methods of hormonal
1. Studying the effect from the combined applications of SYNCRO-PART sponges and SYNCRO-PART PMSG.

The first experiment was carried out in the sheep farm at the Agricultural Institute of Stara Zagora with ewes from the Thracian Merino breed for a period of 3 farming years – 2009, 2010 и 2012. The animals were assigned into two groups. The first group of animals was treated with hormonal preparations during April and May. The following hormonal treatment scheme was used: inserting intravaginal sponges Sincro-part (30mg flurogestone acetate-FGA) into the ewes, taking the sponges out after 12 days and administering PMSG injection in a dose of 500UI, artificial insemination on the 50th-55th hour.

The second group of animals was artificially inseminated during the months of July and August without the use of any hormonal preparations.

The groups were set up in accordance with the time interval between the previous lambing and weaning of the lambs. This means that the ewes from the first group gave birth in November and December, and those from the second group-in January and February. Treated with intravaginal sponges were 271 ewes and the
370 броя.

2. роувч не н ефект от прил г нето н мел тонинови импл нти Melovine (CEVA ANIM. HEALTH).

торият експеримент бе проведен с овце от пород т р кийск тънкорунн в овцевъдн т ферм н - т р гор през 2 стоп нски години – 2011 и 2013г.

рез месец феврури бяях пост вени импл нти н овцете-м йки и н кочовете в и през м рт- прил животните бяях осеменени изкуствено ( - в груп ) – 90 броя. рез месец м й бяях осеменени нетретир - ните животни ( - р груп ) – 277 броя.

росложен бе з плодяемостт и плодовитостт през от- делните стоп нски години. он- тролир ни бяях следните пок - з тели: брой осеменени, з пло- дени, о гнени и бортири ли овце, брой родени гнет (живо родени, мъртво родени и недоносени) и биологичн плодовитост.

плодяемостт при поро- д т р кийск тънкорунн след прил г не н хормон лн сти- мул ция с т мпони и PMSG е предст вен н блиц 1.

рез първ т годин з плодяемостт при опитн т групп е 63,00%, през втор т годин – 77,08%, през трет т

untreated animals included in the trial were 370.

2. Studying the effect from the application of melatonin implants Melovine (CEVA ANIM. HEALTH).

The second experiment was carried out in the sheep farm at the Agricultural Institute of Stara Zagora with ewes from the Thracian Merino breed for a period of 2 farming years – 2011 and 2013.

In February there were placed implants ewes and rams into and through March-April animals were artificially inseminated (Group I) – 90. In May they were inseminated untreated animals (Group II) – 277.

Both fertility and prolificacy were observed over the reported farming years. The following parameters were monitored: number of artificially inseminated animals, number of ewes that gave birth to lambs, number of abortions, number of lambs born (live born, stillborn) and fertility and prolificacy.

RESULTS AND DISCUSSION

Fertility in the breed Thracian Merino after administration of hormonal stimulation pads and PMSG is presented in Table 1.

The concepted ewes from the trial group represent 63,00% during the first year, 77,08% during the second year, and a lower
годин е по-ниск - 69,33%.

бри овцете от контролн т груп тя е съответно 62,84%, 87,29% и 79,81 %. Олучените резултати с близки до уст новен т от ловови и сътр. (2013) за плодяемост на овце от същ т пород – 72,92% при трети ните животни и 83,58% при нетрити ните.

при опитн т груп с т мпони плодяемостта е незн чително по-висок от т зи н контролн т груп с 0,16% първ т годин, и по-ниск втор и трет годин, съответно с 10,21% и 10,48%.

рез първ т годин с се о гнили 63,00% от опитните животни и 60,13% от контролните, през втор т съответно 76,04%, и 84,75%.

рез трет т годин от първ т груп с се о гнили 69,33% от трети ните овце, и 79,81% от нетрити ните животни.

рез рез рез 1. плодяемост при пород т р кийск тънкорунн –хормон ли стимул ция с т мпони

<table>
<thead>
<tr>
<th>Traits</th>
<th>I годин / I year</th>
<th>II годин / II year</th>
<th>III годин / III year</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>питн Experimental</td>
<td>контролн Control</td>
<td>питн Experimental</td>
</tr>
<tr>
<td></td>
<td>n %</td>
<td>n %</td>
<td>n %</td>
</tr>
<tr>
<td>семенени Inseminated</td>
<td>100</td>
<td>100</td>
<td>148</td>
</tr>
<tr>
<td>плодени Mated</td>
<td>63</td>
<td>63,00</td>
<td>93</td>
</tr>
<tr>
<td>гниени Lambed ewes</td>
<td>63</td>
<td>63,00</td>
<td>89</td>
</tr>
<tr>
<td>борти ли Aborted</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>

The obtained results are similar in ewes from the same breed quoted by Slavova et al. 2013, i.e. 72,92% in the animals treated and 83,58% in the animals untreated. During the first year, fertility in the animals from the trial group for which tampons were used, is insignificantly higher than that of the control group with 0,16%, and during the second and the third year, it decreases by 10,21% and respectively 10,48% in the animals treated.

During the first year, 63,00% of the trial animals and 60,13% of the control animals gave birth to lambs. During the second year this rate is respectively 76,04% and 84,75%. During the third year, 69,33% of the animals from the first group and 79,81% of the animals from the second group gave birth to lambs.
The reproduction performance is presented in Table 2. Prollificacy of experimental group is 142.86; 156.16 and 151.92% for the three years studied. In the control group, productivity is significantly lower, i.e. 124.72%, 120.00% and 138.55%.

The applied hormonal stimulation on the basis of progesterone sponges results in increased prolificacy in ewes from the Thracian Merino breed during all three studied years (increase by 18.14%; 36.16% and 13.37% respectively). Prolificacy is higher after hormone stimulation of animals from the same breed also according to the studies of Slavova et al. (2012) (increase by 37.26%) and Slavova et al. (2013) (increase by 42.97%).

<table>
<thead>
<tr>
<th>traits</th>
<th>I year</th>
<th>II year</th>
<th>III year</th>
</tr>
</thead>
<tbody>
<tr>
<td>n %</td>
<td>n %</td>
<td>n %</td>
<td>n %</td>
</tr>
<tr>
<td>Born lambs</td>
<td>90 100</td>
<td>111 100</td>
<td>114 100</td>
</tr>
<tr>
<td>Live borns</td>
<td>86 95.55</td>
<td>96 86.49</td>
<td>108 94.74</td>
</tr>
<tr>
<td>Still borns</td>
<td>4 4.44</td>
<td>15 131,51</td>
<td>6 5.26</td>
</tr>
<tr>
<td>Плодовитост</td>
<td>- 142.86</td>
<td>- 124.72</td>
<td>- 156.16</td>
</tr>
</tbody>
</table>

Table 3 shows the results after applying hormonal stimulation with melatonine implants in animals from the Thracian Merino breed.
Fertility in the trial group is 31.67% during the first year and 43.33% during the second year. In the control group, the percent of concepted ewes is significantly higher, i.e. representing 81.48% and 76.76%.

The animals into which implants were inserted, show significantly lower fertility than that of the control animals. During the first year the difference is 49.81% and during the second year - 33.43%.

Table 3. Fertility of Thracian Merino-hormonal stimulation with implants

<table>
<thead>
<tr>
<th>Traits</th>
<th>Experimental / I year</th>
<th>Control / I year</th>
<th>Experimental / II year</th>
<th>Control / II year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inseminated</td>
<td>60 100</td>
<td>135 100</td>
<td>30 100</td>
<td>142 100</td>
</tr>
<tr>
<td>Mated</td>
<td>19 31.67</td>
<td>110 81.48</td>
<td>13 43.33</td>
<td>109 76.76</td>
</tr>
<tr>
<td>Lambed ewes</td>
<td>19 31.67</td>
<td>110 81.48</td>
<td>13 43.33</td>
<td>109 76.76</td>
</tr>
<tr>
<td>Aborted</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
</tr>
</tbody>
</table>

Effect of implants on reproduction performance is shown on Table 4. Prolificacy of the animals from the experimental group during the first and the second year is respectively 152.63% and 138.46%, and 135.45% and 133.94% in the animals from the control group.

Hormonal stimulation with melatoinine implants results in increased prolificacy by 17.18%.
during the first year and 4.52% during the second year.

Similar are the results from the meta-analyses carried out by Palacin et al. (2011), stating that the melatonine implants increase prolificacy by 10.0% in the Merino sheep breed.

Table 4. Reproductive performance of Thracian Merino-hormonal stimulation with implants

<table>
<thead>
<tr>
<th>Traits</th>
<th>I години / I year</th>
<th>II години / II year</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Experimental</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>n   %</td>
<td>n   %</td>
</tr>
<tr>
<td>Родени агнета</td>
<td>29 100</td>
<td>149 100</td>
</tr>
<tr>
<td>Живо родени</td>
<td>29 100</td>
<td>146 97,99</td>
</tr>
<tr>
<td>Мъртво родени</td>
<td>0 0</td>
<td>3 2,01</td>
</tr>
<tr>
<td>Плодовитост</td>
<td>- 152,63</td>
<td>- 135,45</td>
</tr>
</tbody>
</table>

Figure 1 shows fertility with the two methods of hormonal stimulation. The average fertility for all three years after intravaginal sponges is 69.74%, and 75.68% for the control group animals. The experimental group animals show lower fertility (decrease by 5.94%) in comparison with the control group animals.

For the animals treated with melatonin implants, the average fertility for the two study years is 35.55% and for the untreated animals – 79.06%. The impregnation rate in the trial group animals is lower than that of the
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poni ovce s c 34,19% po-visok
z plodiamost v sr vnieie c
treti nite s mel toninoi
impl ntiживотни.

tcontrol group by 43,51%. The
animals treated with intravaginal
sponges show higher fertility
(increase by 34,19%) in
comparison with the animals
treated with melatonine implants.

**Fig. 1.** Fertility of Tracian Merino breed after hormonal stimulation (%)

<table>
<thead>
<tr>
<th></th>
<th>Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sponges</td>
<td>69.74</td>
</tr>
<tr>
<td>Implants</td>
<td>35.55</td>
</tr>
</tbody>
</table>

Figure 2 shows the effect from the two methods for hormonal stimulation. The animals from the experimental group treated with intravaginal sponges show average prolificacy for the three years of 134,57%, and 127,21% for the control group animals. The trial group shows increased prolificacy by 7,36%.

The average prolificacy of the animals treated with melatonine implants for the two years is 146,88%, and that of the control group-134,7%. The trial
12,18% по-висок плодовитост от тази на контролната група. Овцете, хормонално стимулирани с вагинални тампони, са с 12,31% по-ниска плодовитост от тези с мелатонинови импланти.

The application of hormonal stimulation increase productivity in animals from the Thracian Merino breed. Fertility is higher after hormonal stimulation with progesterone sponges, but prolificacy is higher in animals treated with melatonin implants.
CONCLUSIONS

Fertility of treated with intravaginal sponges ewes is slightly higher than that of the untreated with 0.16% in the first year and lower in the second and third year, respectively 10.21% and 10.48%.

Progesterone sponges increase prolificacy in ewes during the three years studied, respectively 18.14%, 36.16% and 13.37%.

Fertility of the ewes treated with implants is lower by 49.81% the first year and by 33.43% in the second year. Melatonin implants increase prolificacy by 17.18% and 4.52%.

Prolificacy in animals fine fleece is increased by an average of 7.36% using the intravaginal progestone sponges and to 12.18% when using implants compared with untreated hormonal stimulation.

Treated with intravaginal sponges sheep are 34.19% higher prolificacy, but with 12.31% lower fertility compared to treatment with melatonin implants animals.

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Tenderization effect of plant proteases bromelain and papain on buffalo meat

Maria Doneva, Svetla Dyankova*, Daniela Miteva, Petya Metodieva

Institute of Cryobiology and Food Technologies, 53 Cherni Vrah Blvd., 1407 Sofia, Bulgaria

SUMMARY
Tenderness is one of the most important flavor characteristics of the meat. The aim of this study is to investigate tenderization effect of plant proteases bromelain and papain on buffalo meat. Experiments are conducted with samples of raw meat in 3 different concentration levels of the enzyme solutions (50 U/ml, 100 U/ml and 200 U/ml) and in 3 different time periods (duration) of treatment (24 h, 48 h, 72h). Upon treatment with solutions of 50 U/ml and 100 U/ml caseinolytic activity, the water retention rate is higher, while the degree of hydrolysis is lower. The processing of buffalo meat with papain preserves higher level native texture, color and moisture of fresh meat compared to variants tenderized with bromelain. The optimal conditions for hydrolysis with minimal loss of protein and highest retention of organoleptic qualities of the meat samples are established.

Keywords: tenderization, buffalo meat, bromelain, papain
INTRODUCTION

The quality of the meat is defined as a combination of sensory and technological characteristics, such as color, tenderness, water-holding capacity and texture (Istrati et al., 2014).

Tenderness is one of most important meat texture attributes which affects the perception of buffalo meat, by the customers (Brooks et al., 2000; Morgan et al., 1991).

Fragility depends on the structural integrity of the myofibrils and of connective tissue which surrounds the muscle fibers. It has been found that in aged animals, buffalo meat becomes more resilient, which structurally is due to the formation of multiple cross links between collagen molecules (Ionescu et al., 2008).

In the recent years interest is the development of better methods to produce meat with improved tenderness whilst preserving its nutritional qualities (Koohmariae, 1996; Georgieva & Nacheva, 2007).

There are various chemical and physical methods for meat processing, but the use of proteolytic enzymes is one of the most popular methods for tenderization (Naveena at al., 2004).

In the meat processing, enzymes are interesting technological tools because they
enabling the conduction of highly specific chemical reactions.

Sources of enzymes for tenderization with practical importance are: natural proteolytic enzymes in meat, enzymes of microbial origin and enzymes of vegetable or animal origin.

Meat tenderization in postmortem maturation is a result of endogenous enzyme activity in the muscles.

However, the ensuing biochemical process of autolysis causes enzyme inhibition. Therefore, the proteolytic hydrolysis of the endogenous enzymes in the postmortem period is of reduced efficacy (Taylor et al. 1995).

Proteolytic enzymes derived from plants such as papain, bromelain, ficin, etc. have been widely used as meat tenderizers in most parts of the world (Sunantha and Saroat, 2011). Plant proteases are superior to bacterial derived enzymes mainly because of safety problems such as pathogenicity or other disadvantageous effects (Qihe et al. 2006).

The aim of presented study is evaluating the tenderization effect of the plant proteases bromelain and papain over raw buffalo meat and determining the optimal conditions for the process of hydrolysis.
MATERIAL AND METHODS

Materials
Meat – Biceps femoris buffalo muscle, breed Bulgarian Murrah.

Enzymes – papain (Merck), bromelain (Merck).

Methods
Enzymatic processing of buffalo meat samples – The meat samples are treated with bromelain or papain with alternating enzyme concentration and duration of the process.

Enzyme solutions
Both enzyme solutions are with the following caseinolytic activity – I (50 U/ml), II (100 U/ml), III (200 U/ml). The enzymes are dissolved in a solvent containing 0,9% NaCl, sodium hydrogen carbonate and citric acid. The active acidity of the enzyme solutions is pH 6,30.

Measuring the Water Retention Capacities
Meat samples of 3-5 grams are wiped with filter-paper to remove surface water and to weigh accurately in milligrams. This value is noted as raw meat weight (starting weight). The samples are then treated with bromelain and papain solutions at 4ºC for 24, 48 and 72 hrs.

Then, the surface water is removed with filter-paper. Alongside the samples, controls are assigned every full hour of treatment, in which the meat is
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Enzymatic activity
The caseinolytic activity of
the proteases papain and
bromelain is measured by the
substrate casein in a 50mM Tris/
HCl buffer at pH 8.0 with 1mM
CaCl₂, in accordance with Chen et
al. (2003) method. One unit of
enzyme activity is defined as the
amount of enzyme needed to
release 1 µg tyrosine from casein
for 1 minute.

Quantity assessment of free
amino acids – ninhydrin test
(Murariu et al., 2003).

Samples of 2.0 g muscle or
connective tissue are flooded with
40 ml of the enzyme solution and
incubate at room temperature for
72 hours, then determine the
concentration of free amino acids
in soluble fractions after enzymatic
hydrolysis.

Statistical analysis
All data are presented as
means ±SD (standard deviation)
for at least three replications for
each prepared sample. Statistical
analysis was performed using two-
sample t-test. The results are
considered to be significant when
P<0,05. All statistical analyses
RESULTS AND DISCUSSION

Preservation of juicy and fresh look of buffalo meat is an important indicator for consumers. Therefore, in our study had traced the change in capacity for water retention of the meat, incubated in enzyme solutions type of marinade. It was reported the weight of the control and experimental variants processed with corresponding solutions of bromelain and papain and left for the forthcoming 4°C for 24, 48 and 72 hours. Based on the accounting differences in the weight of each option is calculated processing capacity for water retention in percentages (Fig. 1, 2)
The above results indicate that, under treatment with solutions having the 50 U/ml and 100 U/ml caseinolytic activity, the rate of water retention increases with increasing time of treatment and the concentration of the enzyme. Upon hydrolysis with 200 U/ml, with a rise time of treatment with the enzyme bromelain is reported weight loss of experimental variants. This is due to the higher degree of hydrolysis of meat proteins, respectively, of the gelatinization of samples and release the terminal peptides and amino acids. Lighter effect is observed with the enzyme papain. In these experimental variants have seen a rise in the percentage of water retention, even at the highest concentration of enzyme and working up to 72 hours. Also
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добър външен вид, в ср вние с пробите, третир ни с бромел ин.
ромел инът и п п инът с р стителни цистеинови ендо-
пептид зи. ромел инът е не-
специфичн проте з , която
t кув с повишен финитет
пептидните връзки между ми-
нокиселините лизин и ргинин.
п инът хидролизир пептид-
ните връзки в белтъчните моле-
кули, с изключение н тези, в
чието изгр жд не уч ств т ми-
nокиселините пролин и глут ми-
nов киселин с дисоциир н
к рбоксил груп .
лиянието н ензимите при
окрехкотяв не върху външния
вид н месото е в ж ен ф ктор
при избор н ензим и условия
н обр ботк .
samples tenderized with papain
have crisp color and better
appearance, in comparison with
the samples treated with the
bromelain.
Bromelain and papain are
vegetable cysteine
endopeptidases. Bromelain is a
non-specific protease, which
attacks the peptide bonds between
lysine and arginine in protein
molecules with high affinity.
Papain hydrolyzes the peptide
bonds in protein molecules with
the exception of those composed
of amino acids proline and
glutamic acid with a dissociated
carboxyl group.
The influence of enzymes in
tenderization on the appearance of
the meat is an important factor in
the choice of enzyme and
conditions of processing.
Fig. 4. Control and experimental variants processed with papain

In the following visual materials (Fig.3 and 4) it is clear that the meat samples processed with 50 and 100 U/ml bromelain and papain for 24 hours retain their color and fresh look. In appearance, the structure of these experimental variants is similar to both control and monitor arranged muscle fibers. The samples from 48 and 72 hour especially muscle fibers start to deform and break down, the color fades and the surface of the samples it slimy. This change in the types of meat samples is more intense in the treatment with the enzyme bromelain. Dissociation of muscle fibers in such a degree is not desirable, since the appearance of the product is of particular importance for the consumers.

The degree of activity of proteolytic enzymes papain and bromelain on meat proteins in samples raw buffalo meat was determined by analyses of the amount of free amino acid in the
reactive liquid. The analysis was carried out after a stay of the meat samples from 72 hours in enzymatic solutions. The results obtained are presented in Table 1.

Table 1. Free amino acid content in the reactive liquid after enzyme hydrolysis of samples meat (±SD)

<table>
<thead>
<tr>
<th>Варианти</th>
<th>Концентрация mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bromelain</td>
</tr>
<tr>
<td>Контрол / Control</td>
<td>1,040±0,060</td>
</tr>
<tr>
<td>I</td>
<td>1,900±0,130*</td>
</tr>
<tr>
<td>II</td>
<td>2,170±0,082**</td>
</tr>
<tr>
<td>III</td>
<td>2,430±0,030**</td>
</tr>
</tbody>
</table>

1 т титъчески значими различия спрямо контролната група при: *p<0,05; **p<0,01

Parallel test was held for research of proteases surveyed on the connective tissue of the buffalo meat. The results of the analysis are given in Table 2.

Table 2. Content of free amino acids in the reaction liquid after the enzymatic hydrolysis of samples connective tissue (±SD)

<table>
<thead>
<tr>
<th>Варианти</th>
<th>Концентрация mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bromelain</td>
</tr>
<tr>
<td>Контрол / Control</td>
<td>0,730±0,016</td>
</tr>
<tr>
<td>I</td>
<td>1,952±0,047**</td>
</tr>
<tr>
<td>II</td>
<td>2,926±0,033**</td>
</tr>
<tr>
<td>III</td>
<td>5,081±0,094**</td>
</tr>
</tbody>
</table>

1 т титъчески значими различия спрямо контролната група при: *p<0,05; **p<0,01
Анализът на резултатите от Таблица 1 и 2 показва, че като екзогенни ензими броме- лин и папаин имат протеолитична активност към местото от субстрат и хидролизират отделни компоненти от месните протеини. Що ток тези ръцетелни протеази показват и значителна хидролитична активност към съединителна тъкан, което води до по-ефективно окрехкотяване на биволското месо. О-високо количество свободни аминокиселини се отчита и в двата проведени експерименти при връзката от броя на свободни компоненти от месните протеини. Получените резултати са статистически значими (P < 0,05).

Hydrolysis in such an extent is non-desired, as this leads to a higher level of destruction of the structure of the meat and muscle fibers and therefore deterioration of its texture. In order to achieve tenderization, but without structural degradation of buffalo meat processing with enzyme solutions type marinade should be carefully dosed and monitored.

It is recommended that treatment of buffalo meat to be
извърши с ензим п п ин при
следните п р метри: концентра-
ция н ензим в м ривов ция
р зтвор 50 U/ml, времетр ене
do 24 ч с , при 4° .., последв -
но от термичн обр ботк , при
което се получ в пълно ин кти-
вир не н ензимн т компонент .

made with the enzyme papain in
the following parameters:
concentration of the enzyme in the
marinating solution 50 U/ml,
lasting up to 24 hours at 4°C,
followed by heat treatment, in
which case it gets full inactivation
of the enzyme components.

CONCLUSIONS
Proper timing and
temperature of enzymatic
hydrolysis, are the conditions for
effective tenderization of buffalo
meat. In the experiments been
varied 3 concentrations of the
enzyme solutions (50 U/ml 100
U/ml, and 200 U/ml and duration of
treatment 3 options (24h, 48h, and
72h).

In processing test variants
with solutions having the 50 U/ml
100U/ml caseinolytic activity, the
rate of water retention increases
with increasing time of treatment
and the concentration of the
enzyme.

The analysis of the content of
free amino acids showed that both
enzymes (bromelain and papain)
hydrolyze protein complexes
present in the meat (and
connective tissue). The processing
of buffalo meat with enzyme
papain have better preservation
ability, the fresh color and moisture
of the meat, which makes this
enzyme suitable for inclusion in the
solutions type marinade to improve
fragility of buffalo meat.
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