Вестник Российского университета дружбы народов.

Серия: АГРОНОМИЯ И ЖИВОТНОВОДСТВО

2018 Том 13 № 4
DOI: 10.22363/2312-797X-2018-13-4
agrojournal.rudn.ru

Научный журнал
Издается с 2006 г.

Издание зарегистрировано Федеральной службой по надзору в сфере связи, информационных технологий и массовых коммуникаций (Роскомнадзор)
Свидетельство о регистрации ПИ № ФС 77-61171 от 30.03.2015 г.
Учредитель: Федеральное государственное автономное образовательное учреждение высшего образования «Российский университет дружбы народов»

Вестник Российского университета дружбы народов.

Серия: АГРОНОМИЯ И ЖИВОТНОВОДСТВО

ISSN 2312-7988 (online); 2312-797X (print)
4 выпуска в год.
http://agrojournal.rudn.ru/agronomy/index
Языки: русский, английский.
Материалы журнала размещаются на платформе РИНЦ Российской научной электронной библиотеки, Electronic Journals Library Cyberleninka, DOAJ.

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Подписано в печать 03.12.2018. Выход в свет 15.12.2018. Формат 70×100/16.
Бумага офсетная. Печать офсетная. Гарнитура «Times New Roman».
Усл. печ. л. 18,14. Тираж 500 экз. Заказ № 1660. Цена свободная.
Федеральное государственное автономное образовательное учреждение высшего образования
«Российский университет дружбы народов» (РУДН)
117198, Москва, Россия, ул. Миклухо-Маклая, д. 6

Отпечатано в типографии ИПК РУДН
115419, Москва, Россия, ул. Орджоникидзе, д. 3,
тел. (495) 952-04-41; ipk@rudn.university

RUDN JOURNAL OF AGRONOMY

AND ANIMAL INDUSTRIES

2018 VOLUME 13 No. 4
DOI: 10.22363/2312-797X-2018-13-4
agrojournal.rudn.ru

Founded in 2006

Founder: PEOPLES' FRIENDSHIP UNIVERSITY OF RUSSIA

RUDN JOURNAL OF AGRONOMY AND ANIMAL INDUSTRIES

Published by the RUDN University

(Peoples' Friendship University of Russia),

Moscow, Russian Federation

ISSN 2312-7988 (online); 2312-797X (print)
4 issues per year
http://agrojournal.rudn.ru/agronomy/index
Languages: Russian, English

Aims and Scope

RUDN Journal of Agronomy and Animal Industries is a international peer-reviewed periodical covering the latest research in the field of Agricultural Sciences. The journal is international both in terms of the editorial structure and expert board and authors and subjects of publications.

The journal is intended to publish results of the fundamental and applied scientific researches of the Russian and foreign scientists in the form of scientific articles, review scientific material, bibliographical reviews on specific topics of scientific researches. The journal may publish the materials with the scientific value and suitability for publication valued by the journal editorial board.

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The editors are open to thematic issue initiatives with guest editors.

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Review editor *K.V. Zenkin*
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8/2 Miklukho-Maklaya str., 117198 Moscow, Russia
Ph. +7 (495) 434-70-07;
e-mail: agroj@rudn.university

Printing run 500 copies. Open price

Peoples' Friendship University of Russia (RUDN University), Moscow, Russian Federation
6 Miklukho-Maklaya str., 117198 Moscow, Russia

Printed at RUDN Publishing House:
3 Ordzhonikidze str., 115419 Moscow, Russia,
Ph. +7 (495) 952-04-41;
e-mail: ipk@rudn.university

CONTENTS

СОДЕРЖАНИЕ

RUDN Journal of Agronomy and Animal Industries 2018 Vol. 13 No. 4 257—286
Вестник РУДН. Серия: АГРОНОМИЯ И ЖИВОТНОВОДСТВО http://journals.rudn.ru/agronomy

VETERINARY SANITARY EXPERTISE

DOI: 10.22363/2312-797X-2018-13-4-257-286

RECENT ADVANCES ON STABILITY OF ANTHOCYANINS

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Abstract. Since Neolithic era, natural pigments have been added to foods and colour of food products is still one of the major concerns of food industry. Anthocyanins are the most noticeable group among coloured flavonoids, widely existing in the roots, stems and leaves as well as flowers and fruits of the vascular plants. They have a high potential for use as natural colorants instead of synthetic pigments because of their attractive colour and pharmacological properties. Stable and attractive colours are a highly valued attribute in competitive food industry. Considerable studies have been done on the effects of the most important chemical and physical factors involved in the stability of anthocyanins (temperature, light, pH, SO_2, metal, sugar, ascorbic acid and oxygen), their concentrations, chemical structures, and matrix food compositions. Furthermore, the effects of separation technologies including microwave/ultrasound assisted extraction (MAE, UAE), and Colloidal Gaz Aphron (CGA) fractionation on the stability of anthocyanins are reviewed.

Keywords: anthocyanins, stability, ascorbic acid, MAE, UAE, CGA

1. INTRODUCTION

The study of natural colorants is an extensive and active area of investigation due to the growing interest of substituting synthetic colorants with toxic effects in humans [1]. Anthocyanins are natural pigments present in fruits and vegetables and widely distributed in nature. They are pigments brilliant responsible for shiny orange, pink, red, violet and blue colours in roots, stems, flowers and fruit of many plants (e.g., orchids, grapes) [2]. Incorporating anthocyanins as food colorants is not only valuable for improving overall appearance but also very beneficial to health [3]. However, research on regulation of anthocyanins biosynthesis under in vitro conditions is still limited, restricting the commercial application of their production by plant cell cultures [4]. Anthocyanins are differentiated from one another by the number and position of hydroxyl and/or methyl groups and by the nature, number, position and acylation of the sugars present in their structure. Due to this enormous variety, there are reports of more than 550 anthocyanins and 23 anthocyanidins, of which only 6 are frequently found distributed in nature and in foods: cyanidin (50%), delphinidin, pelargonidin and peonidin (12%) and petunidin and malvidin (7%) [5].

Anthocyanins are highly unstable and very susceptible to degradation. The colour stability of anthocyanins is affected by several factors such as pH, their own chemical structure, concentration, storage temperature, light, oxygen, ascorbic acid, moisture content, and the presence of enzymes, flavonoids, proteins and metal ions [6]. But also, the technologies employed as well as microwave and ultrasound extraction, solvent extraction, and micellar encapsulation influence strongly the stability of anthocyanins.

During the last six decades, general interest and research activities focusing on anthocyanins have considerably increased. This increased interest is not only based on knowledge that these pigments can be used as possible alternatives to artificial food colorants, and it relates to their bioactive properties, but also it relates to their stability, extraction and purification. When searching the Web-of-science database in the title-field for the word anthocyanin, 5,763 articles were found. The last two decades show an increased interest in anthocyanins studies, mainly because of their potential health-promoting properties and their use as natural food colorants, as well as their appearance in cultivars and plant mutants with new colours and shapes.

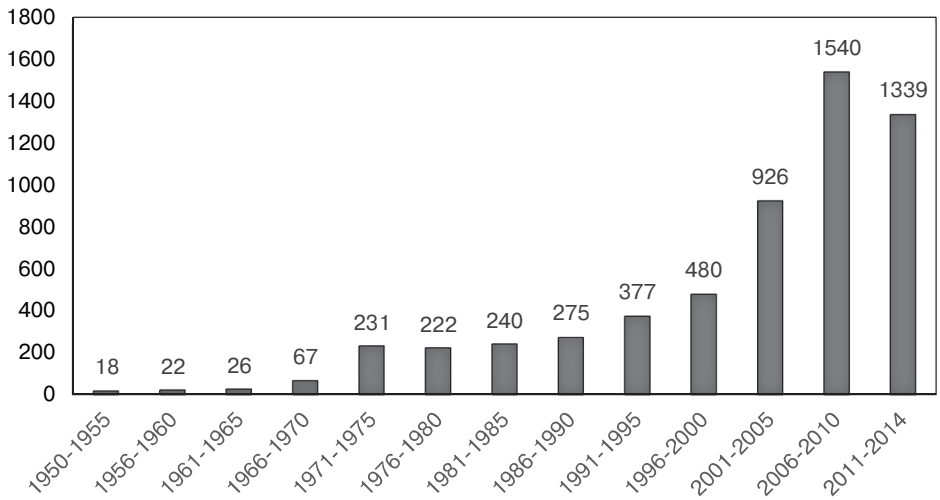


Fig. 1. Records in the literature search within the database Web of Science giving the numbers of articles containing the word 'anthocyanins' in the period 1950—2014

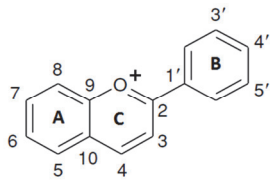
2. ANTHOCYANIN CHEMISTRY

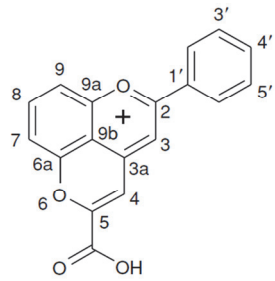
Some of phenolic compounds are directly related to colours in plants, and in particular anthocyanidins are natural pigments present as anthocyanins in plant tissues in glycoside forms with a C6-C3-C6 skeleton [7]. Anthocyanins are part of the plant-derived flavonoid compounds and are responsible for colours ranging from pale pink to red to purple and deep blue [8]. They are the largest group of water-soluble pigments widespread in the plant kingdom, these natural pigments are usually associated with red fruits but also occur in vegetables, roots, legumes and cereals [9]. The variation of anthocyanins are due to: (i) the number and position of hydroxyl and methoxy groups on the basic anthocyanidin skeleton; (ii) the identity, number, and positions at which sugars are attached; and (iii) the extent of sugar acylation and the identity of the acylating agent [10].

Table 1

The structures of naturally occurring anthocyanidin

[a]The numbering of the structure on the left is used for all anthocyanins;
The numbering for the pyranoanthocyanins is given in the structure on the right [13].





	Substitution Pattern						
	3	**5 (6a)[b]**	**6 (7)[b]**	**7(8)[b]**	**3′**	**4′**	**5′**
Common anthocyanidins							
Pelargonidin (Pg)	OH	OH	H	OH	H	OH	H
Cyanidin (Cy)	OH	OH	H	OH	H	OH	H
Delphinidin (Dp)	OH	OH	H	OH	OH	OH	OH
Peonidin (Pn)	OH	OH	H	OH	OMe	OH	H
Petunidin (Pt)	OH	OH	H	OH	OMe	OH	OH
Malvidin (Mv)	OH	OH	H	OH	OMe	OH	OMe
Rare methylated anthocyanidins							
5-MethylCy	OH	OMe	H	OH	OH	OH	H
7-MethylPn (rosinidin)	OH	OH	H	OMe	OMe	OH	H
5-MethylDp (pulchellidin)	OH	OMe	H	OH	OH	OH	OH
5-MethylPt (europinidin)	OH	OMe	H	OH	OMe	OH	OH
5-MethylMv (capensinidin)	OH	OMe	H	OH	OMe	OH	OMe
7-MethylMv (hirsutidin)	OH	OH	H	OMe	OMe	OH	OMe
6-Hydroxylated anthocyanidins							
6-HydroxyPg	OH	OH	OH	OH	H	OH	H
6-HydroxyCy	OH	OH	OH	OH	OH	OH	H
6-HydroxyDp	OH	OH	OH	OH	OH	OH	OH
3-Desoxyanthocyanidins							
Apigeninidin (Ap)	H	OH	H	OH	H	OH	H
Luteolinidin (Lt)	H	OH	H	OH	OH	OH	H
Tricetinidin (Tr)	H	OH	H	OH	OH	OH	OH
7-MelhylAp[c]	H	OH	H	OMe	H	OH	H
5-MethylLt[c]	H	OMe	H	OH	OH	OH	H
5-Methyl-6-hydroxyAp (carajurone)[c]	H	OMe	OH	OH	H	OH	H
5,4′-Dimethyl-6-hydroxyAp (carajurin)	H	OMe	OH	OH	H	OMe	H
5-Melhyl-6-hydroxyLt[c]	H	OMe	OH	OH	OH	OH	H
5,4′-Dimethyl-6-hydroxyLt[c]	OH	OMe	OH	OH	OH	OMe	H
Riccionidin A[c,d]	OH	H	OH	OH	H	OH	H
Pyranoanthocyanidins							
5-CarboxypyranoPg[c]	OH	O–	H	OH	H	OH	H
5-CarboxypyranoCy[c,e]	OH	O–	H	OH	OH	OH	H

[a] Sphagnorubins A—C from peat moss, Sphagnum, have not been included (Fig. 1).

[b] The numbers in parentheses correspond to the pyranoanthocyanidins.

[c] New anthocyanidins (reported between 1992 and 2004).

[d] Ring closure on the basis of ether linkage between the 3- and 6'-positions. Riccionidin A and its dimer, riccionidin B, have an additional OH-group in the 2'-position (Fig. 1).

[e] Rosacyanin B (Fig. 1).

Chemically, these flavonoids naturally occur as glycosides of flavylium or 2-phenylbenzopyrylium salts [9]. The anthocyanidins consist basically of this structure with some hydroxyl groups through which sugar molecules are conjugated. The anthocyanidin refers to the molecule without the glycoside residue [11]. The core of the anthocyanidin is a 15-carbon (C15) structure of two aromatic rings (the A and B rings) joined by a third ring of C_3O_1 (the C-ring; Table 1). The degree of oxidation of the C-ring defines the various flavonoid types. Anthocyanidins have two double bonds in the C-ring — and hence carry a positive charge [12].

The sugar moieties vary but are commonly, glucose, rhamnose, galactose or arabinose. The sugar moiety may be a mono or disaccharide unit, and it may be acylated with a phenolic or aliphatic acid. These compounds differ in the methoxyl and hydroxyl substitution pattern of ring B [9]. The 3-desoxyanthocyanidins, sphagnorubins and rosacyanin B are the only anthocyanidins found in their non-glycosylated form in plants. Nearly all reports on anthocyanins specifying the D or L configuration of the anthocyanin sugar moieties (monosaccharides), lack experimental evidence for this type of assignments [14].

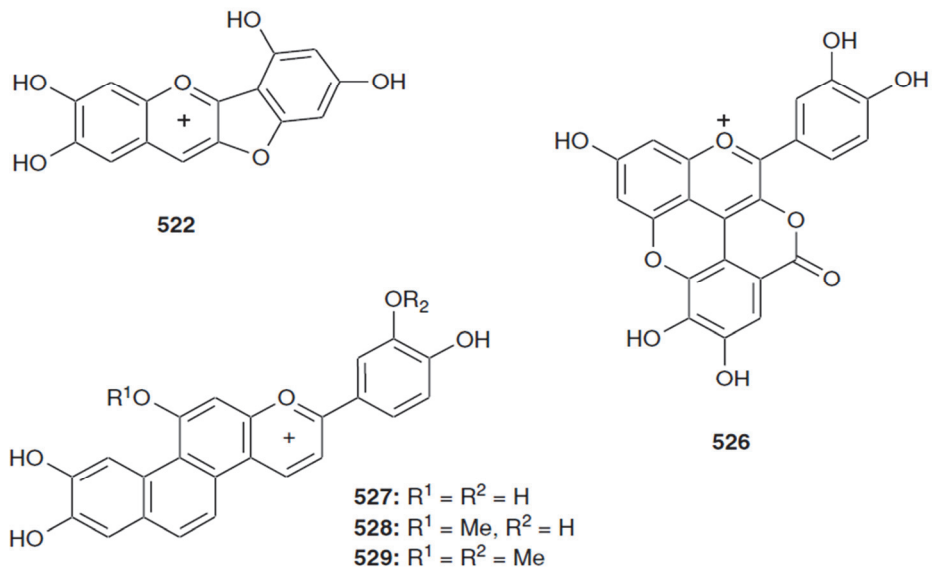


Fig. 2. Some anthocyanidins with unusual structures:
522, riccionidin A; 526, rosacyanin B; 527—529, sphagnorubins A—C [13]

2.1. Source and distribution of anthocyanins in foods

In general, the anthocyanin concentration in most of the fruits and vegetables ranges from 0.1 to 1% dry weight (d.w.) [15], and the most widespread anthocyanidin in foods is cyanidin [16]. Anthocyanins are dissolute in fruit and vegetable vacuolar juice (Table 2), mainly in mature epidermal cells (apple, apricot, artichoke, asparagus, eggplant, fig, red-lettuce, nectarine, red onion, peach, pear, plum, pomegranate, red-skinned potato, radish, etc.), although they can also accumulate in flesh tissues [7].

Table 2

Average amount of anthocyanins in some foodstuffs [17]

Anthocyanin source	Amount (mg.litre^{-1} or mg.kg^{-1})
Blackberry	1 150
Blueberry	825—4 200
Boisenberry	1 609
Cherry	20—4 500
Chokeberry	5 060—10 000
Cranberry	600—2 000
Cowberry	1 000
Currant (black)	1 300—4000
Elderberry	2000—10 000
Red grapes	300—7 500
Blood orange	2 000
Plum	20—250
Sloe	1 600
Strawberry	150—350
Raspberry (black)	1 700—4 277
Eggplant	7 500
Onion	up to 250
Rhubarb	up to 2 000
Red cabbage	250
Red wine	240—350
Port wine	140—1 100

2.2. Acylation, Hydroxylation and Glycosylation

The core anthocyanidin structure is modified by the addition of a wide range of chemical groups, in particular through hydroxylation, acylation and methylation. Hydroxylation and methylation usually, but not exclusively, occur on the anthocyanidin prior to further modifications. Thus, there are a small number of anthocyanidin types that have been identified as the basis of the subsequent large number of known anthocyanins with differing glycosylation and acylation patterns, which include anthocyanidins with additional rings incorporated, for example the pyranoanthocyanidins and riccionidin A (Table 1) [12].

Presently, it is known that anthocyanins have some positions sensitive to react with different nucleophilic and electrophilic compounds (Fig. 3). Anthocyanins can undergo a nucleophilic attack in the positively charged carbons 2 and 4 of the pyranic ring (anthocyanin hydration in carbon 2 gives rise to the colourless hemiacetal form). Additionally, and despite its positive charge, anthocyanins were shown to be able to react with electrophilic compounds through its hydroxyl groups and carbons 6 and 8 of the phloroglucinol ring probably involving the uncharged hemiacetal form. The existence of the 5-OH group is very important for the reactivity of these pigments with other compounds that occur during anthocyanin-rich food processing and aging [1].

Generally, di-, tri-, or poly-acylated anthocyanins are more stable in neutral and slightly acidic conditions than mono-acylatedanthocyanins [18]. It is well known that there is a strong correlation between the colour and the predominant type of anthocyanin

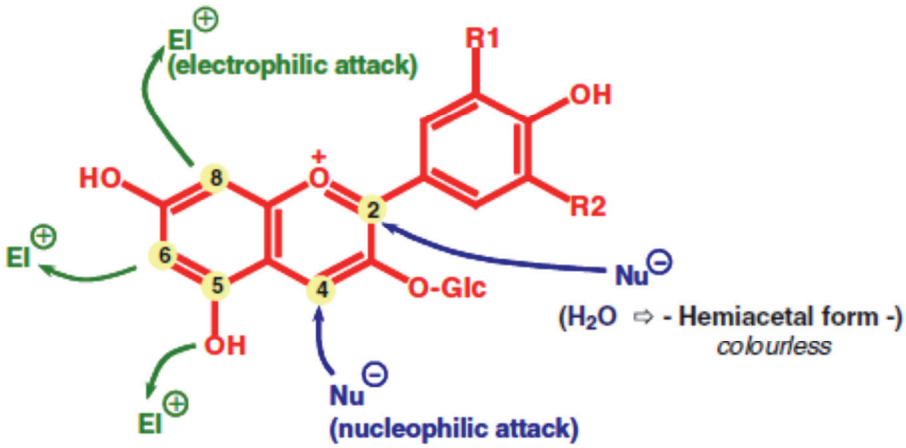


Fig. 3. Schematic representation of the main reactive position of anthocyanin structures [1]

that accumulates. A hydroxyl group or rather the lack thereof at the C-3 position in the C-ring also dramatically influences the colour of the pigment. The common anthocyanidins have 3-hydroxylation. 3-Deoxyanthocyanidins lack this hydroxyl group, and show a marked difference in wavelength absorbance, with the derived pigments giving yellow, orange and bright red flower colours. 3-Deoxyanthocyanins are relatively rare [19]. Furthermore, the glycosylation confers increased stability and water solubility to the anthocyanins compared with the anthocyanidins. Increasing the number of sugar residues seems to further increase stability of the anthocyanin [18], explained by a net of intramolecular H-bondings within the sugar moiety and between the sugar and the chromophore [16].

2.3. Functional properties of anthocyanins and their applications

In view of the considerable consumption of anthocyanin, toxicological as well as mutagenic studies of the pigment have been carried out. Anthocyanin pigments have been shown to have a number of potential health benefits including improved visual acuity, reduction of incidence of coronary heart-disease and stroke, and anticancer and antiviral activities. Therefore, find increasing application in not just the food area but in the medical field as well [20].

In practice, the anthocyanins used as food colorants supply colours going from purplish red to cherry red and can be used at a pH of between 3.5 and 5.5 [21]. The stability of anthocyanin in the lower pH range means that anthocyanins are best suited for use in food of low pH. Successful application of the anthocyanins includes the colouring of canned fruit, fruit syrups, yogurt, and soft drinks [20]. Anthocyanins have the advantage of being relatively insensitive to heat and light. The anthocyanins used in food (E 163) can only be obtained from edible fruits and vegetables such as strawberries, blackberries, cherries, plums, raspberries, blackcurrants, redcurrants, red cabbage, red onions, bilberries, eggplants, grapes, elderberries, etc. [21].

3. FACTORS AFFECTING THE STABILITY OF ANTHOCYANINS

Considerable studies have been done on the effects of the most important chemical and physical factors involved in the degradation of anthocyanins (temperature, light, pH, SO_2, metal, sugar, and oxygen) in model systems and food extracts. Stabilities and colours of anthocyanins are dependent on the nature and number of sugars attached to the flavylium ion and the nature and number of acids linked to the glycosylic moiety. Tint and hue, however, are related to the numbers and positions of hydroxyl and methoxyl substituents in the flavylium ion [16].

3.1. Effect of physicochemical parameters

Application of anthocyanins in food is restricted due to their ability to participate in a number of reactions, resulting in its decolourisation [20]. Anthocyanins would be the ideal substitutes for synthetic red colorants based on their bright colours varying from orange red to blue, water solubility, and non-toxicity. Nevertheless, the use of these pigments in foods has been hampered by poor stability [16].

Factors influencing anthocyanin stability are diverse and widely discussed in the literature. The influence of the specific structures of anthocyanins (glycosylation, acylation with aliphatic or aromatic acids, pH, temperature, light, presence of metal ions, oxygen and sugar content), and effects of sulfur dioxide have been covered and partially clarified [22]. For that, intense research has been done on stabilisation of anthocyanins and elucidation of the high stability of the colour [16].

3.1.1. pH

Anthocyanins are sensitive to changes in pH. When a given anthocyanin is dissolved in water, a series of secondary structures are formed from the flavylium cation according to different acid-base, hydration, and tautomeric reactions [14]. The flavylium cation is the predominant equilibrium form in strongly acidic solutions. In addition, the existence of tautomeric quinonoidal bases derived from the flavylium cation by deprotonation, and hemiacetal and chalcone forms related to the flavylium form by nucleophilic reaction with water, under various pH conditions, have been described [23].

In addition, the anthocyanin colours vary with changes in the pH: at pH 1 and below, the anthocyanin pigment gives an intense red but becomes colourless or purple when the pH is increased to between 4 and 6. Meanwhile, the pigment turns a deep blue when the pH is between 7 and 8. Further increase in pH sees the anthocyanin pigment turning from blue to green and then to yellow. Such variation in colour has been attributed to structural transformation in response to changes in pH [20], as illustrated in Figure 4.

The major species and therefore the colour of the solution are determined by the equilibrium constant values. If the deprotonation equilibrium constant, K_a, is higher than the hydration constant, K_h, the equilibrium is displaced toward the coloured quinonoidal base (A), and if $K_h > K_a$ the equilibrium shifts toward the hemiacetalic or pseudobase form (B) that is in equilibrium with the chalcone species (C), both colorless (Fig. 4). Therefore, the structure of an anthocyanin is strongly dependent on the solution pH, and as a consequence so is its colour stability, which is highly related to the deprotonation and hydration equilibrium reaction constant values (K_a and K_h) [16].

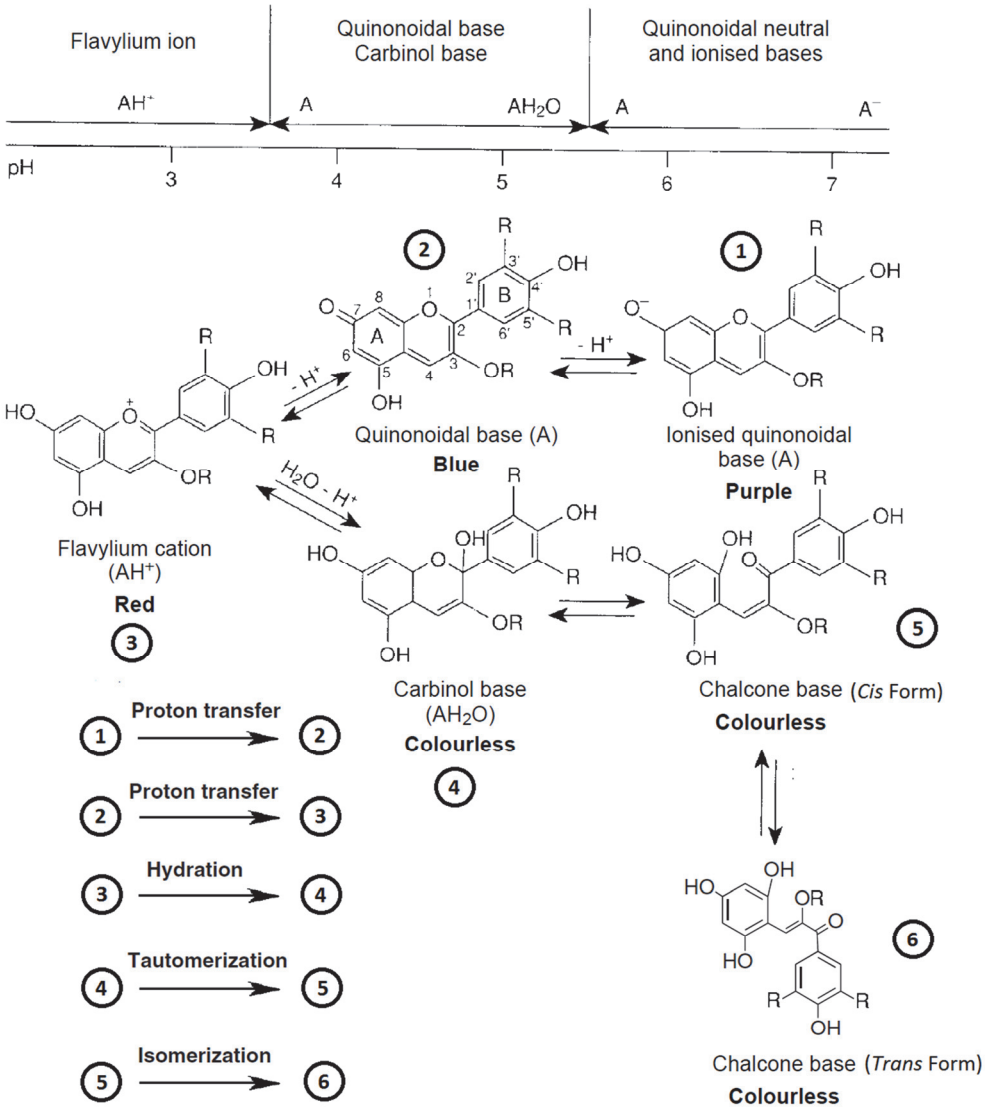


Fig. 4. Chemical reactivity of the anthocyanins depending on the pH

3.1.2. Temperature

The thermal degradation of anthocyanins, both in extracts and model systems, was reported to follow first-order reaction kinetics in all studies. The stability of anthocyanins and all pigments found in foods decreased with increases in temperature [16]. Studies on the effect of temperature on anthocyanin have indicated that the stability is dependent on the structure of anthocyanin, with the sugar moiety playing a significant role [20]. Monomer anthocyanins and the corresponding colorant intensity decreased with the time—temperature combination, whereas the polymer fraction (brown pigments) exhibited the reverse [22].

Storage stabilities of blood orange juices and concentrates with different total soluble solids concentrations and at different temperatures were investigated under

identical conditions. The half-life values of juices and concentrates decreased with increases of °Brix value and temperature [16]. High-temperature short time processing is recommended for maximum anthocyanin retention of foods containing anthocyanins [18].

3.1.3. Oxygen

Anthocyanin content decreased for all atmospheres tested; however, high oxygen caused a higher decrement [24]. Oxygen has a deleterious effect on anthocyanins, and it is known that anthocyanins stored under vacuum, nitrogen or argon atmosphere are more stable than anthocyanins exposed to molecular oxygen [18].

This effect was attributed to the reaction of anthocyanins to inhibit radical activity at high oxygen concentrations, causing depletion of the antioxidant pigments [24]. The oxygen may degrade anthocyanins either directly or indirectly by oxidizing compounds, which in turn, may degrade the anthocyanins; this deteriorating effect is most pronounced when both oxygen levels and ascorbic acid concentrations are high [18].

3.1.4. Light

The anthocyanins are good absorbers of visible light, thus appearing as coloured substances, responsible for the characteristic orange/red/bluish colours of grapes and berries. This reflects the origin of the term anthocyanin, which is derived from Greek, and means flower and blue. The colour is largely determined by the substitution pattern of the B-ring of the aglycon compared with the pattern of glycosylation of the flavan structure, which to a smaller extent, influences colour formation [18]. Light-induced degradation is dependent on the concentration of molecular oxygen present. The most vigorous anthocyanin loss can be experienced when the pigments are exposed to florescent light [25].

Protection toward light may be achieved by selecting packaging material with proper light barriers in the visible and particularly in the ultraviolet range of the spectrum. Glycosylation, acylation and co-pigmentation of anthocyanins have been reported to improve light stability [25].

3.1.5. Enzyme

A number of authors have linked browning of the pericarp to a degradation of anthocyanins by the action of polyphenol oxidase [1, 18, 19, 25]. Enzymes such as polyphenol oxidase (PPO) and peroxydase (POD) are primarily membrane-bound, whereas anthocyanins are vacuolar [19].

An enzymatic system capable of degrading cyanidin-3-glucoside in the absence of phenols is present in the skin of sweet cherries; contrarily, the pulp homogenate degraded the anthocyanin only in the presence of phenols. The decoloration was influenced by the anthocyanin structure at different pH and by the nature of the quinone obtained by enzymatic oxidation. The anhydrobase appeared to be the form of the anthocyanin most susceptible to oxidation. The degradation occurred according to the oxidation kinetics of the phenol substrate and was inhibited by ascorbic acid, indicating that the quinone's degradation of the anthocyanin occurred by a consecutive-type mechanism [1].

Under certain conditions enzyme preparations may degrade anthocyanins and other pigments present in fruit by hydrolysing glycoside substituents. Hydrolysed anthocyanins in their pure aglycon form are extremely unstable and degrade quickly, losing their colouring properties [25]. Anthocyanin losses also occur due to the action of degrading enzymes (i.e., polyphenol oxidase) that are endogenous to the fruits [18].

3.1.6. Sulfur dioxide (SO_2)

Sulfur dioxide has been used extensively in the fruit and vegetable industry, chiefly as an inhibitor of microbial growth and of enzymatic and non-enzymatic browning [25]. The sulfonation of flavonoids improved their abilities to complex with anthocyanins, hindering the hydration reaction and shifting the equilibria toward the coloured quinonoidal base [16].

Anthocyanins react with sulfur dioxide to form colourless components. The reaction is reversible, and heating will release some of the sulfur dioxide from the anthocyanins, thus partially regenerating the colour. Also, acidifying to a low pH regenerates the anthocyanins by liberation of the SO_2 [18]. The presence of sulfur dioxide can also lead to a nucleophilic attack on the oxonium ion's (flavylium cation) C-4 position by the negatively charged bisulfate ion, resulting in colour loss of anthocyanins by a reversible bleaching mechanism, which generally occurs when fruits are treated with 500 to 3000 ppm of SO_2 [25].

3.1.7. Effect of concentration

At high concentrations, the anthocyanins may associate with themselves. Anthocyanins are more stable at higher concentrations. The effect of concentration has been shown to be more important than the variation in stability caused by differences in anthocyanin structure [18].

3.1.8. Impact of water activity (aw)

Several studies have shown that anthocyanin stability increases with decreased water content, i.e., decreasing water activity (aw). Dry anthocyanin powders ($aw \leq 0.3$) are stable for several years when stored in hermetically sealed containers. This property is advantageous when anthocyanins are produced into dry products and powders [18].

3.2. Effect of food matrix

Over the last years, the structure of several groups of anthocyanin-derived pigments found in wines was elucidated by spectroscopic techniques, which have helped to understand some physical-chemical properties of anthocyanins. Some of those pigments were described to result from [1]:

A. Reaction between anthocyanins and small compounds (e.g. pyruvic and phenolic acids, acetaldehyde, *p*-vinylphenol) giving rise to other pyranoanthocyanin pigments:
   — Anthocyanin-pyruvic acid adducts (carboxypyranoanthocyanin);
   — Pyranoanthocyanins (Vitisin B);
   — Pyranoanthocyanin-phenol pigments.

B. Condensation between anthocyanins and flavanols mediated by aldehydes (*e.g.* acetaldehyde):
   — Anthocyanin-alkyl-flavanol pigments;
   — Pyranoanthocyanin-flavanol pigments;
   — Vinylpyranoanthocyanin-flavanol pigments.

C. Direct condensation between anthocyanins and flavanols.

3.2.1. Co-pigmentation

Co-pigmentation is a valuable and natural tool for enhancing and stabilizing the colour of anthocyanin-rich products [25], by hindering the addition of water at position 2 of the chromophore through hydrogen bonding [16]. Co-pigmentation can take place through several interactions: intermolecular complex formations, intramolecular complex formations, self-association mechanisms and metal complexation. Co-pigmentation is observed as a bathochromic shift in the visible range towards higher wavelength (increase in intensity of absorbance to the molecule's λ_{max}) [16]; which is also called the bluing effect, since the colour of an anthocyanin changes from red to a more blue hue or as a hyperchromic shift in which the intensity of the anthocyanin colour increases (shift from λ_{max} to higher wavelengths) [25].

Co-pigmentation involves complexation phenomena, generally at low energy, to the formation of a complex by juxtaposition of the pigment and co-pigment and stabilization by π-π ring interaction, H-bonding, and ionic bonding [16], either between the various forms of anthocyanins or between anthocyanins and other, mostly colourless, phenolic compounds (coumarins, phenolic acids, flavonols, flavanols, etc.). These bulky complexes modify the cation resonance and prevent the substitution of carbons 2 and 4 [25].

Table 3

Co-pigmenting effect of different type of compounds on cyanidin 3,5-diglucoside (2×10^{-3} M, pH 3.31, λ_{max} 508 nm) [15]

Compound / Chemical Class	λ_{max} (nm)	$\Delta\lambda_{max}$ (nm)	% Absorbance increase at λ_{max} (nm)
Aureusidin/aurone	540	32	327
Brucine/alkaloid	512	4	122
Proline/amino acids	508	0	25
Procatechuic acid/benzoic acid	510	2	23
Esculin/coumarin	514	6	66
Sinapic acid/cinnamic acid	519	11	117
Phloridizin/dihydrochalcone	517	9	101
(+) Catechin/flavan 3-ols	514	6	78
Apigenin 7-glucoside/flavone	517	9	68
6-C-Glucosylgenkwanin (swertsin)/C-glycosil flavones	541	33	467
Hesperidin/flavanone	521	13	119
Quercetin 3-galactoside/flavanols	531	23	282

The magnitude of the co-pigmentation is influenced by pH value, pigment and co-pigment concentrations, chemical structure of anthocyanin, temperature, and ionic strength of the medium. As to the effect of the solvent, the important issue is the hydrogen-bonded molecular structure of the liquid water, not the polarity of the medium [16].

The best cofactors are typically flavonoid derivatives that contain many hydroxyl groups, the most favourable at position 3 of the flavones. The strongest cofactors have electron-rich systems that associate with electron-poor compounds such as the flavylium cation [16].

Finally, regardless of the cofactor, anthocyanin stabilization during storage via the phenomenon of co-pigmentation was not revealed, as similar degradation ratios were observed in the presence or absence of these co-factors [22].

3.2.1.1. Intramolecular co-pigmentation

The other type of co-pigmentation is intramolecular, which is due to anthocyanin acylation. This co-pigmentation is more effective than intermolecular, and it is suggested that acyl groups interact with the basic anthocyanin structure avoiding formation of the hydrated species [15].

Intramolecular co-pigmentation (sometimes referred to as intramolecular stacking) is a mechanism for stabilising more complex anthocyanins such as those that are polyacylated with aromatic acids. The tertiary structure is formed by either the sandwiching of the anthocyanin nucleus between two of its acyl groups or by the anthocyanin nucleus being covered by one acyl group [19].

Intramolecular co-pigmentation is thought to be stronger and more effective in stabilizing anthocyanin colour, probably due to the strength of the covalent bonds present. This type of co-pigmentation is mostly associated with anthocyanins derived from flowers and vegetables, which generally contain acylation [25].

3.2.1.2. Intermolecular co-pigmentation

The poor stability of anthocyanins with simple structures can be overcome by intermolecular co-pigmentation reaction [16], (which contains non-acylated anthocyanins), that is, an association with different compounds that is not bound covalently to the anthocyanin molecule [25], especially polyphenolic ones. Stabilized by hydrogen bonding, hydrophobic interactions and electrostatic interactions, resulting in a 1 : 1 complex formation [25]. However, this complex dissociates at high temperatures [16].

Intermolecular co-pigmentation may take place between anthocyanins and catechin, amino acids, polysaccharides and metal ions. Flavonols and flavones are always found in conjunction with anthocyanins in fruits and fruit juices; it appears that they may contribute to the stabilization of the anthocyanins [18].

3.2.1.3. Self-association

Stability of anthocyanins can be attained by self-association, that is, when two or more anthocyanin molecules are associated [16]. Self-association requires high anthocyanin concentrations. It involves the vertical stacking of anthocyanin molecules, and is deduced to play at least some role in flower colour [19].

This effect was verified by increasing the concentration of the cyanidin 3,5-diglucoside solution from 10^{-4} M to 10^{-2} M with a consequent bathochromic shift in maximum wavelength absorption in the visible region [16]. The colour of malvidin 3-glucoside in aqueous solution can also be stabilized by self-association or co-pigmentation with the *cis*-chalcone form, according to the pH of the solution [16].

Self-associations of anthocyanins have been observed to take place during wine aging and it is assumed that they may partially contribute to the colour of aged wines (Fig. 5) [25], the monomeric anthocyanins are irreversibly transformed into polymeric compounds through self-association reactions. The polymeric material is less pH-sensitive and is less susceptible to degradation by ascorbic acid and light [18].

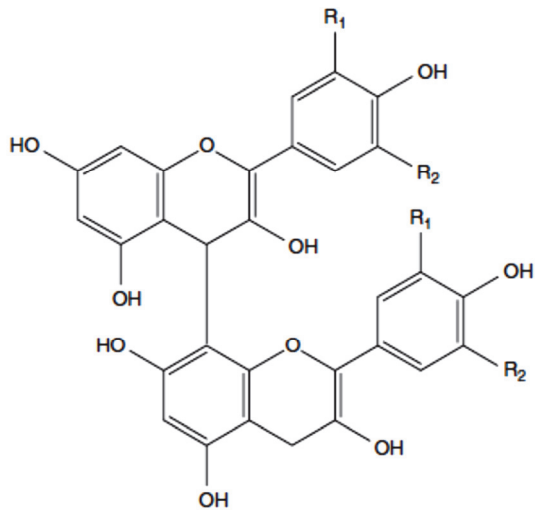


Fig. 5. Self-association of anthocyanins at the C-4 position [25]

3.2.1.3.1. Complexation with metals

The precise colour of anthocyanins depends on the substitution pattern, the pH (red in acid, blue in base), but also on the formation of metal complexes [27]; mainly with Al^{3+}, Fe^{3+}, Cu^{2+}, Mg^{2+}, which form complexes with anthocyanins that have two phenols in the *ortho* position on the B nucleus (delphinidin, petunidin and cyanidin) (Fig. 6). These are responsible for bathochrome effects of varying intensity. Two types of chelates, either directly with both phenol functions (flavylium A^+ form) or with the aromatic ketone in carbon 4´ (AO quinoid base), stabilize the molecules and prevent formation of colourless carbinol base (AOH). The colour becomes intensely blue, even at pH values around 3. These bonds generally break in a strong acid medium [26].

Only cyanidin, delphinidin and petunidin based anthocyanins, which have more than one free hydroxyl group in the B-ring are capable of metal chelation on the aglycon [25].

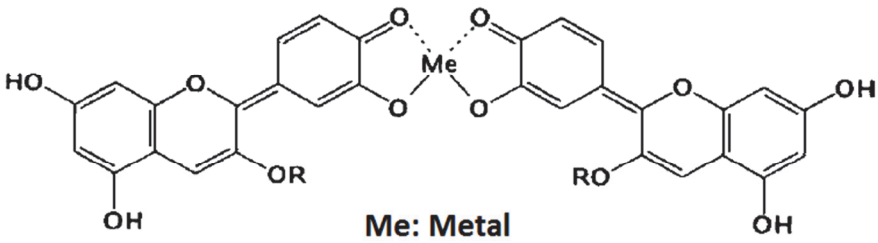


Fig. 6. Mechanism of formation of the complex anthocyanin—metal [28]

3.2.2. Interaction with Vitamin C

The addition of ascorbic acid brought about a notable acceleration in degradation of anthocyanins under model conditions, as in the analyzed matrix [22].

The deteriorating effect is most pronounced when both oxygen levels and ascorbic acid concentrations are high. The reactions are known to be accelerated by copper ions [18] (Fig. 7).

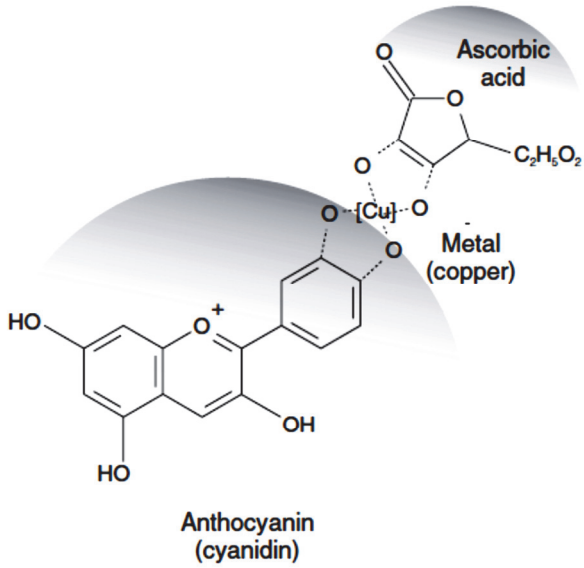


Fig. 7. Suggested mechanism of formation of the complex anthocyanin—metal—ascorbic acid [15]

In general, the effects of ascorbic acid on anthocyanins are complex and not easily predictable. In the absence of oxygen, ascorbic acid may condense with anthocyanins to form unstable products that degrade into colourless compounds. It is assumed that condensation of anthocyanins with flavonols prevents formation of complexes between anthocyanins and ascorbic acid [18], probably by competition with the anthocyanins in the preference for condensation reactions [16], so that the deteriorating effect of ascorbic acid is diminished [18].

Ascorbic acid retention and loss of anthocyanin contents in blood orange juices during refrigerated storage were investigated using CIELAB parameters. Concurrent losses of anthocyanins and ascorbic acid were observed, but the rate of reactivity depended on the anthocyanin structure. When no anthocyanin was present, ascorbic acid degraded 100% after 9 days, while in the presence of malvidin 3-glucoside and malvidin 3,5-diglucoside after 9 days, 15 and 23% of ascorbic acid, respectively, remained. This effect may have been provided by the antioxidant properties attributed to anthocyanins [16].

3.2.3. Redoxpotential

From a chemical viewpoint, anthocyanins and in general flavonoids can be considered as fairly reactive compounds. They typically display π-electron-rich aromatic nuclei and labile phenolic —OH groups that confer on them a reducing (electron- and hydrogen-donating) character. This property largely underlies the well-known *in vitro* antioxidant properties of flavonoids, or regenerate endogenous amphiphilic antioxidants (bound to membranes or lipoproteins) such as α-tocopherol. In addition, through their catechol (1,2-dihydroxybenzene) or hydroxyketo groups, flavonoids bind iron and copper ions, whose redox cycling is typically involved in ROS production. A third property of flavonoids especially critical to their biological effects from plants to humans is their affinity for a wide variety of proteins, including enzymes and receptors [29].

Among the common chemical groups, the phenolic nucleus is probably the one that is most prone to developing molecular interactions with proteins. Indeed, the phenolic –OH group is both a hydrogen bond donor and acceptor and the aromatic ring can develop strong dispersion (van der Waals) interactions with non-polar amino-acid residues or polarizable cofactors [29]. Additionally, the keto group frequently found on the C-ring — as well as the glycosyl residues — can also be involved in hydrogen bonding [29].

3.2.4. Interaction with proteins

It has long been known that flavonoids can interact with proteins in vitro. On the basis of recent studies it appears that the concept of tertiary structures can be expanded to include complexes formed with or interactions between flavonoid pigments and proteins (Fig. 8). In some species, stable or unstable complexes containing anthocyanins and protein are formed in the vacuole. These are termed anthocyanic vacuolar inclusions (AVIs). Their formation can alter flower colour, but their biological function is not yet known [19].

The redox and binding properties of flavonoids are combined in the process of flavonoid—protein covalent coupling (Fig. 9). In a first step, flavonoids are enzymatically or chemically oxidized into highly electrophilic *ortho*-quinones and/or *para*-quinonemethides. In a second step, nucleophilic thiol or amino groups of the protein side-chains add to these electrophiles and form the covalent bonds [29].

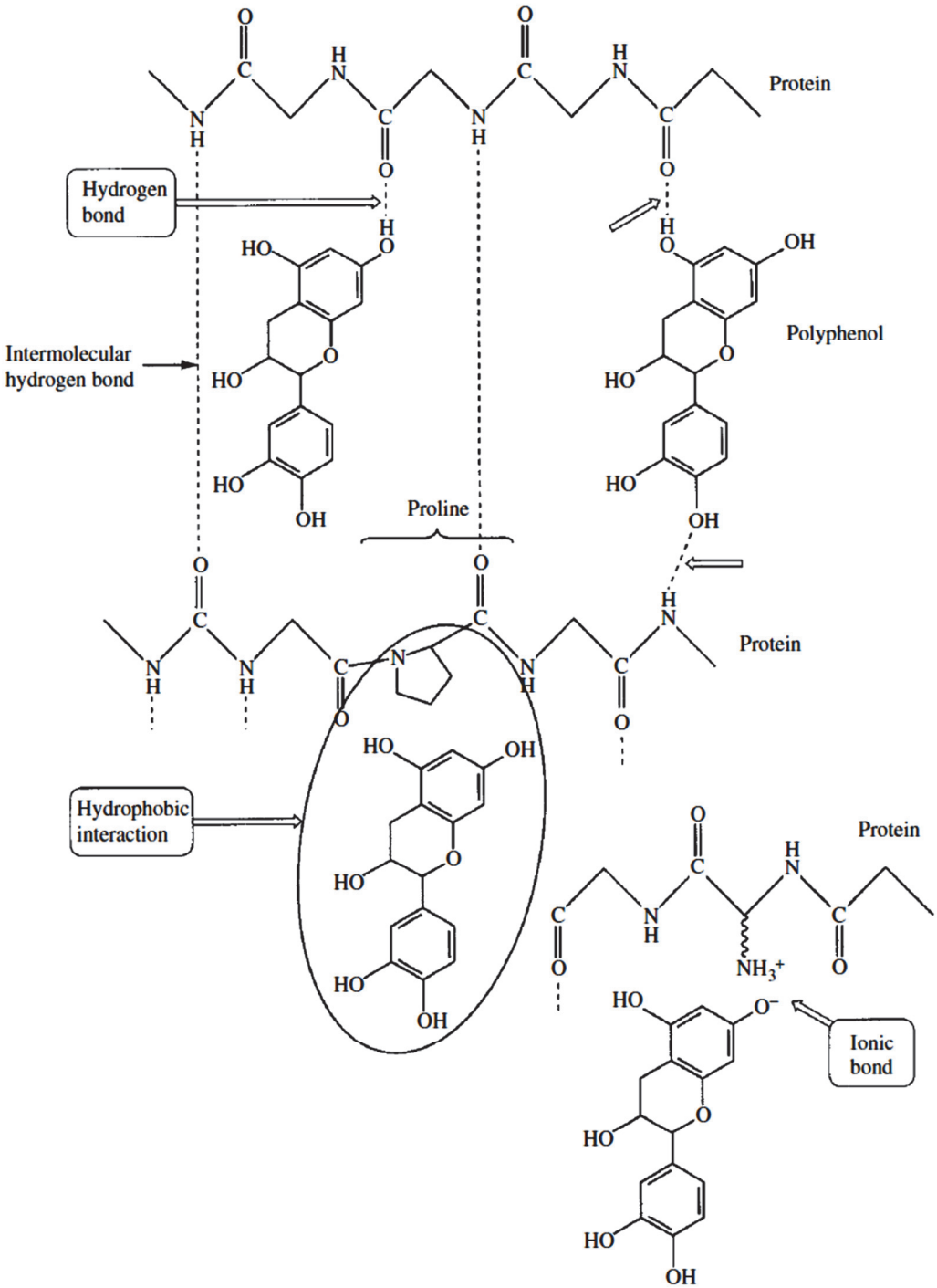


Fig. 8. Interaction between proteins and polyphenols [25]

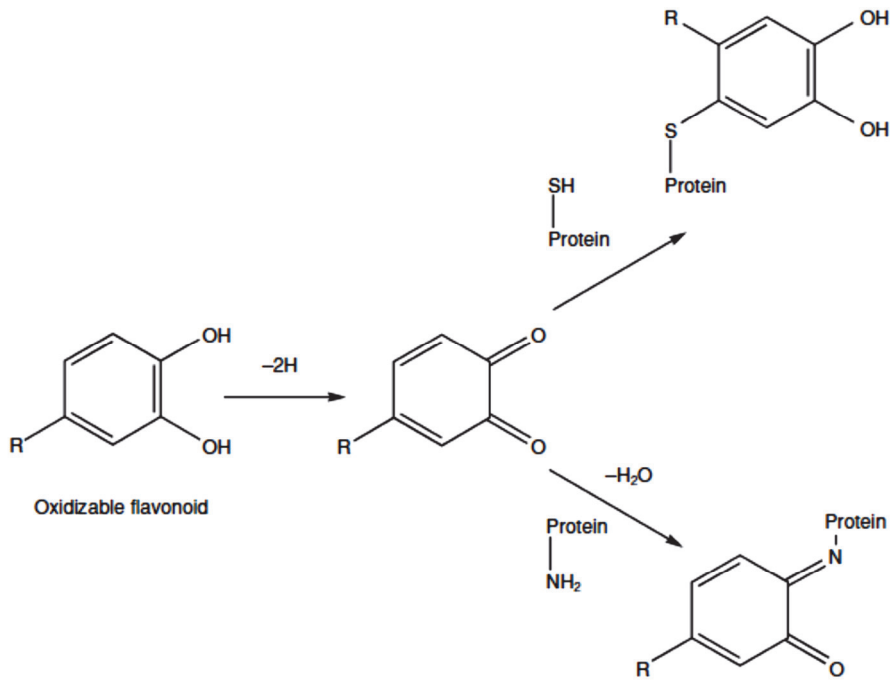


Fig. 9. Oxidative flavonoid—protein covalent coupling [29]

In summary, the binding of flavonoids to redox-inactive enzymes such as HSA does not necessarily depress their antioxidant activity. In fact, the influence of proteins on the redox properties of flavonoids could well be specific to each flavonoid—protein couple under consideration. Ultimately, the fate of quercetin undergoing oxidation in the presence of HSA could be covalent coupling to HSA as evidenced in the case of oxidation by H_2O_2 catalysed by horseradish peroxidase [29].

3.2.5. Effect of aldehydes

In the intermolecular reactions between anthocyanins and flavonoids mediated by acetaldehyde, new compounds linked by an ethyl bridge are formed. Three new compounds were detected by the reaction of malvidin 3-glucoside and proanthocyanidin B mediated by acetaldehyde under wine-like conditions. Two were assigned to enantiomers containing malvidin 3-glucoside covalently bonded to proanthocyanidin linked through C-8 by an H—C—CH_3 ethyl bridge [16].

A pigment derived from the acetaldehyde-mediated condensation between (+)-catechin and malvidin 3-glucoside was prepared and its properties were compared to those of malvidin 3-glucoside in aqueous solution. With a pH increase from 2.2 to 5.5, the colour of the formed pigment solution shifted toward violet at pH 5.5, whereas similar solutions of malvidin 3-glucoside were almost colourless at pH 4.0. This behaviour indicated that the anthocyanin moiety of the new pigment was more protected against water attack. The new pigment was more stable with regard to bleaching by SO_2 than malvidin glycoside, but was more sensitive to degradation in aqueous solution. The cleavage of the bridge linking the anthocyanin to the catechin is the first step in its degradation; malvidin-glycoside is the major product formed [16].

3.2.6. Effect of sugars

Sugar solutions also help to stabilize anthocyanins during frozen storage by the inhibition of enzymatic reactions [25].

The effect of added sugar on anthocyanin stability depends on the anthocyanin structure, concentration, and type of sugar. Reducing and non-reducing sugars had destructive effects on the stabilities of anthocyanins from black currants [16].

The influence of sugars on anthocyanin stability remains a controversial subject. Certain authors do not mention any effect with a model solution of commercial anthocyanidin-based pigments with or without sugar (10 °Brix), whereas anthocyanidin degradation in the presence of sugar is frequently mentioned in the literature [22]. The glycosylation confers increased stability and water solubility to the anthocyanins compared with the anthocyanidins. Increasing the number of sugar residues seems to further increase stability of the anthocyanin [18].

The anthocyanin thermostability was reduced when sucrose concentration increased from 0 to 20%, while further concentration increases to 40% had positive effects on the stabilities of pigments [16]. Fructose, arabinose, lactose and sorbose are more detrimental than glucose, sucrose and maltose, but the degradation of anthocyanins by sugar is enhanced by oxygen [18]. On the other hand, the thermostabilities of pigments decreased linearly with increases of fructose concentration, probably due to the formation of furaldehydes [16]. Although most anthocyanic extracts showed lower stabilities in sugar-added systems, no statistical analysis was carried out to verify the significance of this difference [16].

3.2.7. Effect of other factors

The stability of cyanidin 3-glucosyl-arabinoside was investigated in different solvents, water, and dimethyl sulfoxide (DMSO, an aprotic solvent) under the same conditions. The k_{obs} values unexpectedly showed that the anthocyanin was more stable in water solution. DMSO was chosen as a solvent so that it would make the addition of DMSO to C-2 difficult and thus the anthocyanin would be more stable in DMSO than in a water solution. However, since the experiment took place in acidic solution ($pK_a > pK_h$), the preferential addition at C-2 or C-4 of the flavylium ion probably took place by a protonated molecule of DMSO [16].

The degradation kinetics of malvidin 3-glucoside in ethanolic solutions under conditions simulating wine accelerated with the increase of ethanol concentration, probably because the extent of anthocyanin self-association decreased with elevated ethanol concentration [16].

Strawberries and raspberries were submitted to high hydrostatic pressures from 200 to 800 mPa at 18 and 21 °C, followed by storage at 4, 20, and 30 °C. The greatest stability of the anthocyanins was observed in raspberries submitted to 200 mPa pressure followed by 800 mPa, both stored at 4 °C [16].

Attempts to stabilize anthocyanins by complex inclusion with α- and β-cyclodextrins failed; on the contrary, a discoloration of anthocyanin solutions was observed. Thermodynamic and kinetic investigations demonstrated that inclusion and co-pigmentation had opposite effects. In the anthocyanins, the *cis*-chalcone colorless structure is the best species adapted to inclusion into the β-dextrin cavity, shifting the equilibrium toward colourless forms [16].

4. ANTHOCYANINS IN SEPARATION TECHNOLOGIES

The increasing number of investigation published in this area highlights unyielding interests in the application of microwave, ultrasound and colloid gas aphron technologies in the colour-making process. Therefore, the main objective of this part is to summarizing review the current state-of-the-art of the application of microwave, ultrasound and colloid gas aphron to accelerate natural colorant production.

4.1. Microwave

Microwave energy is a non-ionizing electromagnetic radiation with frequencies in the range of 300 MHz to 300 GHz. They lie in the electromagnetic spectrum between infrared waves and radio waves with wavelengths between 0.01 and 1 m. Commercial microwave ovens approved for domestic applications operate at a frequency of 2.45 GHz to avoid interference with telecommunication and cellular phone frequencies [30, 31]. Typical bands approved for industrial, science, medicine and instrumentation (ISMI) applications are 915 MHz and 2.45 GHz, which correspond to wavelengths of 33.5 and 12.2 cm, respectively. These frequencies were chosen by international agreement to minimize the interference with communication services [32]. In the past 20 years [33], the microwave oven has become an essential appliance in most kitchens. Faster cooking times and energy savings over conventional cooking methods are the primary benefits. Although the use of microwaves for cooking food is widespread, the application of this technology to the industrial, science, medicine and instrumentation (ISMI) is a relatively new development. The use of microwave energy for processing materials has the potential to offer similar advantages in reduced processing times and energy savings. For this reason, microwave energy is today seeing an exponential increase in acceptance as a technique used to enhance extraction of bioactive compounds. Growing numbers of investigators are adopting microwave extraction as a means to accelerate desorption of high added value compounds from cells and increase their productivity. Microwave heating has gained popularity in green extraction due to its ability to achieve high heating rates, significant reduction in extraction time, more uniform heating, safe handling, ease of operation and low maintenance. Scientists now have access to microwave systems designed for laboratory applications. Advances in microwave technology have allowed the design and development of systems specifically for extraction applications, well beyond what the early investigators envisaged [34].

Energy associated with microwaves is lower than the energy of Brownian motion which is not strong enough to even break chemical bonds as such microwaves cannot induce chemical reactions. The influence of microwave energy on chemical or biochemical reactions is both thermal and non-thermal. Within the frequency domain of microwaves and hyper-frequencies (300 MHz — 300 GHz), the corresponding energies are 1.24×10^{-6} — $1.24\ 10^{-3}$ eV, respectively. These energies are much lower than ionization energies of biological compounds (13,6 eV), of covalent bond energies such as OH (5 eV), hydrogen bonds (2 eV), van der Waals intermolecular interactions (lower than 2 eV) and even lower than the energy associated with Brownian motion at 37 °C ($2{,}7.10^{-3}$ eV) [35]. Microwaves, as an energy source, produce heat by their interaction with the materials at molecular level without altering the molecular structure.

4.1.1. Microwave role in anthocyanins extraction

The application of microwave assisted extraction (MAE) technology in bioactive extraction has attracted large interest in the last decade not only as a new non-thermal preservation technology but it offers a number of other useful applications in the food industry improvement of intracellular colorant extraction.

The first step for analysis and exploitation of colorant constituents is their extraction from the cellular matrix. Efficient extraction methods should maximize anthocyanins recovery with minimal amount of adjuncts and minimal degradation or alteration of its natural state. Currently, anthocyanins/colorant production processes are based on either conventional or recent methods. Commonly used methods are: conventional solvent extraction [36, 37], supercritical heating [38—41], ultrasound [42, 43], microwave [44, 45]. Among these methods, the state of the art in these fields has shown that microwave radiation could accelerate the extracting process improving anthocyanins compounds extraction. Microwave is attractive because it allows for rapid heating of aqueous samples and presents advantages over conventional extraction techniques, such as improved efficiency, reduced extraction time, lower solvent consumption, higher selectivity toward target molecules and higher level of automation [46].

As shown in Table 4, most of irradiation processes led to a preservation of stability of the anthocyanins.

Table 4

List of microwave assisted extraction studies from the literature on various food anthocyanin components

Product / anthocyanins	Microwave Process	Processing conditions	Performance	References
Prunuscerasus var. Marasca	Microwave reactor (Milestone Start S Microwave Labstation for Synthesis, Italy)	40 mL of 0.1% HCl in 80% methanol, temperature (60 °C), time (6—9 min) and 400 W	Lower temperature and shorter time of exposure is more convenient for anthocyanins extraction	Elez Garofulić, Dragović-Uzelac, Režek Jambrak, and Jukić (2013)
blueberry	Advanced Microwave Digestion System (Ethos1, Milestone Inc., Italy)	34 mL of ethanol concentration (v/v) of 55.5%, time (7 min) and temperature (47 °C)	Increase to achieve the highest anthocyanin extraction rate of 73.73%	Zheng et al. (2013)
grape peel	Microwave reactor workstation (Model MAS-II, SINEO Microwave Chemistry Technology Co Ltd., Shanghai, China)	20 mL water acidified 0.79 mol/L citric acid, energy density: 32 W/mL, and time: 50 s.	Anthocyanins yield was more sensitive to high energy density and microwave power	Y. Li et al. (2012)
purple corn (Zea mays L.)	Microwave extractor (Model NJL07-3, Jiequan microwave equipment Co., Ltd., Nanjing, China)	20 mL of 1.5 M HCl — 95% ethanol, time of 19 min, and a microwave power of 555 W	The highest total anthocyainins content (185.1 mg/100 g, 98.85%) obtained at short time. MAE was highly efficient and rapid in extracting anthocyanins in comparison with the conventional solvent extraction	Yang & Zhai (2010)
grape skins	Microwaves Ethos 1600 microwave extractor (Milestone, Shelton, CT, USA)	25 mL of 40% methanol, time of 5 min, temperature of 100 °C and 500 W	12% decreasing of anthocyanins content (100 °C than 75 °C with 25 mL volume). Quantify in the samples the compounds malvidin 3-coumaroylglucoside (cis), malvidin 3-caffeoylglucoside, petunidin 3-p-coumaroylglucoside with MAE which do not reach the quantification limit using the classical method	Liazid et al. (2011)

Table 4 (continued)

Product / anthocyanins	Microwave Process	Processing conditions	Performance	References
Sweet cherries	A Milestone Micro SYNTH microwave oven (Milestone, Sorisole, Italy)	Solvent-free	Reduction in extraction time (45 s).	Grigoras, Destandau, Zubrzycki, & Elfakir (2012)
Rosmarinus officinalis	Modified domestic microwave oven (LG Electronics)	70% ethanol 1% acetic acid, 2 extraction cycles lasting 5 min and 320 W	2 times increase of the anthocyanis content extracted by microwave compared to ultrasound method	Švarc-Gajić et al. (2013)
grape juice	Microwave hydrodiffusion and gravity, Milestone EOS-G microwave laboratory oven (Sorisole Bergamo, Italy)	20 min at atmospheric pressure and at a power density of 1 W/g without solvent	Stability of anthocyanins content during extraction	Al Bittar, Périno-Issartier, Dangles, & Chemat (2013)

4.1.2. Ultrasound based anthocyanins colorant

The number of papers published in the last decades related with this topic has suffered an exponential increase. It was reported that an ultrasonic extraction method could increase the recovery and purity of bioactive compounds extracted from different tissues.

Intensification of extraction efficiency using ultrasounds has been attributed to the propagation of ultrasound pressure waves through the solvent and resulting cavitation phenomena. The controlling mechanism of UAE is generally attributed to mechanical, cavitation, and thermal effects which can result in disruption of cell walls, particle size reduction, and enhanced mass transfer across cell membranes. The implosion of cavitation bubbles generates micro-turbulence, high-velocity inter-particle collisions and perturbation on particles of the matrix which accelerates the eddy diffusion and internal diffusion [53]. Due to limited “space” for the bubbles to expand, most of the bubbles collapse asymmetrically in the vessels, resulting in significant liquid circulation currents coupled with intense turbulence. Also cavitation on the surface of the source material causes impingement by micro-jets resulting in surface peeling, erosion and particle breakdown [54, 55]. The dominant mechanical effects of ultrasounds are well evident from scanning electron microscopy of the extracted samples as reported for specific cases of basil for extraction of essential oil. It has been reported that micro fractures appeared in the basil tissue after application of ultrasound and the surface morphology of basil tissue changed giving more porous nature [56]. Similar results were obtained for oil extraction from autoclaved almond powder and extraction of gingerols from ginger [53].

Ultrasound, as relatively low-cost, non-hazardous and environmental friendly technology, is commonly utilized in food industry [57, 58]. Studies have been conducted on the use of ultrasound as a simple and rapid extraction method for the anthocyanins colour pigment. The advantages of using over other extraction techniques are higher reproducibility and possibility of simultaneous extraction of several samples, which makes the ultrasonic-assisted extraction an interesting alternative for the analysis of the anthocyanins colour compounds.

Table 5

List of ultrasound assisted extraction studies from the literature on various food anthocyanin components

Product / anthocyanins	Ultrasoun Process	Processing conditions	Performance	References
blackberry cultivar "Caˇ canskaˇ Bestrna"	Rectangular ultrasonic cleaner bath (Bandelin Sonorex RK 52, BANDELON electronic, 35 kHz, 60 W, volume of 1.8 L, internal dimensions: 150 mm × 140 mm × 100 mm) with a useful	Absolute ethanol with 0.01% (v/v) of HCl (solvent-to-solid ratio of 2.5 ml/g of the purée at for 15 min or 30 min at 25 °C and 40 °C	Increase of 5.3—6.3% of anthocyanins content (15—30 min Increase of anthocyanins content with increasing of temperature from room at 40 °C. Increase of the antioxidant activity with sonication time and temperature. Increase of cyanidin content with increasing a sonication and temperature	Ivanovic et al. (2014)
wine lees	An ultrasonic bath system (MC300, Elma Hans Schmidbauer GmbH & Co. KG, Singen, Germany)	60 mL of Ethanol 51.5%, 36.3 min at 59.9 °C	High stability of total anthocyanins, monomeric anthocyanins and polymeric anthocyanins at 4 °C. Less stability of total anthocyanins, monomeric anthocyanins and polymeric anthocyanins at 20 °C	Tao et al. (2014)
jussara pulp (Euterpe edulis)	Ultrasonic cleaning bath (USC-2800-A model, Thorton, São Paulo, Brazil)	Different ethanol concentrations (0, 30, 50, 70 and 90) and adjust to a pH of 3.0 with 0.35%. Citric acid (w/v), temperature (25, 35, 45 and 55) and ratio liq/sol of 5, 10, 15, 20, 25 and 30	Increase of anthocyanins from 7.12 to 15.72 mg anthocyanin/g dry pulp with time increasing (5 to 20min). Increase of the antioxidant activities (FRAP and DPPH). Use of a 30—70% (v/v) ethanol solution promoted the biggest anthocyanin yields. The anthocyanins are not sensitive to heat tested 15 mL/g pulp is the best for anthocyanins extraction	Vieira, Cavalcanti, Meireles, & Hubinger (2013)
Aronia melanocarpa (black chokeberry) wastes	Ultrasounds generator (SinapTec, France)	Ethanol—water (25—50%), ratio liquid-to-solid (40 : 1), Temperature at 20, 45 and 70 °C, frequency of 30.8 kHz and power of 50 or 100 W and time of 5, 10, 15, 25, 45, 60, 120, 180 and 240 min	Increase of extraction yields using 50% ethanol were about 3-fold higher than aqueous extractions. Anthocyanins are not stable at high temperatures. Decrease of the anthocyanins at 20 °C. Yields of 90% of the extractable anthocyanins under 70 °C, 34% ethanol, 17 min and 100 W	Galván D'Alessandro, Dimitrov, Vauchel, & Nikov
red raspberry puree	A 400 W capacity batch sonication system (Branson Sonifier, S-450A, Danbury, CT) with a 7 cm vibrating titanium tip with the probe immersed half way in the liquid. Custom-made ultrasound generator (APC-841, American Piezo Ceramics, Mackeyville, PA)	50% output power, 20 kHz, time of 0, 10, 20, 30 min. 490 and 986 kHz, time of 0, 10, 20, 30 min	12.6% at 20 kHz increase. Maximum increase of anthocyanins yield (40 °C, 20 min). Time saving of ultrasound methods compared to conventional techniques. 6.7% at 490 kHz after 10 min sonication increase	Golmohamadi et al. (2013)
Delonixregia tree flowers	PEX 3 Sonifier (R.E.U.S., Contes, France)	Water—sulphuric (0.01 N), acid, water—citric acid (0.01 N) or methanol—water acidified with HCl, ratio liquid-to-solid (100 : 1), for 1 h and power of 150 W	More stability of total anthocyanins in citric acidified-water than sulphuric acid-water. More stability of anthocyanins in water acidified than less polar solvent (methanol)	Adjé et al. (2010)

Table 5 (continued)

Product / anthocyanins	Ultrasoun Process	Processing conditions	Performance	References
Red grape juice	A 1500 W ultrasonic processor (VC 1500, Sonics and Materials Inc., Newtown, USA)	80 mL water at 32 to 45 °C, Amplitude level (24.4—61 μm), time (2—10 min) and pulse durations of 5 s on and 5 s off	Stability of cyanidin-3-*O*-glucosides (97.5%), malvanidin-3-*O*-glucosides (48.2%) and delphinidin-3-*O*-glucosides (80.9%) during sonication. Significant effects of sonication colour values and colour index (CI)	Tiwari, Patras, Brunton, Cullen, & O'Donnell (2010)
Jabuticaba *(Myrciaria cauliflora)* skins	ultrasonicator bath 40 kHz (81 W) (model T 1440, Thornton, São Paulo, Brazil)	10 mL ethanol 99.5% at room temperature for 2 hours	Ultrasound method resulted higher extraction efficiency than agitated bed technique and soxhlet	Veggi, Santos, & Meireles (2011)
Canna indica flower	The ultrasound chamber (35 kHz, JULABO, USR3)	50 ml 0.1% HCl (v/v) in methanol for 2 h at room temperature	Efficiency of ultrasonication method to extracting the anthocyanins. Stability of total anthocyanins content. Increase of the antioxidant activity	Srivastava & Vankar (2010)
fruit pulp of *Euterpe edulis*	Unique 1400A ultrasonic bath (Unique, São Paulo, Brazil)	Methanol/1.5 M HCl, solid to liquid ratio (1 : 30) and (1 : 50) and extraction time of 24 h	Stability of total anthocyanins extracted during ultrasonication	Borges, Vieira, Copetti, Gonzaga, & Fett (2011)
Nephelium lappaceum L. fruit peel	Ultrasonic bath (Power sonic, Korea) equipped with digital sonication power, time and temperature controller with a useful volume of 10 L (internal dimensions: 30 · 24 · 15 cm)	Water 18.6 : 1 mL/g, 50 °C, ultrasound power of 20 W, time of 20 min	Stability of anthocyanins content (30 to 50 °C). Increase of anthocyanins with solvent-to-solid ratio from 1 : 10 to 1 : 20 (g/ml). Adequacy of ultrasonication method for anthocyanins extraction	Prakash Maran, Manikandan, Vigna Nivetha, & Dinesh
Grapes	Ultrasonic UP200S sonifier (200W, 24 kHz) (Hielscher Ultrasonics, Teltow, Germany)	Water—ethanol acidified (50 : 50) (HCl, pH: 2.0), (0—75 °C), output amplitude (20, 50 and 100%), duty cycle (0.2 s, 0.6 s and 1 s), the quantity of sample (0.5—2 g) and the extraction time (3—15 min)	High recovery of anthocyanins obtained with ultrasound at 6 min. Anthocyanins more sensitive to ultrasonication time. Increase and high stability of anthocyanins 10 °C. Decrease of anthocyanins at 30—40 °C	Carrera, Ruiz-Rodríguez, Palma, & Barroso (2012)
Garcinia indica	UP 200S from Dr. Hielscher GmbH (Teltow, Germany)	Water-to-powder ratio 10 (v/g), 35 min, cycle ranging from 0.44 to 0.48 s^{-1}, amplitude from 10 to 14%	Stability of total anthocyanins. Increase of antioxidant activity with ultrasound irradiation	Nayak & Rastogi (2013)

4.1.3. Micellar effect (Colloidal Gaz Aphron)

In these last years, considerable interest in replacing synthetic colorants with natural pigments as like anthocyanins has developed, nevertheless the main problem related to their utilisation is the very low stability in aqueous media at pH values above 2.0 [69, 70].

Nowadays, only a few researches have been made to verify the ability of micellar systems to stabilise anthocyanins compounds. Micellar solutions are widely used as host systems for synthetic and natural organic compounds and basically three differently charged surfactants can be used to produce micelles, anionic, cationic and non-ionic.

The outer surface of the micro bubble may be positively charged, negatively charged or neutral, to which oppositely charged or non-charged molecules will adsorb, resulting in their effective separation from the bulk liquid, and consequently the selectivity of adsorption can be controlled [71]. The use of surfactant-based methods for the treatment of aqueous streams and solid matrices to remove organic and inorganic contaminants, and for the recovery of natural pigment products, are promising new areas of great environmental and technological importance. In particular, colloidal gas aphrons (CGA) are surfactant-stabilized microbubbles (10—100 μm) generated by intense stirring of a surfactant solution at high speeds (> 8.000 rpm). They were firstly postulated by Sebba (1987) to consist of a microbubble encapsulated in a thin aqueous film ("soapy shell"). CGA have been used for many separation processes of bio-products such as protein, enzyme, carotenoids and dyes recovery [72]. The most striking feature of CGA is their stability, which lets them generated externally to their point of use, and then to be transported by pumping. Generally, the stability of aqueous foam is determined by two different phenomena: the rate at which liquid drains from foam, and the rate at which the body of the foam breaks down. In the case of CGA, there is no perceptible breakdown of the microbubble until the great majority of the liquid has drained. Spigno et al. (2010), first put forward a recovery of gallic acid with colloidal gas aphrons generated from a cationic surfactant to explain the possibility of CGA to separate the gallic acid from aqueous solution [71].

Dahmounea et al. (2013) demonstrated that an equilibrium colour stabilization of extract rich in anthocyanins occurred in micellar solution of non-ionic surfactant Tween 20 [46]. It was hypothesized that the presence a surfactant could increase the stability of natural pigment (anthocyanins) (Fig. 10). Further research is also required to get an insight into the type and stability of the molecular association between the phenolic compounds and the surfactants.

Fig. 10. Picture of agglomerates formed in the aphron phase recovered from the separation trials carried out with undiluted extract [46]

5. SUMMARY

Anthocyanins are the most noticeable group among coloured flavonoids, widely existing in the roots, stems and leaves as well as flowers and fruits of the vascular plants. They have a high potential for use as natural colorants instead of synthetic pigments because of their attractive colour and pharmacological properties. Considerable studies have been done on the effects of the most important chemical and physical factors involved in the stability of anthocyanins (temperature, light, pH, SO_2, metal, sugar, ascorbic acid and oxygen), their concentrations, chemical structures, and matrix food compositions. Furthermore, the effects of separation technologies including microwave/ ultrasound assisted extraction (MAE, UAE), and Colloidal GazAphron (CGA) fractionation on the stability of anthocyanins are reviewed.

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For citation:
Remini H, Dahmoune F, Sahraoui Y, Madani K., Kapranov V.N., Kiselev E.F. Recent advances on stability of anthocyanins. *RUDN Journal of Agronomy and Animal Industries*, 2018, 13 (4), 257—286. doi: 10.22363/2312-797X-2018-13-4-257-286.

DOI: 10.22363/2312-797X-2018-13-4-257-286

ИСТОЧНИКИ, ВЛИЯЮЩИЕ НА СТАБИЛЬНОСТЬ АНТОЦИАНОВ

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Начиная с эпохи неолита натуральные пигменты добавлялись в продукты питания, а цвет пищевых продуктов по-прежнему остается одной из основных проблем пищевой промышленности. Антоцианины являются наиболее заметной группой среди цветных флавоноидов, широко представленных в корнях, стеблях и листьях, а также цветках и плодах сосудистых растений. Они обладают высоким потенциалом для использования в качестве натуральных красителей вместо синтетических пигментов из-за их привлекательных цветовых и фармакологических свойств. Стабильные и привлекательные цвета являются высокоценными атрибутами в конкурентной пищевой промышленности. Проведены обширные исследования влияния наиболее важных химических и физических факторов, связанных с устойчивостью антоцианинов (температура, свет, pH, SO_2, металлы, сахар, аскорбиновая кислота и кислород), их концентрацией, химической структурой и составом продуктов питания. Кроме того, рассмотрено влияние технологий разделения, включая микроволновое/ультразвуковое извлечение (MAE, UAE), и фракционирование коллоидного газа Афрона (CGA) на стабильность антоцианов.

Ключевые слова: антоцианины, стабильность, аскорбиновая кислота, MAE (микроволновое вспомогательное извлечение), UAE (ультразвуковое вспомогательное извлечение), CGA (коллоидный газ Афрон)

RUDN Journal of Agronomy and Animal Industries 2018 Vol. 13 No. 4 287—293
Вестник РУДН. Серия: АГРОНОМИЯ И ЖИВОТНОВОДСТВО http://journals.rudn.ru/agronomy

DOI: 10.22363/2312-797X-2018-13-4-287-293

PREGNANCY-ASSOCIATED GLYCOPROTEIN CONCENTRATIONS IN NON-PREGNANT COWS: A CASE STUDY

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Abstract. The aim of the present work was undertaken to describe three cases of the bPAG concentration measure by three RIA systems during the post-partum period in non-pregnantcow. Three Holstein-Friesian cows of mixed age and parity were diagnosed as non-pregnant. Blood samples were removed from the coccygeal vessel into EDTA-coated tubes. Samples were collected every 2 days during a stabling period of two months in the absence of males. Plasma was obtained by centrifugation immediately after collection and was stored at –20 °C until assay. Plasmatic PAG concentration was measured by radioimmunoassay technique with some modifications. In RIA-780 and RIA-809, there were high peak of PAG concentration that reached 2.56 ng/ml and 0.89 ng/ml, respectively. These peaks lasted longer than 3 days (two successive samples were positive). The other RIA systems gave the values of PAG concentrations below cut-off of pregnancy diagnosis (> 0.8 ng/ml) and remains always non-pregnant. Our data shows clearly that there is another source of glycoproteins expression (e.g. ovarian) apart from the placenta in cow. The present study could be a field for future larger studies on the same subject by exploring deeply other extra structures placental.

Key words: PAG, radioimmunoassay, non-pregnant, cow

INTRODUCTION

The pregnancy-associated glycoproteins (PAG) constitute a large family of glycoproteins specifically expressed in the outer epithelial cell layer (chorion/trophectoderm) of the placenta in eutherian species [1, 2]. They are members of the aspartic proteinase (AP) family having high sequence homology to each other as well as with pepsin, pepsinogen, chymosin, cathepsin D and E and renin [3, 4].

The molecular biology researches estimated that cattle, sheep, and most probably all ruminants possess many, possibly 100 or more, PAG genes [1, 5]. The investigations have also demonstrated that different PAG cDNA are not expressed coordinately throughout pregnancy [1, 6]. Some, for example, are expressed early, others only when pregnancy progresses.

Radioimmunoassay for PAG detection in serum or plasma samples is currently used as a specific serological method for pregnancy diagnosis in cattle from days 28 [7] to 30 [8, 9] after breeding, with a threshold level for pregnancy of 0.8 ng/ml [7].

The present study was undertaken to describe three cases of the bPAG measurement by different radioimmunoassay systems during the post-partum period in three non-pregnant cows from condition of stabling period and the absence of males.

MATERIALS AND METHODS

Three Holstein Friesian cows (N° 7927, 7678 and 5279) of mixed age and parity were diagnosed as non-pregnant by PAG RIA-497 analysis. These females were checked by a mean of ultrasonography (Concept/MCV equipped with a 7.5 MHz Linear array, Dynamic Imaging Limited, Livingston, UK) and rectal exploration by the same veterinary.

Blood samples (7.5 to 9.0 ml) from non-pregnant females were removed from the coccygeal vein or artery into EDTA-coated tubes (Sarstedt, Numbrecht, Germany). Samples were collected every two days for 2 months. Plasma was obtained by centrifugation (1,500 × *g* for 15 min) immediately after collection and stored at –20 °C until assay.

As regards the ethical aspects, the experimental procedure was performed completely *in vitro* except for blood sampling of the animals, which was performed according to good veterinary practice.

Bovine PAG 67kDa preparation ($boPAG_{67}$; Accession number A61232) was used as standard and tracer for all assays [10]. The iodination (Na-I^{125}, Amersham Pharmacia Biotech, Uppsala, Sweden) was carried out according to the Chloramine T method [11].

Antisera were raised in rabbits immunized (R#) against different PAG preparations according to the technique of Vaitukaitiset [12]: R#497 was raised against $boPAG_{67}$ [13]; R#780 was raised against $ovPAG_{57+59}$ [14]; R#809 was raised against $ovPAG_{55}$ [15].

The measurement of plasma PAG concentrations was carried out by three distinct RIA systems (RIA-497, RIA-780 and RIA-809) differing in theantiserum, as described previously [16]. Briefly, standard and plasma samples (0.1 mL) were diluted respectively in 0.2 mL and 0.3 mL of Tris-BSA buffer.

The standard curve ranged from 0.2 ng/ml to 25 ng/ml. In order to minimize nonspecific interference of plasma proteins, 0.1 ml virgin heifer serum was added to each tube of the standard curve. After the addition of appropriate dilution of antisera (0.1 ml), the serum samples and the standard tubes were incubated overnight at room temperature (20—22 °C). The following day, 0.1 ml of I^{125}-PAG (≈ 25.000 cpm) was added and the tubes were incubated for 4 hours at room temperature.

After the tubes had been incubated for 30 min at room temperature with 1.0 ml the second antibody, a volume 2.0 ml of Tris-BSA buffer was added and the tubes were centrifuged (20 min at 1,500 × g). The supernatant was aspirated, and a second wash was done with 3.0 ml of Tris-BSA buffer. After centrifugation (20 min at 1,500 × g), the tubes were aspirated and the pellet containing the ^{125}I-PAG bound to the antibodies was counted using a gamma counter (LKB Wallac 126 Multigamma counter, Turku, Finland).

The minimal detection limit (MDL) calculated for RIA-497, RIA-780 and RIA-809 systems were 0.20 ng/ml, 0.18 ng/ml and 0.16 ng/ml, respectively. Concerning the reproducibility of the five RIA systems, the intra-assay coefficients of variation RIA-497, RIA-780 and RIA-809 were 3.5%, 6.1% and 10.6%, respectively. For the inter-assay coefficient of variation were 6.8%, 14.4% and 20.8%, respectively.

Assay pregnancy associated glycoprotein concentrations were performed in laboratory of endocrinology and animal reproduction in Liege (Faculty of Veterinary Medicine, University of Liege, Belgium).

Statistical analyses were carried out in STATVIEW (Version 4.55). The PAG concentrations measured in the non-pregnant cows were used to calculated mean ± SE.

RESULTS AND DISCUSSION

The PAG concentrations (mean ± SE) determined in plasma samples from non-pregnant (n = 3) during two months of observation are presented in Table 1. In three cows, mean PAG concentrations measured by RIA-497 remained under the 0.8 ng/ml threshold used for pregnancy diagnosis (maximal concentrations of 0.57 ng/ml).

Table 1

PAG concentrations (mean ± SE) obtained by three PAG-RIA systems in three non-pregnant cows during the period observation. Minimal and maximal values (ng/ml) are indicated in parenthesis

	PAG concentration (ng/ml)		
	RIA-497	RIA-780	RIA-809
Cow 7927 (n = 31)	0.20 ± 0.00 (0.20—0.20)	0.39 ± 0.06 (0.16—2.56)	0.18 ± 0.02 (0.18—0.31)
Cow 5227 (n = 31)	0.20 ± 0.00 (0.20—0.20)	0.17 ± 0.02 (0.16—0.47)	0.20 ± 0.04 (0.18—0.89)
Cow 7678 (n = 31)	0.22 ± 0.03 (0.20—0.57)	0.33 ± 0.25 (0.16—1.69)	0.19 ± 0.01 (0.18—0.29)

In RIA-780 and RIA-809, there were high peaks of PAG concentration that reached 2.56 ng/ml and 0.89 ng/ml, respectively. These peaks lasted for duration longer than 3 days in cows 7927 and cow 7678 (Fig. 1 and Fig. 2, respectively). As well, we observed alone high PAG concentration determined by RIA-809 system above level of positive (0.89 ng/ml) in cow 5279 (Fig. 3).

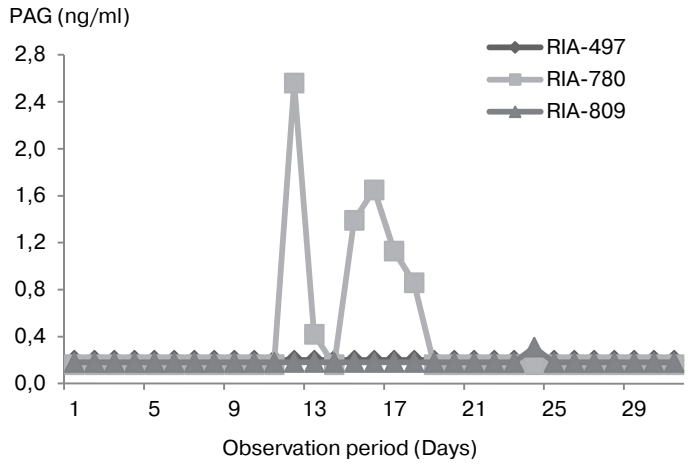

Fig. 1. Plasma concentrations of PAG (ng/ml) measured by three PAG-RIA systems during the period post-partum in cow 7927

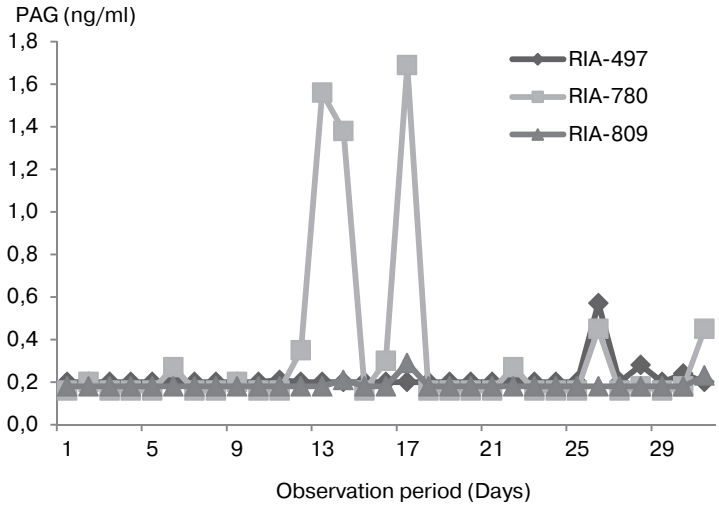

Fig. 2. Plasma concentrations of PAG (ng/ml) measured by three PAG-RIA systems during the period post-partum in cow 7678

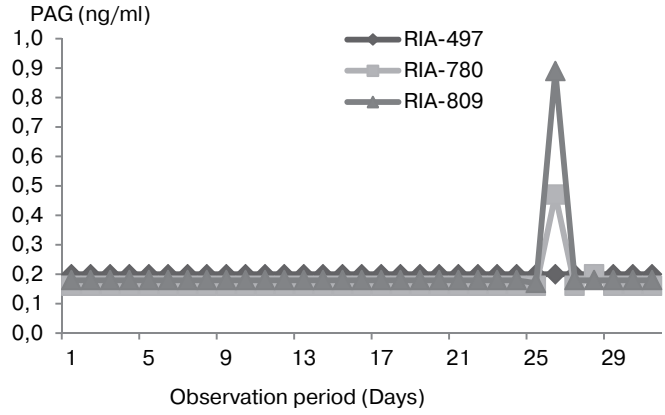

Fig. 3. Plasma concentrations of PAG (ng/ml) measured by three PAG-RIA systems during the period post-partum in cow 5279

In human and various animal species, a number of hormones and proteins appear in the maternal circulation by different party of reproduction tracts (e.g. maternal ovary). Many of these molecules are fetal-placental origin more than of maternal origin [17].

A long time ago, a research team identified a new protein from sera of pregnant women that was later purified from extracts of full-term placenta [18]. This placental protein, named pregnancy-specific (SP1), was considered to have no analog in the non-pregnant adult and to be strictly specific to the placenta. However, further studies have shown that the glycoprotein is not restricted to placenta or even to pregnant women.

Equal, glycoproteins expressed in the ruminant placenta have been isolated and characterized during the last three decades: PSPB [19]; Pregnancy-associated glycoproteins PAG; (PAG I_{67}, bovPAG 1) [10]. Several ovPAG have been purified from 100 Day-ovine placental [5], as well as from cotyledonary tissue collected between Day 60

to 100 [15] and after Day 100 of pregnancy [14]. Caprine PAG showed high sequence identity (from 30 to 81%) with proteins of the aspartic proteinase family like boPAG1, ovPAG1 and boPAG2 [20].

Molecular biology investigations showed that there are probably mores 100 PAG genes in ruminant genome [5] most of them being expressed in the superficial layers of the placenta. The conclusions by molecular biology explain also, that during certain stages of pregnancy some PAGs were expressed, whereas others were absent [1].

In this investigation, the RIA systems of PAG measurement were based of antisera raised against various PAG molecules differing in their molecular masses and species origin (bovine and ovine).

All non-pregnant cows were under condition of stabling period and the absence of males. As shown table 1, the PAG concentrations obtained by the use of RIA-497 system during the period of observation were normal values with minimal and maximal concentration below of threshold 0.8 ng/ml (0.20 ng/ml and 0.57 ng/ml, respectively). The results presented here are in agreement with the authors' previous finding [13], in which the same RIA systems were used to measure PAG concentrations in plasma samples collected.

Concerning both RIA systems heterologous (RIA-780 and RIA-809), some the PAG concentrations obtained exceeded the level of positivity (> 0.8 ng/ml) in three cows, which really does not correspond to the state of reproduction in experimental cows (i.e. post-partum period)

The period of observation of the cows exceeded 100 days after parturition [13]. The presence of the PAG concentrations in maternal blood could be explained by another extra-placental origin of secretion proteins (e.g. ovary). Knowing that the females were old, i.e. cows of reform, their ovaries could be at the origin of molecules secretions by the presence of new structures ovarian such as a prolonged luteal phase and the presence of a luteal cyst. Besides, in abstract Zoliet [19] reached to same observations which the antigen immunologically similar to boPAG67 has also been demonstrated in testicular tissue and an ovarian extract justifying the objective associated and not specific given to this glycoprotein. The explanation of PAG concentrations in plasma might be found in the fact exogenous proteinsmolecular express are recognized by both heterologous tests.

In conclusion, the data of the present report shows differences RIA systems when plasma issued from pregnant females were tested over a long period of observation. Likewise, our results show clearly that there is another source of glycoproteins expression apart from the placenta in cow. The present study could be a field for future larger studies on the same subject by exploring deeply other extra structures placental.

ACKNOWLEDGEMENT

Dr. A. Ayad wishes to thank Professor J.F. Beckers (Laboratory of Physiology of Animal Reproduction, Faculty of Veterinary Medicine, ULg, Belgium) for furnishing of reagents, technical assistance and reception in his laboratory. The authors thank Dr.C. Harrats (U. of Mostaganem, Algeria) for assistance with the English correction.

COMPETING INTERESTS
The authors declare that they have no competing interests.

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For citation:
Ayad A, Touati K. Pregnancy-associated glycoprotein concentrations in non-pregnant cows: a case study. *RUDN Journal of Agronomy and Animal Industries*, 2018, 13 (4), 287—293. doi: 10.22363/2312-797X-2018-13-4-287-293.

DOI: 10.22363/2312-797X-2018-13-4-287-293

СТЕЛЬНОСТЬ-АССОЦИИРОВАННЫЕ КОНЦЕНТРАЦИИ ГЛИКОПРОТЕИНА У НЕСТЕЛЬНЫХ КОРОВ

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Целью настоящей работы было сравнительное описание трех вариантов измерения концентрации bPAGc использованием трех систем RIA в послеродовом периоде у нестельных коров. Три коровы голштинско-фризской породы смешанного возраста были диагностированы как нестельные. Образцы крови отбирались из хвостовой артерии в пробирки, с покрытием EDTA. Образцы собирали каждые 2 дня в 2-месячный стойловый период в отсутствии быков. Плазму получали центрифугированием сразу после сбора и хранили до начала анализа при температуре –20 °C. Концентрацию PAG в плазме измеряли с помощью радиоиммунологического анализа с некоторыми изменениями. В RIA-780 и RIA-809 наблюдался высокий пик концентрации PAG, достигающий 2,56 нг/мл и 0,89 нг/мл соответственно. Эти пики продолжались более 3 дней (два последовательных образца были положительными). Другие системы RIA показывали концентрацию PAG ниже минимальной границы для периода стельности (> 0,8 нг/мл) и всегда оставались такими. Наши данные ясно показывают, что есть другой источник экспрессии гликопротеинов (например, яичники), кроме плаценты у коровы. Настоящее исследование могло бы стать предметом для будущих более масштабных исследований по этому же вопросу, основываясь на более глубоком изучении других структурных элементов плаценты.

Ключевые слова: ПАГ, радиоиммунологический анализ, нестельная, корова

RUDN Journal of Agronomy and Animal Industries 2018 Vol. 13 No. 4 294—302
Вестник РУДН. Серия: АГРОНОМИЯ И ЖИВОТНОВОДСТВО http://journals.rudn.ru/agronomy

УДК: 636.4:611.4
DOI: 10.22363/2312-797X-2018-13-4-294-302

HISTOARCHITECTURE OF THE THYROID GLAND IN PIGLETS WITH HYPOTROPHY AND AFTER THE COURSE OF MEDICAL CORRECTION

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Abstract. Antenatal malnutrition is a condition characterized by both a weight loss in animals and a change in the morphofunctional characteristics of the internal organs, particularly the incompleteness of the thyroid gland structure that will have a direct impact on the further growth and development of animals. Therefore, the aim of the research was to establish the patterns of histostructure of the thyroid gland inhypotrophic piglets and piglets after prenatal correction of hypotrophy considering their age. The study was performed on piglets of the large white breed. The study material was the thyroid gland of hypotrophic piglets and piglets after prenatal correction with Sedimin at 1, 5, 15 and 30 days of age. The main methods of study: histological, morphometric and statistical data processing.

According to the research results, a thickening of the connective tissue capsule of the thyroid gland in newborns and five-day-old hypotrophic piglets was established in relation to fifteen and thirty-day-old ones. The average diameter of the follicle is at a relatively equal level during all periods of the study, the colloid acquired a “foamy” appearance on the fifth and fifteenth day. The form of thyrocytes and their nuclei changed with age from flattened to cubic.

Cell-tissue composition of the thyroid gland of piglets after prenatal correction of malnutrition is functionally active. On the first and fifth days the capsule of the organ was thinned, by the end of the study its thickness increased markedly. The diameter of the follicles of the thyroid gland decreased from the neonatal period to the thirtieth day. Throughout the experiment, the structure of the colloid changed in the follicle lumen from homogeneous consistency to “frothy” by the end of the experiment. At the beginning of the experiment, both flattened and cubic-shape epithelial cells were observed in the parenchyma of the thyroid gland, by the thirtieth day it was stably cubic. The nuclei of thyrocytes are mostly spherical and hypochromic.

In conclusion, it is worth noting that in hypotrophic piglets in the neonatal period and in the five-day age, the incompleteness of the structure of the thyroid gland was observed, the further morphological reorganization reached by the thirtieth day. The medication “Sedimin” had a positive effect on the body of pigs, reducing the degree of development of malnutrition and contributing to the adequate organo- and histogenesis, including the thyroid gland.

Key words: thyroid gland, follicle, colloid, thyrocyte, Sedimin, large white breed, hypotrophic piglets, prevention of hypotrophy

INTRODUCTION

Hypotrophy is a disease of newborn piglets, characterized by underdevelopment, low live weight and low resistance to adverse environmental factors. The etiology of antenatal malnutrition is associated with a violation of the norms of feeding pregnant sows, the occurrence of metabolic disturbances in them causes toxicosis of gestation and results in fetal toxicosis and hypoxia, metabolic disorders in fetus, weakening of the

differentiation of the tissues and organs of the fetus, which all in all lead to morphological and functional immaturity of piglets [1, 2]. Antenatal hypotrophy is characterized by a lag in the growth of individual organs, the underdevelopment of internal organs can manifest as a decrease in their absolute mass, and anstructural inferiority of their parenchyma (cell immaturity, insufficient differentiation), which leads to a decrease in reactivity, metabolic pathologies and toxicosis [3—5]. One of the important organs responsible for the growth and development of the fetus is thyroid gland, a cell-tissue composition of which is directly expressed in its functional ability [4, 6—8]. Prenatal prevention of malnutrition is timely formation of tissues and organs, with their further inclusion in metabolic processes, such organs include the thyroid gland, whose thyroid hormones directly affect metabolism [9—11].

The aim of the study is to establish the patterns of histostructures of the thyroid gland in hypotrophic piglets after prenatal correction of hypotrophy considering age.

MATERIALS AND METHODS

Scientific research was carried out on large white breed piglets on the basis of APC "Pokrovsky" of the Orenburg region and the Department of morphology, physiology and pathology of Orenburg State Agrarian University. Two groups of animals were formed: the first is the control of malnutrition, and the second is the piglets after the prenatal prevention of malnutrition with Sedimin. The object of the study was the thyroid gland from piglets at the age of 1, 5, 15 and 30 days. Histological, morphometric and statistical research methods were used.

RESULTS AND DISCUSSION

The histoarchitecture of the thyroid gland of day-old hypotrophic piglets is relatively homogeneous (Fig. 1). The thickness of the connective tissue organ capsule was — 42.03 ± 12.60 microns. In the parenchyma of the gland, spherical follicles were viewed, with a diameter (Ø — diameter) — 5.98 ± 0.48 μm. In the follicle lumen colloid is pink, layered, homogeneous consistency. Locally, resorptive vacuoles were observed in the colloid. Thyrocytes (height — 0.70 ± 0.06 μm) are flattened, the structure of the cytoplasm is not visualized, the hyperchromic nuclei are flat, the nucleoli are not visualized (Ø — 0.38 ± 0.04 μm, NPR = 0.54). The vascular bed is filled with blood.

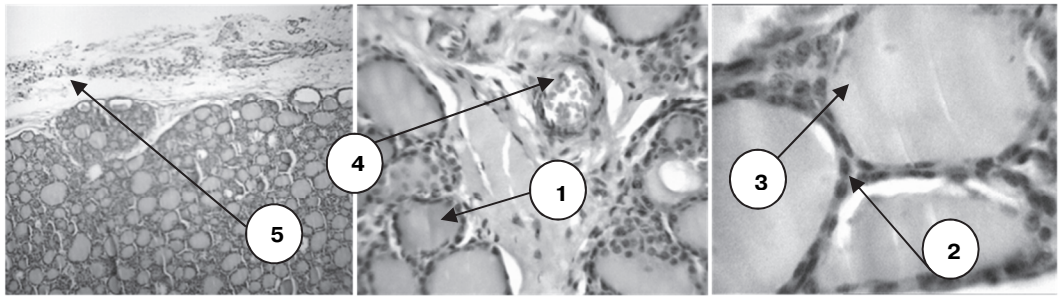

Fig. 1. Thyroid gland of day-old piglets of the first experimental group:
A — ×150; B — ×600; C — ×1500; Mayer's hematoxylin and eosin:
1 — follicles; 2 — thyrocytes; 3 — colloid; 4 — arteriole; 5 — capsule

Histoarchitecture of the thyroid gland of five-day-old hypotrophic piglets is isomorphic. The thickness of the surrounding parenchyma of the thyroid gland capsule is 48.33 ± 0.837 microns. In the center of the organ there are spherical shape and smaller in size follicles (Ø — 6.16 ± 0.76 μm), increasing in diameter and acquiring an ovoid shape to the periphery of the organ. Folliculogenesis processes are observed in the central part of the parenchyma of the gland. In the cavities of the follicles, the colloidal mass is red-pink, with the presence of significant resorption zones. Thyrocytes has a flattened shape (height — 0.64 ± 0.06 μm). The nuclei of thyrocytes are flat (Ø — 0.34 ± 0.04 μm, NPR = 0.55), hyperchromic, and the nucleoli are not visualized. The lumens of the blood vessels of the microvascular bed (MVB) are weakly filled or empty (Fig. 2).

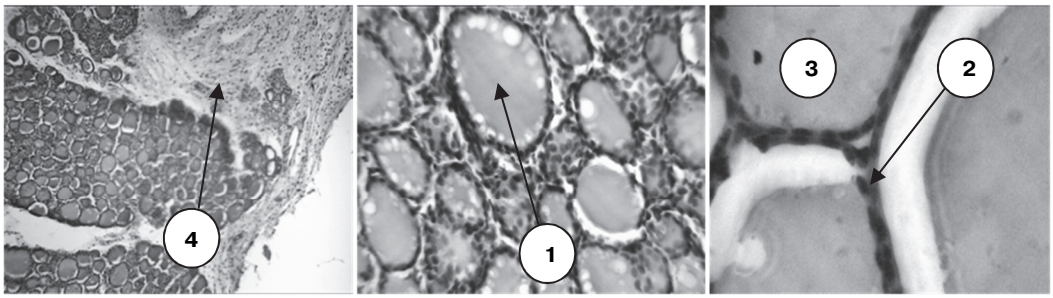

Fig. 2. Thyroid gland of 5-day-old piglets of the first experimental group:
A — ×150; B — ×600; C — ×1500; Mayer's hematoxylin and eosin:
1 — follicles; 2 — thyrocytes; 3 — colloid; 4 — capsule

The connective tissue capsule of the thyroid gland of fifteen-day-old hypotrophic piglets is prominent throughout — 27.0 ± 5.67 μm, the organ parenchyma is distinctly divided into lobules by trabeculae (Figure 3). Follicles of both spherical and ovoid shape (Ø 6.88 ± 0.58 μm) are encountered. The colloid filling the follicles has a color from pale pink to pink, "foamy" consistency, with resorption zones. Half-empty and empty follicles on the periphery of the organ are visualized. The follicular epithelium is from flat to cubic form (height — 0.98 ± 0.06 μm) with a weakly-oxophilic cytoplasm. Spherical nuclei of thyrocytesare hypochromic, visualization of the nucleoli is noted (Ø — 0.58 ± ± 0.056, NPR = 0.59). Intense blood filling is observed in microvascular bed.

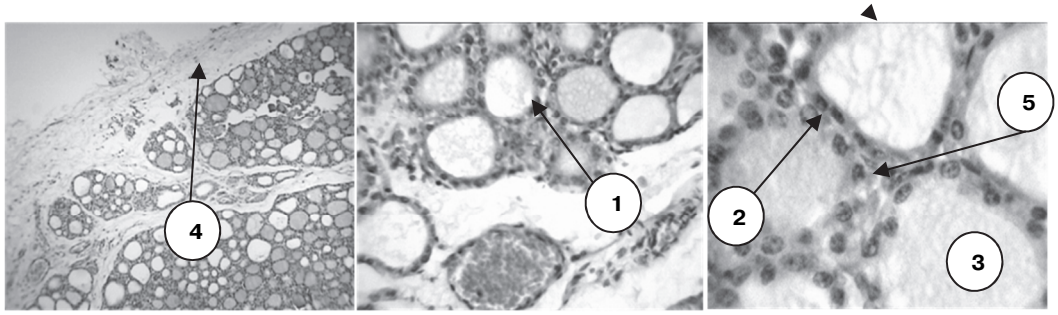

Fig. 3. Thyroid gland of 15-day-old piglets of the first experimental group:
A — ×150; B — ×600; C — ×1500; Mayer's hematoxylin and eosin:
1 — follicles; 2 — thyrocytes; 3 — colloid; 4 — capsule; 5 — capillary

On the thirtieth day a moderately developed connective tissue capsule is observed in the histostructure of the thyroid gland — 40.95 ± 14.94 microns. The shape and size of the follicles are variable over the entire area of the specimen, most of them are spherical and ovoid shape (Ø — 6.02 ± 0.64 μm), with rarely observed triangular shaped structures. The colloidal component is from pale pink to red-pink in color, the consistency is homogeneous, lamination is noted. In low prismatic thyrocytes (height — 1.20 ± ± 0.08 μm), a weakly oxidizing cytoplasm is observed, with the presence of vacuolization. The nuclei of the thyrocytes has a spherical shape, the color is hypochromic, the nucleoli are visible (Ø — 0.76 ± 0.042 μm, NPR = 0.62). The intensive metabolism is observed in the vascular bed (Fig. 4).

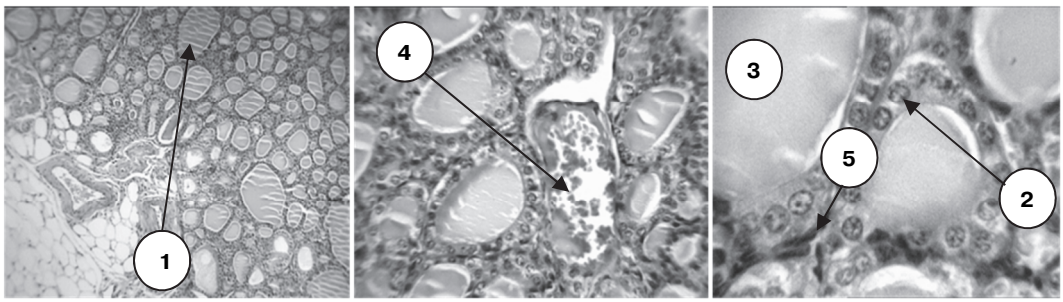

Fig. 4. Thyroid gland of 30-day-old piglets of the first experimental group:
A — ×150; B — ×600; C — ×1500; Mayer's hematoxylin and eosin:
1 — follicles; 2 — thyrocytes; 3 — colloid; 4 — venule; 5 — capillary

Histoarchitecture of the thyroid gland of day-old piglets after prenatal correction with complex medication "Sedimin" is heteromorphic (Fig. 5). The connective tissue capsule is changed throughout the organ, either thickening or gradually becoming thinner, an average of 22.86 ± 4.77, whose trabeculae divide the parenchyma into lobules. The follicles are variable, spherical in shape and small in size (Ø — 7.56 ± 0.42 μm), the formation of new follicles is continued. The colloid is oxyphilic, homogeneous, layered. Thyrocytes range from flat to cubic cells (height — 0.92 ± 0.076 μm), moderately colored, hypochromic, spherical (Ø — 0.64 ± 0.04 μm, NPR = 0.69), locally flattened, nucleoli are well visualized. MVB is moderately filled with blood.

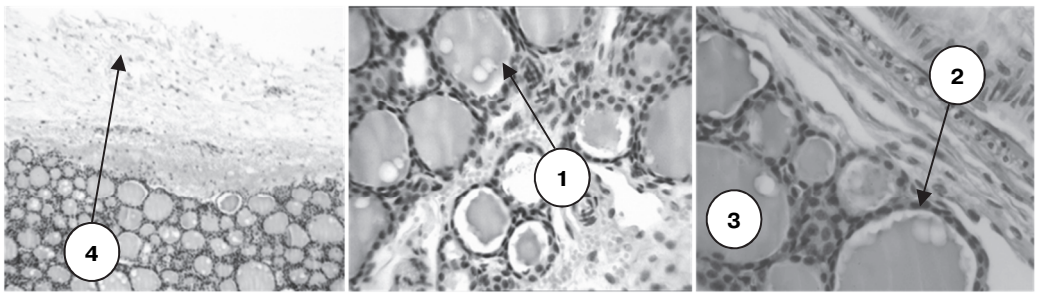

Fig. 5. Thyroid gland daily piglets of the second experimental group:
A — ×150; B — × 600; C — ×1500; Mayer's hematoxylin and eosin:
1 — follicles; 2 — thyrocytes; 3 — colloid; 4 — capsule

The micromorphology of the thyroid gland of piglets of the second group on the fifth day is heterogeneous (Fig. 6). The thickness of the connective tissue capsule is 10.53 ± 5.40 microns. The follicles predominate in spherical shape, smaller sizes in the center and large on the periphery (Ø — 6.94 ± 0.94 microns). In addition, follicles of polygonal form, including stellate, are observed. The follicle cavities are filled with pink, homogeneous, layered colloid. Resorption sites along the periphery of the follicle cavity are observed. Proliferation zones of the follicular epithelium indicate offolliculogenesis. Thyrocytes are from flat to cubic form (height — 0.78 ± 0.064), cytoplasm is weakly oxyphilous, nucleus of thyrocytes has from flattened to spherical configuration (Ø — 0.50 ± 0.048 μm, NPR = 0.64), hyperchromic, nucleoli are not visible. The vascular bed is moderately filled with blood.

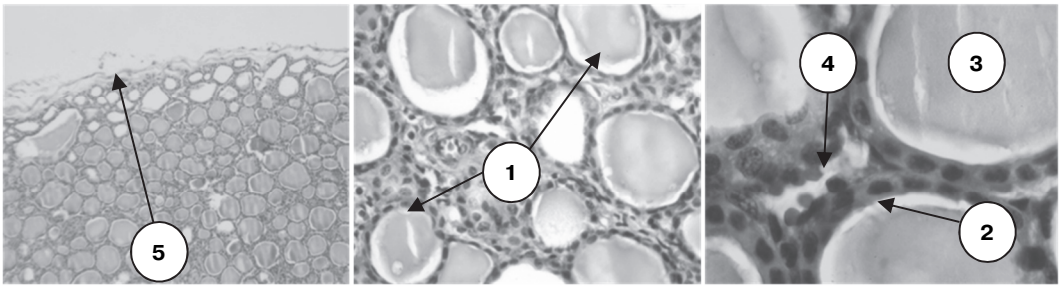

Fig. 6. Thyroid gland of 5-day pigs of the second experimental group:
A — ×150; B — ×600; C — ×1500; Mayer's hematoxylin and eosin:
1 — follicles; 2 — thyrocytes; 3 — colloid; 4 — capillary; 5 — capsule

On the fifteenth day, the thickness of the capsule is 46.53 ± 7.65 microns. The shape of the follicle is characterized by variability (Ø — 7.02 ± 0.42 μm), in the center of the thyroid parenchyma tissue clusters are small and spherical, large in size and ovoid shape at the periphery. Colloid is pink, granular consistency, with resorption zones. Processes of folliculogenesis are observed. Thyrocytes (height — 0.70 ± 0.04 μm) are variable in shape from flat to low prismatic, cytoplasm is oxyphilic, hypochromic nuclei of spherical thyrocytes (Ø — 0.50 ± 0.038 μm, NPR = 0.72), nucleoli are visualized. The vascular bedis characterized by intense metabolic processes in the thyrohematic barrier (Fig. 7).

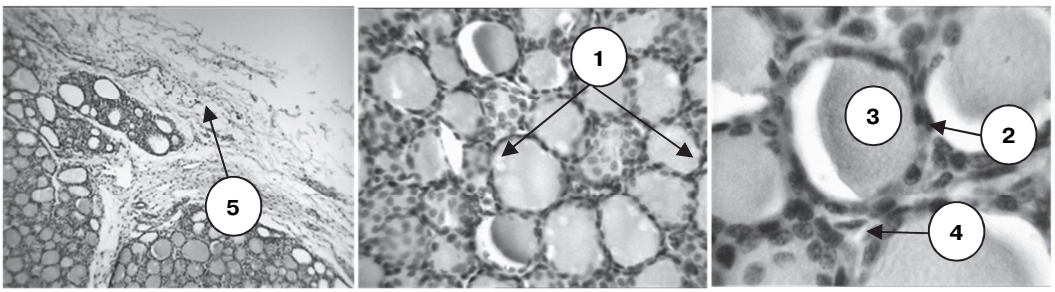

Fig. 7. Thyroid gland of 15-day pigs of the second experimental group:
A — ×150; B — ×600; C — ×1500; Mayer's hematoxylin and eosin:
1 — follicles; 2 — thyrocytes; 3 — colloid; 4 — capillary; 5 — capsule

The histostructure of the thyroid gland of piglets on the thirtieth day is characterized by the presence of a moderately developed capsule (10.58 ± 11.25 μm). Follicles (Ø — 5.42 ± 0.38 μm) are spherical in shape and smaller in the center, oval and large at the periphery of the parenchyma of the organ. The colloid is pink, "foamy", layered, with the presence of resorptive vacuoles. Thyrocytes (height — 0.90 ± 0.10 μm) are cubic inshape, cytoplasm is weakly acidic, the nucleus of thyrocytes (Ø — 0.66 ± 0.044 μm, JAPO = 0.73) are spherical, hypochromic, nucleoli are visualized. The MCBis intensively filled with blood (Fig. 8).

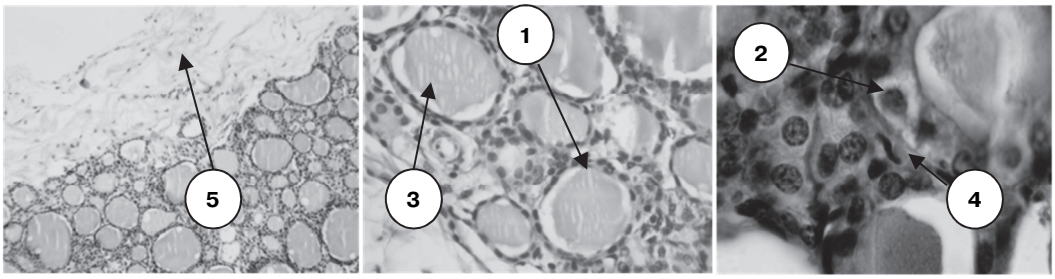

Fig. 8. Thyroid gland of 30-day pigs of the second experimental group:
A — ×150; B — ×600; C — ×1500; Mayer's hematoxylin and eosin:
1 — follicles; 2 — thyrocytes; 3 — colloid; 4 — capillary; 5 — capsule

CONCLUSIONS

Thus, the analysis showed that on the first and fifth days the hypotrophic piglets thyroid gland had a thickening of the capsule, a decrease in the diameter of the follicles and thyrocytes and the flattening of their nuclei in comparisonwith the piglets after the use of the Sedimin complex medication. By the fifteenth and thirtieth days, the micromorphology of the thyroid gland of piglets of both groups was characterized as functionally active, the morphometry of the structural components of the thyroid gland showed a decrease in the thickness of the capsule and an increase in the size of follicles, thyrocytes and their nuclei.

The drug "Sedimin" had a positive effect on the formation of the parenchyma of the thyroid gland of piglets in all age periods. Histoarchitecture of the thyroid gland of piglets in a state of hypotrophy was characterized as hypofunctionalon the first and fifth days, but histophysiology was restored to thirty-day age.

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For citation:
Bilzhanova GZ. Histoarchitecture of the thyroid gland in piglets with hypotrophy and after the course of medical correction. *RUDN Journal of Agronomy and Animal Industries*, 2018, 14 (4), 294—302. doi: 10.22363/2312-797X-2018-13-4-294-302.

УДК: 636.4:611.4
DOI: 10.22363/2312-797X-2018-13-4-294-302

ГИСТОАРХИТЕКТОНИКА ЩИТОВИДНОЙ ЖЕЛЕЗЫ ПОРОСЯТ ПРИ ГИПОТРОФИИ И НА ФОНЕ ЕЕ КОРРЕКЦИИ

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Недостаточное поступление питательных веществ является условием, характеризующимся как потерей веса у животных, так и изменением морфофункциональных характеристик внутренних органов, особенно неполнотой структуры щитовидной железы, которая будет иметь прямое влияние на дальнейший рост и развитие животных. Поэтому целью исследования было установить закономерности гистоструктуры щитовидной железы у гипотрофических поросят и поросят после пренаталь-

ной коррекции гипотрофии с учетом их возраста. Исследование проводилось на поросятах большой белой породы. Изучаемым материалом была щитовидная железа гипотрофических поросят и поросят после пренатальной коррекции с Седимином в возрасте 1, 5, 15 и 30 дней. Основные методы исследования: гистологическая, морфометрическая и статистическая обработка данных.

По результатам исследования было установлено утолщение капсулы соединительной ткани щитовидной железы у новорожденных и пятидневных гипотрофических поросят в отношении пятнадцати и тридцатидневных. Средний диаметр фолликула находится на относительно равном уровне в течение всех периодов исследования, коллоид приобрел «пенистую» внешность на пятый и пятнадцатый день. Форма тироцитов и их ядер менялась с возрастом от сплющенного до кубического.

Функционально активна клеточная ткань щитовидной железы поросят после пренатальной коррекции недоедания. В первый и пятый дни капсула органа была разбавлена, и к концу исследования ее толщина заметно увеличилась. Диаметр фолликулов щитовидной железы снижался с неонатального периода до тридцатого дня. На протяжении всего эксперимента структура коллоида изменялась в просвете фолликула от однородной консистенции до «пенистого» к концу эксперимента. В начале эксперимента в паренхиме щитовидной железы наблюдались как сплющенные, так и кубические эпителиальные клетки, на тридцатый день они были стабильно кубическими. Ядры тироцитов в основном сферические и гипохромные.

В заключение следует отметить, что у гипотрофических поросят в неонатальном периоде и в пятидневный период наблюдалась неполнота структуры щитовидной железы, дальнейшая морфологическая реорганизация достигалась к тридцатому дню. Лекарство «Седимин» оказало положительное влияние на организм свиней, уменьшило степень развития недоедания и способствовало адекватному органо-гистогенезу, включая щитовидную железу.

Ключевые слова: поросята-гипотрофики, щитовидная железа, фолликул, тироцит, «Седимин»

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Для цитирования:
Бильжанова Г.Ж. Гистоархитектоника щитовидной железы поросят при гипотрофии и на фоне ее коррекции // Вестник Российского университета дружбы народов. Серия: Агрономия и животноводство. 2018. Т. 13. № 4. С. 294—302. doi: 10.22363/2312-797X-2018-13-4-294-302.

RUDN Journal of Agronomy and Animal Industries 2018 Vol. 13 No. 4 303—316
Вестник РУДН. Серия: АГРОНОМИЯ И ЖИВОТНОВОДСТВО http://journals.rudn.ru/agronomy

DOI: 10.22363/2312-797X-2018-13-4-303-316

RADIATING PATHOLOGY IN AGRICULTURAL ANIMALS WITH A DISEASED THYROID GLAND

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Abstract. Radioactive iodine is present in atmospheric fallout during the first hours of accidental emissions at nuclear power plants. It causes damage to the thyroid gland of varying severity. As a result of lesions with radioactive iodine, pathological changes develop in the thyroid gland, which can affect the productive indicators and reproduction of farm animals. Physiological changes are also diagnosed in animals that do not receive a sufficient amount of iodine compound with food or water. This can lead to pathology of the thyroid gland. Studies of the pathology of the thyroid gland should be carried out on the basis of structural modeling methods and data obtained experimentally. Analysis of the comparison of pathological effects allows to systematize information and use it in scientific and practical purposes.

Depending on the intensity and duration of external and internal radiation exposure in farm animals acute or chronic radiation diseases can be diagnosed. Farm animals with or without signs of radiation disease of mild severity are left in farms and used for its intended purpose. Long-term effects of radiation can be manifested as temporary or permanent sterility, metabolic and endocrine status disorders, immunodeficiency, increased sensitivity to infectious diseases, the emergence of tumors. Monitoring of the radiological situation on the territory of the livestock complex is of particular importance, as its products are transported to different regions. Livestock products must meet regulatory requirements. Timely and full implementation of general and specific rules in the field of radiation safety is aimed at ensuring the safety of animals and preserving the quality of animal products.

Keywords: thyroid gland, radiating pathology, farm animals, modeling

INTRODUCTION

In 1986, as a result of the Chernobyl accident, a significant part of Belarus, Ukraine and part of the Russian Federation were affected by radionuclide contamination. The problem of radiation exposure to farm animals was studied before the Chernobyl accident, thus a large experimental material was already collected, which allowed to diagnose radiation pathology of the thyroid gland of different severity [1—4]. Scientific fundamentals of diagnosis, methods of maintenance and therapy were in demand after the Chernobyl accident [3, 5—9].

MATERIALS AND METHODS

The aim of the study was to systematize the data of radiobiological studies obtained as a result of irradiation of sheep and physiological parameters in cattle, which were in the area of radioactive contamination and evaluate the productivity of irradiated animals.

Material for the study:

1. The experimental data obtained following exposure of different doses to sheep.

Under experimental conditions, the conditions of radiation exposure were set and the dose dependence was determined. Experiments were conducted on the basis of Federal Center for Toxicological, Radiation, and Biological Safety — All-Russian Research Veterinary Institute (FCTRBS-ARRVI, Kazan). For simulation of radiation exposure in experiments on sheep, internal Iodine-131 irradiation (single or multiple), external gamma irradiation and combined external gamma irradiation with Iodine-131 or with a three-component mixture of melted radioactive particles were used as a radiation factor. Priming with iodine was carried out in the morning before feeding, in the form of an aqueous solution of potassium iodide without a carrier, into the esophagus using a polyvinyl tube and a syringe, daily in equal portions for 30 days or once, on the basis that the greatest intake of it with food in the "young" fission products is noted in the first 30 days after falling out on the terrain. Doses were selected according to the norms of contamination of feed by "young" fission products and norms of feed consumption by sheep.

Sheep were given contaminated with radioactive Iodine-131 through feed, the doses and conditions were different:

1.5 microcurie\kg\days' 30
4.5 microcurie\kg\days' 30
45 microcurie\kg\days' 30
45 microcurie\kg\days' 30
450.0 microcurie\kg\l days'
1.0 microcurie\kg
300 x-rays (γ)
600 x-rays (γ)
1000 x-rays (γ) + hunger (7 days')
300 x-rays + 4,5 microcurie\kg\day 30 days'
600 x-rays + 1 microcurie\kg\day + fasting 7 days
1.0 microcurie\kg\day +7 day fasting

Study material: wool; blood (hematological and biochemical parameters); thyroid hormones. The immunological status and reproduction indices were also studied.

2. Information obtained during monitoring on the radioactively contaminated territory of Belarus. Cows were examined after irradiation with doses 27—29 krad and 16—18 krad at the farm «Strelichevo» in Hoyniksky region.

Methods:

Experiment. The functional activity of the thyroid gland in sheep was assessed by the ability of serum proteins (α- and β-globulins) to bind exogenous thyroxine labeled with radioiodus-125 during electrophoretic separation on paper tapes. Indicator-1/F assesses the functionality of the thyroid gland and indicates a direct relationship: its high rates indicate high activity of the gland; low rates indicate low activity.

Methods of system analysis. A block approach was used for system analysis of information. The information was analyzed in accordance with the structural scheme.

Methods of logic and information modeling. Using the methods of system analysis and modeling, the radiobiological parameters of cattle located on the territory of livestock complexes in the Gomel region were studied.

RESULTS AND DISCUSSION

Radioactive Iodine is present in atmospheric deposition in the first hours of accidental emissions at nuclear power plants. It causes thyroid lesions of varying severity. The thyroid gland is a part of the neuro-hormonal regulation system. As a result of radioactive iodine damage, pathological changes develop in the thyroid gland, which can be manifested as a result of direct irradiation of the exocrineepithelium and mediated — through the hypothalamic-pituitary system. The damage of the thyroid gland causes a violation in the functional activity in other parts of the neuro-endocrine system. The changes have a phase character and are manifested as a compensatory-adaptive response to hormonal disorders because of radiation exposure. Changes in the metabolic activity of the thyroid gland are also diagnosed in farm animals kept in areas with low concentrations of stable iodine in soil and feed. Iodine pathology is diagnosed on the physiological level in affected animals (Fig. 1, Fig. 2). Endemic diseases of the thyroid gland are typical for Belarus. After the Chernobyl accident, it was necessary to determine what pathology of the thyroid gland and what methods of therapy should be used. On the basis of the designed structural and model schemes, information can be not only systematized, but also the cause of pathology can be modeled and identified (Fig. 1, Fig. 2).

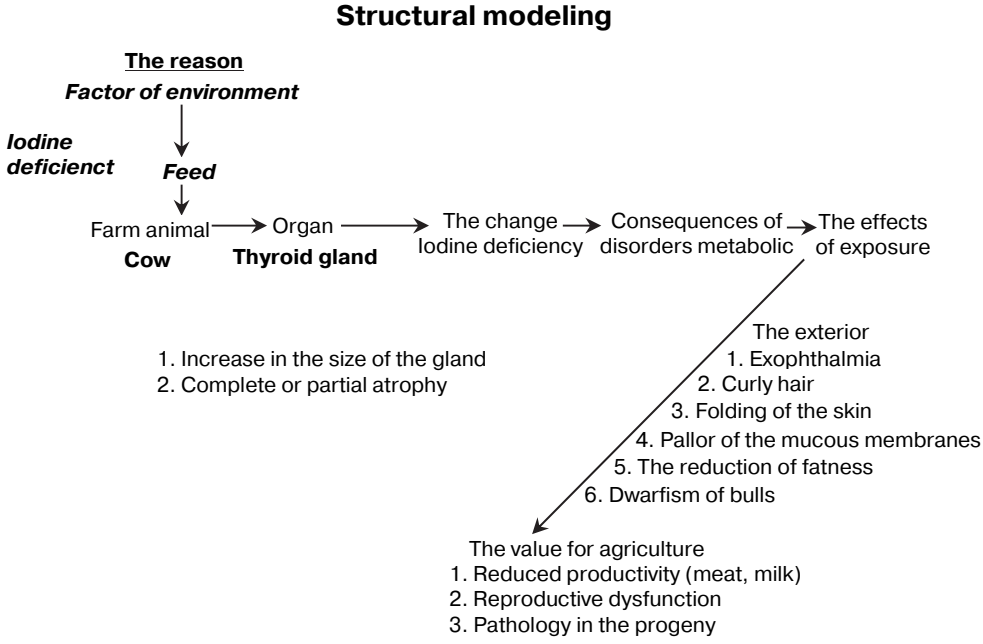

Fig. 1. Model structural scheme of the study of the effects of Iodine deficiency in cattle

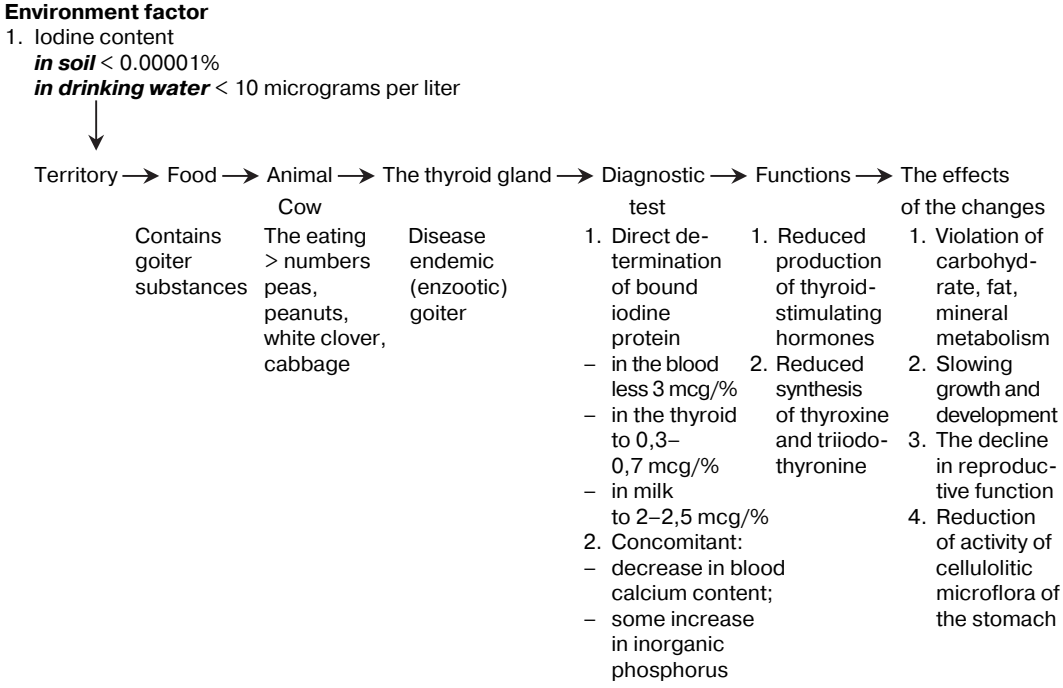

Fig. 2. Model structural diagram of the influence of iodine deficiency on the physiological indexes of agricultural animals

Results of studies of animals with affected thyroid gland

Chronic radiation disease, which is diagnosed in sheep with Iodine-131, develops slowly, early diagnosis of severity is difficult. When feeding with contaminated feed radiation pathologycan is diagnosed in sheep, for example:

— if there is Iodine-131 microcurie 2.0 and above in a feed of the daily diet of adult sheep;

— in the first 10 days of feeding bymore than 500 microcurie contaminated feed.

In farm animals diagnosed symptoms of general lethargy, increased intestinal motility, severe diarrhea. The decrease in appetite is observed in the first 5—15 days from the beginning of feeding with contaminated feed, and there is a significant decrease in body weight after 15—30 days. Subsequently, leuko-, lympho- and erythropenia is diagnosed.

Experimental studies on the kinetics of Iodine-131 in the thyroid gland were carried out in ARRVI, Kazan (Fig. 3) [1]. The kinetics of Iodine-131 in sheep in the critical organ depended on the dose and multiplicity of seeding. As a result of the conducted experiments, the dependence of the thyroid activity index on the effective dose was found and the peculiarities of their dynamics were determined (Table. 1, Table 2, Fig. 3). As a result, on the basis of experimental studies, changes in the functional activity of the thyroid gland in time for all acting doses were established. The optimal absorbed dose, causing thyroid tumor in experimental animals is 2.3…16.0 krad; minimum — 0.2 krad. During starvation in experimental animals there is a decrease in the activity of the thyroid gland. The dynamics of combined effect is similar to the dynamics during

starvation. In calf-bearing cows, there is an increase in thyroid activity at the current dose of 45 microcurie\kg\days' 30 for the entire duration of the study, which corresponds to chronic radiation disease of II severity. Reduced activity is diagnosed at a dose of 600 rad + 1 microcurie\kg\once a day for adults + 7 days of srarvation, while developing an extremely severe degree of acute radiation disease for the period from 2 days to 15 days. Increases in activity of 15 days is noted with I degree of chronic radiation illness in influencing the dose of 4.5 microcurie\kg\day' 30. For the rest of the treatment doses that cause radiation disease is characterized by reduction in activity.

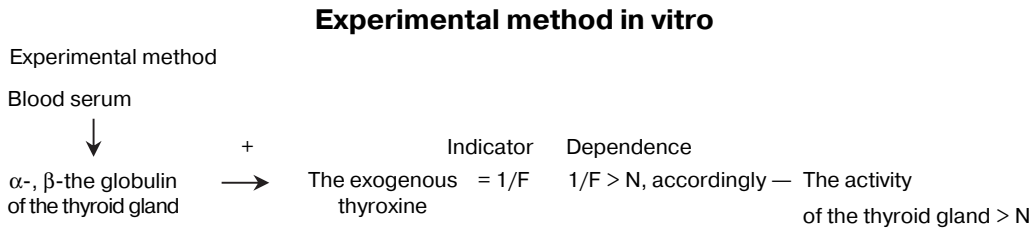

Fig. 3. Model scheme of studies increased functional activity of the thyroid gland in experimental research methodology

Table 1

Indicators of functional activity of the thyroid gland in vitro, irradiated by different doses: Iodine-131 — seed (inter), 300 x-ray radiation dose and 600 x-ray radiation dose — external gamma-irradiation of sheep, in indicator of 1/F (description N — an indicator of the physiological norm)

Dose Type Duration Organism	Min	N (norm)	Tendency	Effects of radiation. Degree of radiation disease
1.5 microcurie\kg\days' 30 chronic 30 days' lambs (5—6 months')	30 days'	no data	15 days' ← to 30 days' → increase up to control to 1.5—2 months'	3—5 months' — oscillation < N 9—12 months' > N Chronic, I degree
4.5 microcurie\kg\days'30 chronic	outcome	no data	15 days' → (→1.5 years)	> N Chronic, II degree
45 microcurie\kg\days' 30 chronic adults	outcome	no data	←to 1.5 months' For all. 3 months' >N; 4 months' N; 5—9 months' >N	Chronic, II degree
45 microcurie\kg\days' 30 chronic pregnant	4 months'	no data	1.5 years ←. outcome > N 2 months' > N; 3 months'.> N; 4 months' < N 5—9 months'.> N; 1.5 years ←	4 months-abortion and non-viable lambs
450.0 microcurie\kg\day chronic adults	3 months' (<< N in 3 times more)	outcome	← with the outcome	< N
1.0 microcurie\kg singly adults	2 days'		> N outcome ← from 2 days' → to 15 days' >> N	>> N 15 days' An acute severe disease
300 x-rays (γ) adults	6 months' < N	1.5 months' N	← from 2 months' to 6 months' → to 9— 12 months' to N	N lower control limits. An acute illness of mode- rate severity

Table 1 (continued)

Dose Type Duration Organism	Min	N (norm)	Tendency	Effects of radiation. Degree of radiation disease
600 x-rays (γ)	2 days' (> N)	to 15 days'	≈ N to 15 days'	Individual differences are characteristic. An acute illness, severe severity
1000 x-rays (γ) + hunger (7 days') adults	5 days'	no	← from 2 days' to death < N ← from 2 days', from 15 days' →	Death 13 days' increase < N ≈ control, < outcome's an acute illness of moderate severity
300 x-rays + 4.5 microcurie\kg\day 30 days' adults	The end of the experiment	no	← from 15 days' (in 2—2.5 more); ← 4—5 months', → from 6—9 months' to control	< N An acute illness of moderate severity
600 x-rays + 1 microcurie\kg\day single adults + fasting 7 days	2 days'	no	→ from 2 days' to 15 days' → with days' (in 2 more)	< N An acute illness, extremely severe disease
To 1.0 microcurie\kg\day +7 day fasting adults	End of the experiment	no	← with 2 days' 2 times more from twice the outcome to the end (in 3.1 more)	< N An acute illness, severe degree
Conclusion: there are temporal dynamics of the activity changes at all dose levels; fasting — lowering activity (decrease in metabolism).				
Designations: 1/F-index reflecting the activity of the thyroid gland, the more 1/F, the higher the activity. ← — decreasing trend from normal (control) N; → — increase				

Table 2

The absorbed thyroid dose β-, γ-radiation of Iodine-131 at 30-day introduction of isotopes of the sheep in the experiment

The dose of iodine-131	The radiation disease	Absorbed thyroid gland dose (rad)
1.5 microcurie\kg ·30 days	Chronic radiation disease of I degree of severity	8 138 ± 236
4.5 microcurie\kg ·30 days	Chronic radiation disease of I degree of severity	14 814 ± 849
45 microcurie\kg ·30 days	Chronic radiation disease of II degree of severity	25 673 ± 1090
450 microcurie\kg ·30 days	Chronic radiation disease of III degree of severity	29 346 ± 1640
300 x-rays + 4,5 microcurie\kg 30 days	Acute radiation disease of moderate severity	4 812 ± 773
300 P+45 microcurie\kg 30 days	An acute radiation disease of severe degree of severity	28 948 ± 2414

Metabolic features

The decrease in thyroid activity is recorded for high doses and in chronic radiation disease of II and III severity [6, 7, 9, 10]. Features of the functional activity of the thyroid gland depend on severity of radiation disease.

1. Chronic radiation disease of I degree of severity is characterized by increased activity of the gland in lambs and sheep. After 1.5—2 months young indicators of thyroid activity in the contaminated farms are above the control. After 2 years, there was a decrease, and then again an increase in activity.

2. Chronic radiation disease of the II degree of severity: there was instability of activity in the first 2 months, depression with increased activity in the next 7 months and a sharp decline after 1.5 years.

3. Chronic radiation disease of the II degree of severity: a decrease in activity below control until the end of life.

It was determined that small doses cause increased activity, and large — inhibition of activity and destruction of organ parenchyma. On the basis of experimental data, it was found that at high acting doses, a decrease in the sensitivity of the nervous system is characteristic, which is diagnosed as an extension of the latent reflex time. At the same time, there is a decrease in productive indicators (Table 3).

Table 3

Indicators of biological effectiveness of Iodine-131 in a dose dependent manner

The dose of iodine-131	The radiation disease	Absorbed thyroid gland dose (rad)
1.5 microcurie\kg ·30 days	Chronic radiation disease of I degree of severity	8 138 ± 236
4.5 microcurie\kg ·30 days	Chronic radiation disease of I degree of severity	14 814 ± 849
45 microcurie\kg ·30 days	Chronic radiation disease of II degree of severity	25 673 ± 1 090
450 microcurie\kg ·30 days	Chronic radiation disease of III degree of severity	29 346 ± 1 640
300 x-rays +4.5 microcurie\kg · 30 days	Acute radiation disease of moderate severity	4 812 ± 773
300 x-rays +45 microcurie\kg · 30 days	An acute radiation disease of severe degree of severity	28 948 ± 2 414

Radiation has a direct and indirect effect on the activity of the nervous system and the processes of regulation. The experiments on sheep determined [1]:

1) chronic radiation disease of I degree of severity: 1.5 months after seeding, there is a significant acceleration of defensive unconditional reflex reaction;

2) acute radiation disease of moderate severity: unreliable acceleration of defensive unconditional reflex reaction;

3) with all other degrees of severity of acute and chronic radiation disease, there is a slowdown in the unconditional reflex reaction.

The depth and severity of violations directly depends on the dose. With relatively small doses, an increase in the sensitivity of the nervous system is recorded. In the studied animals there are no changes in the productive indicators.

The diagnosed features of hormonal dynamics in cows with radiation damage of thyroid Iodine-131 may be associated with changes in other physiological systems [9]:

1) hematological parameters: no profound changes. In the radiosensitive system of erythroid hematopoiesis, a decrease in the number of erythrocytes is diagnosed and their dose dependence in individual individuals is weakly expressed;

2) the system of hemostasis: activation of coagulation secondary link and the primary inhibition of vascular-platelet link;

3) changes in the level of cyclic nucleotides in blood plasma: more pronounced in the spring and less pronounced in healthy cows in the “clean” area, systematically receiving alcohol-containing feed;

4) hormonal parameters: decrease in the level of T4 and T3 and disturbances in interhormonal relationships, as well as seasonal variability and a significant increase

in the content of biologically active free fraction of cortisol due to a decrease in the binding capacity of blood proteins. The dose direct dependence of changes in the level of T4, T3, insulin and cortisol is determined, the dependence is direct — the higher the dose, the greater the change;

5) Changes in the hormonal status of cows with radiation damage to the thyroid gland cause disorders in the reproductive system (Table 5). In the first group of cows that received a large dose to the thyroid gland in the polluted farms of the Gomel region there is a decrease in reproductive capacity:

a) reduced the index of impregnation capacity (in 1987),

b) reduction of service period duration.

For example, the duration of the service period by day in 1987 increases, and by 1989 in the studied farms decreases, i.e. shortens.

Reproductive performance

The account of reproductive indicators for farm animals is very important for any animal husbandry, and for those who are in the pollution zone they are of particular importance. To calculate the index of reproduction (Iv) for one individual, the calculation of the average value for the lifetime of the individual can be used:

$$\sum Iv = \frac{\sum Fi}{\sum Pj}, \quad (1)$$

where *Fi* — the offspring (lambs); *Pj* — the sheep that produces it's offspring.

∑Iv — can be calculated for a group of individuals both for the year and for the time of observation or life of individuals of the group. Statistical research methods can be applied:

— for carrying out the dispersion analysis between the values of indicators of individuals in the group, under the action of different doses of radiation on them;

— determination of the confidence interval between different indicators of individuals, groups of individuals and indicators of their reproduction, etc.

Time component accounting

The time component is taken into account when studying the time dynamics of both individuals and individuals in the group. At the same time the season, exposure factors, physiological time, etc. can be seen. In the system information analysis time allows to systematize the information considering the time of the study or the time period of the diagnosed changes.

Time dynamics

The change of indicators in time reflects the time dependence of changes in the studied parameters on the physiological norm and characterizes the stage of development of pathology (Table 4).

Table 4

Time dynamics of change of indicators from physiological norm of the hormonal status on groups cattle of the Gomel area: Hojniksky area (2nd pollution degree) I, II group of cows and offspring; state farm Oktyabrskaya of Oktyabrsky (district control) cows

Year	Quarter	Cows I group (2nd pollution degree)	Bulls (86 year of birth) from I group	Heifers from I group	Cows II group (2nd pollution degree)	Cows (4th pollution degree)
Triiodothyronine (T3) (nmol\l)						
1987	II	< N, N, > N				
	III	< N, N		< N		
	IV	< N, N				< N, N, > N
1988	I	< N, N, > N		< N		N
	IV	< N, N, > N	> N			< N, N, > N
1989	I	< N, N, > N	N			< N, N
Result: Changes in the values of the indicator are recorded in groups of cows from farms in all study periods; in young animals there is a decrease in heifers from cows of group I.						
Thyroxine (T4) (nmol\l)						
1986	IV	> N				
1987	II	< N, N, > N		N	< N, N	
	III	< N, N, > N		N	< N, N	IV quarter < N, N
1988	I	< N, N, > N		N	< N, N	< N
	IV	< N, N, > N	> N		> N, N	N, > N
1989	I	< N, N, > N	< N		< N, N	< N, N
	II	< N, N, > N		> N	< N, N IV quarter < N, N, > N	< N, N
Results: Greatest changes are observed in groups of cows in all study periods; for young animals, the values are reduced in bulls in the first quarter of 1988.						
Conclusion: Dynamics in the values of the indicator relative to the norm is typical for cows, for young recorded some deviations, mainly in the group of heifers of group I.						

Physiological indices in the exposed cows from contaminated farms

At the farm "Strelichevo" Hoyniksky district study of cows affected with thyroid revealed some peculiarities [9, 10].

Indicators of immune status:

— No significant differences in the level of infection were found.

— The concentration of lysozyme in the blood serum in the summer was below the control values.

In cows that received a dose of 27—29 krad, the concentration of lysozyme in serum decreased by 24%, and in those who received a lower dose (16—18 krad) by 35% — an inverse dependence on the dose.

— Bactericidal activity of blood serum: in group I cows it was at a sufficiently low level (26.4 ± 5.6%) (< N), and in group II it was higher (> N).

— Immunological response to A_{22}/C_1 anti — vaccination (IV quarter 1988) — positive, but the temporal dynamics is poorly expressed.

Studies of reproductive and productive indicators of irradiated animals have shown that the most pronounced changes in them are diagnosed in farm animals:

— with a higher absorbed dose in the thyroid gland;

— consuming contaminated feed;

— located in an area with a higher density of pollution.

Comparative analysis of productivity in the polluted farm "Vysokoborsky" in Vetkovsky district (the maximum degree of pollution) and the Collective Farm of First of May, Chechersky district, where the animals were fed for 3 years after the accidental release of contaminated feed above $1.0 \cdot 10^{-6}$ Curie\kg, showed that in the long term, 4 years after the accident, there is a restoration of reproductive function and reproductive performance in cows, including cows with signs of thyroid damage (Table 5).

The calculation of the coefficients relative to the physiological norm

The conducted researches were based on comparison of the diagnosed sizes with sizes of physiological norm. The comparison analysis revealed a number of differences, which are reflected in Table 5.

Table 5

The degree of priority of the indicators (maximum of the diagnosed values in the group) of thyroid hormones on the physiological norms for the groups of cattle Hoyniksky district (2nd degree of pollution)

Year	Quarter	Triiodothyronine (T3) (nmol\l)			Thyroxine (T4) (nmol\l)			Bulls from I group cows (born in 1986) (from N)		Sum Indicators'
		< N	> N	N	< N	> N	N	T3	T4	per quarter
1987	II	2	3	1	2	3	1			6 + 6
	III	1	3(0)	2	2	3	1			3 + 6
	IV	1	3(0)	2						3 + no data
1988	I	2	3	1	2	2	1			6 + 5
	IV	1	3	2	1	1	1	> N	> N	6 + 3
1989	I	1	3	2	2	3	1	N	N	6 + 6
1989	II				1	3	2			no data + 6
Conclusion: the spread of values from the norm. (3 (0) — the value was diagnosed, but it is 0)										

Offspring obtained from cows with radiation damage to the thyroid gland are diagnosed with abnormalities [9]:

— 1—2 calves monthly changes in coagulogram — hypercoagulability;

— increase in the level of cyclic nucleotides by 1.9—1.5 times;

— decrease in the level of red blood cells;

— platelet content 2 times higher than the control;

— bactericidal activity of serum in young animals obtained from cows of group I — at a high level (> N): bulls 50.0%; heifers 44.6%; calves 66.6%;

— the dynamics of hormonal parameters does not indicate pathological changes;

— the main causes of mortality of calves received from cows with signs of radiation damage to the thyroid gland (I, II groups): dyspepsia; bronchopneumonia. Calves of first generation (F1) 1989 year birth lagged behind in growth — bulls dwarfs, but in the

future, they gained height and weight, reaching a weight of 400 kg. Cows were resulting offspring in 1990. Calves born in 1990 (F2) from cows born in 1987 did not differ in their physiological development from young control and non-polluted farms, dominated by heifer. 30% of all livestock received in 1989—1990 were bull-calves;

— hormonal parameters in offspring obtained from cows with varying degrees of radiation damage to the thyroid gland, deviate from the norm. Steers were characterized by the excess rates of T3 and T4 in the first quarter of 1989.Heifers had a decrease below normal in the second quarter of 1987 and in the first quarter of 1989 because of incomplete data and the lack of a temporal chronology of diagnosis, based on their changes obtained from the cows and the dose can not be determined.

Studies of the temporal annual variability of the average indicators of triiodothyronine and thyroxine indicate deviations from the norm. In cows of I group, for example, in 1987, in the period from the II quarter to IV, an increase in triiodothyronine was diagnosed, but below the norm (<N). At the same time, the minimum values of deviation from the norm, as well as the detected elevated triiodothyronine values fall above the norm. For thyroxine in the same period, the maximum values (average for the group) are observed for the group — more than the norm (>N), and the minimum values — norms (N).

The results of statistical studies revealed deviations from the physiological norm of hematological parameters in heifers (born in 1987) from the I group of cows with affected thyroid farm «Strelichevo» of Hoyniksky district (2nd degree of pollution) [7, 8]. Deviations were determined in the autumn.

The results of the diagnosis of biochemical blood parameters indicate their deviations from the physiological norm in the offspring obtained from cows with irradiated thyroid gland. In General, according to the diagnosed indicators in the offspring obtained from the I group of cows, it is noted:

— for bulls born in 1986 had a decrease relative to the norm;

— for calves — a small prevalence above the norm, but there is a decrease relative to the norm.

For groups of cows (I and II group) in total, there is a more pronounced decrease in biochemical parameters in the group that received a large dose to the thyroid gland. Biochemical indicators can be informative at radiation pathology, therefore it is necessary to carry out their diagnostics systematically on different age groups of farm animals, in comparison of producers and their posterity.

A comparative analysis of productivity in the polluted state farm "Vysokoborsky" of Vvetkovsky district (the highest degree of pollution) and on the Collective Farm of First of May of Chechersk district, where animals were fed for 3 years after the accidental emissions by contaminated feed above $1.0 \cdot 10^{-6}$ curie\kg, showed that in the long term, 4 years after the accident, there is a positive trend towards the restoration of reproductive function and reproductive performance in cows with signs of thyroid damage.

CONCLUSIONS

To assess the state of the endocrine system of farm animals located in the contaminated area, it is necessary to consider seasonal and age-specific dynamics of hormonal parameters by age groups and acting doses. Changes in endocrine parameters entail

changes in other physiological parameters. For livestock management in contaminated areas, it is important to know how these changes will affect the reproductive and productive features of farm animals.

The most pronounced pathological changes after the Chernobyl accident were diagnosed in farm animals left in polluted farms. They were also diagnosed in cows with an affected thyroid gland. In offspring obtained from cows with the highest dose of thyroid radiation, deviations from the physiological norm were most pronounced in the first generation, and in the second generation of pathology was not noted.

When exposed to radioactive Iodine, thyroid damage is diagnosed, which is the greater the dose absorbed by the gland. It was determined that there are more pronounced the pathology of the thyroid gland, more pronounced the changes in physiological parameters and decrease of reproduction and productivity in farm animals.

Studies of reproductive qualities and productive indicators of irradiated animals showed that the most pronounced changes were diagnosed in farm animals that were on the territory with a higher density of pollution and with a higher absorbed dose in the thyroid gland.

It is advisable to carry out studies to identify changes in hematological, biochemical and hormonal parameters systematically in groups of farm animals, taking into account the radiation background of the area and livestock premises, the degree of contamination of feed. Comparative data analysis is necessary to identify dependencies and patterns. It should be carried out on different groups of farm animals exposed to different doses of radiation on the basis of methods of system analysis and modeling. The use of methods of system analysis and modeling allows not only to systematize the material of research and diagnosis, but also to predict the situation in the livestock complex.

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For citation:
Pavlova SA. Radiating pathology in agricultural animals with a diseased thyroid gland. *RUDN Journal of Agronomy and Animal Industries*, 2018, 13 (4), 303—316. doi: 10.22363/2312-797X-2018-13-4-303-316.

DOI: 10.22363/2312-797X-2018-13-4-303-316

РАДИАЦИОННАЯ ПАТОЛОГИЯ У СЕЛЬСКОХОЗЯЙСТВЕННЫХ ЖИВОТНЫХ С ПОРАЖЕННОЙ ЩИТОВИДНОЙ ЖЕЛЕЗОЙ

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Радиоактивный йод присутствует в атмосферных выпадениях в первые часы аварийных выбросов на АЭС. Он вызывает поражения щитовидной железы различной степени тяжести. В результате поражения радиоактивным йодом в щитовидной железе развиваются патологические изменения, которые могут влиять на продуктивные показатели и воспроизводство сельскохозяйственных животных. Физиологические изменения диагностируют и у животных, которые не получают в достаточном количестве соединения йода с кормом или водой. Это может привести к патологии щитовидной железы.

Экспериментально определено, что малые поглощенные щитовидной железой дозы радиации вызывают повышение ее активности, а большие — угнетение активности и разрушение паренхимы органа, что характерно для хронической лучевой болезни II и III степени тяжести.

Хроническая лучевая болезнь, которая диагностируется у овец при поражении Йодом-131, развивается медленно, ранняя диагностика степени тяжести затруднена. На основе экспериментальных данных установлено, что для средней степени тяжести лучевой болезни отмечается снижение шерстной продуктивности и плодовитости и выживаемости потомства.

Системный анализ данных по радиоактивно загрязненным хозяйствам позволил выявить степень тяжести радиационной патологии у сельскохозяйственных животных животноводческих комплексов. Исследования патологии щитовидной железы следует проводить на основе методов

структурного моделирования и данных, получаемых экспериментально. Анализ сравнения патологических эффектов позволяет систематизировать информацию и использовать ее в научно-практических целях. На основе проведенных исследований установлено, что для оценки состояния эндокринной системы сельскохозяйственных животных, находящихся на загрязненной радионуклидами территории, необходимо учитывать сезонные и возрастные особенности по возрастным группам и с учетом воздействующей дозы. Разработка и практическое использование методов моделирования позволяет не только систематизировать собранную информацию, но и спрогнозировать ситуацию в хозяйствах, подвергнутых радиоактивному загрязнению.

Ключевые слова: щитовидная железа, радиационная патология, сельскохозяйственные животные, моделирование

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Для цитирования:
Павлова С.А. Радиационная патология у сельскохозяйственных животных с пораженной щитовидной железой // Вестник Российского университета дружбы народов. Серия: Агрономия и животноводство. 2018. Т. 13. № 4. С. 303—316. doi: 10.22363/2312-797X-2018-13-4-303-316.

RUDN Journal of Agronomy and Animal Industries 2018 Vol. 13 No. 4 317—335
Вестник РУДН. Серия: АГРОНОМИЯ И ЖИВОТНОВОДСТВО http://journals.rudn.ru/agronomy

DOI: 10.22363/2312-797X-2018-13-4-317-335

DIGITAL SOIL MAPPING FOR SMART AGRICULTURE: THE SOLIM METHOD AND SOFTWARE PLATFORMS

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Abstract. The key challenges faced by many of the existing digital soil mapping (DSM) techniques are the rigid requirements on the size of soil samples to extract the relationships needed and on the stationarity of the extracted relationships. These requirements limit the application of these DSM techniques. This paper provides an overview of the SoLIM approach and an introduction to the operation of SoLIM through the software platforms available. SoLIM is based on the Third Law of Geography, which calls for the comparison of similarity in geographic (environmental) configuration of a prototype and an unsampled location and then use this similarity to predict the value of a soil property at a given location. DSM under SoLIM approach removes requirements on the sample size and the stationarity assumption. In addition, the uncertainty computed based on the similarities can be used to improve the efficiency of error reduction efforts. The SoLIM approach has been implemented in two platforms: SoLIM Solutions and CyberSoLIM. The theoretical foundation and the availability of software platforms under SoLIM make DSM possible and convenient over large and complex geographic regions.

Keywords: digital soil mapping, DSM, SoLIM, First Law of Geography, Second Law of Geography, Third Law of Geography, spatial prediction

1. INTRODUCTION

Smart agriculture, as a way to increase the productivity of agricultural lands while minimizing the negative impacts on the environment, must base its practices on detail information about the status of agricultural land and information on how this status varies over space. Data on the status of soils and its spatial variation across landscape is an essential part of the information about agriculture lands. Among the many methods for acquiring information on soil conditions, digital soil mapping (DSM), an emerging area in this field, is a major approach to soil spatial information gathering [1].

DSM techniques, an application of spatial prediction in soil mapping, are mostly based on three basic principles [2]: spatial autocorrelation principle (also referred to as the First Law of Geography [3]), the statistical principle, and the spatial heterogeneity (also referred to as the Second Law of Geography [4]), or the combination of these principles (such as the various versions of kriging [5] and geographically weighted regression [6]). The key challenges to the techniques based on these principles are: 1) the requirement of samples of sufficient size for the extraction of the spatial relation-

ships or covariate relationships needed for soil prediction; and 2) the stationarity assumption of the extracted relationships [1]. These requirements have limited the application of this type of techniques for DSM over large and complex geographic areas where collecting a sample set sufficient enough is prohibitively expensive and where geographic processes are so complex that the stationarity assumption required often does not hold.

In recognition of these limitations faced by the techniques based on above principles Zhu [7] and Zhu et al. [8] presented a similarity approach to DSM (referred to as the Soil Land Inference Model (SoLIM) approach). The basis behind this similarity approach is what now been referred to as the Third Law of Geography [1]. This paper provides an overview of the theoretical thinking, the implementation and operation of this similarity approach. The next section describes the theoretical grounding of the SoLIM approach which is then followed by a presentation on how this idea is implemented. Software realization and operation of the SoLIM idea are presented in Section 4. Future research issues related to SoLIM are discussed in Section 5.

2. SOLIM AS AN APPLICATION OF THE THIRD LAW OF GEOGRAPHY

The basic idea behind the SoLIM approach is another principle which has been commonly applied and now referred to as the Third Law of Geography [1]. This law states that "The more similar geographic configurations of two points (areas), the more similar the values (processes) of the target variable at these two points (areas)". The SoLIM approach exploits the comparative nature of this law in predicting soil conditions at an unsampled location. With this comparative nature the soil property value at an unsampled location can be estimated by the similarities in the environmental configuration between the unsampled location and a known prototype available (Figure 1). A prototype in the sense of soil mapping can be perceived as the central concepts of a soil class, a representative case of the class, or a field sample [9]. Under this notion, the process of DSM under the SoLIM approach can be accomplished in three steps: First, the similarity in environmental configuration ($S^k_{i,j,E}$) between a prototype (k) and the unsampled location (i, j) is measured. The computed $S^k_{i,j,E}$ is used to approximate the similarity ($S^k_{i,j}$) in soil attribute value between prototype k and the unsampled point (i, j). Finally, the attribute value ($V_{i,j}$) at the unsampled point (i, j) is calculated through Equation 1 below [10]:

$$V_{i,j} = \sum_{i=1}^{n} w^k_{i.j} \bullet v_k, \qquad \text{Equation 1}$$

where n is the number of prototypes involved, v_k is the soil attribute value for prototype k and $w^k_{i.j}$ is the weight assigned to prototype k for the unsampled point (i, j) and is calculated using Equation 2 [11].

$$w^k_{i,j} = \frac{S^k_{i,j}}{\sum_{i=1}^{n} S^l_{i,j}}. \qquad \text{Equation 2}$$

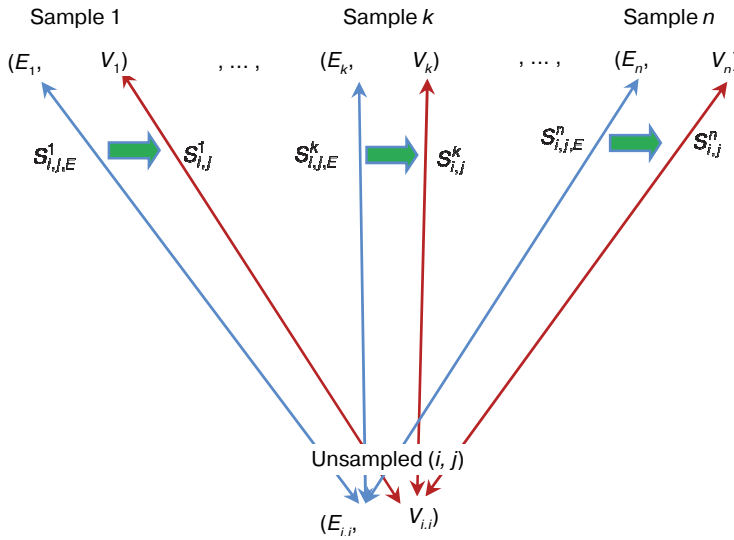

Fig. 1. The comparative nature of the Third Law of Geography used in SoLIM

E_k is the environmental configuration at prototype k and $E_{i,j}$ is the environmental configuration at unsampled point (i, j). V_k is the soil attribute value at prototype k and $V_{i,j}$ is the soil attribute value at unsampled point (i, j). $S^k_{i,j,E}$ is the similarity in environmental configuration between unsampled point (i, j) and prototype k. $S^k_{i,j}$ is the similarity in soil attribute value between unsampled point (i, j) and prototype k. $S^k_{i,j}$ is approximated by $S^k_{i,j,E}$

Figure 1 clearly shows that under the SoLIM approach the weight assigned to each prototype involved is based on the similarity of the unsampled point with each of individual prototypes, which is what the Third Law of Geography calls for. In this notion, no general relationships need to be extracted and quantified. Instead, the similarity in environmental configuration, as an individual representativeness of a prototype to an unsampled location, is captured and thus local variations in soil conditions can be expressed [12]. This similarity can also be used to compute the uncertainty for each prediction [13]. This uncertainty can be used to assess the quality of the so predicted results and be used to allocate future sampling efforts to further improve the quality of the prediction [12, 14, 15]. The use of individual prototypes and the uncertainty measure associated with the prediction using these prototypes under SoLIM removes the requirements on the specific number of prototypes (or samples) needed, the requirement on spatial distribution of these prototypes (or samples), and the stationarity assumption [1]. The impacts of the SoLIM approach on soil mapping have been documented in other studies [16, 17] and are not repeated here.

3. IMPLEMENTATION AND DEVELOPMENT OF SOLIM

The key issues to the success of the SoLIM idea as expressed above are the quantification of prototypes and the characterization of environmental configuration. Thus, the implementation and development of the SoLIM approach have focused on these two areas so far.

3.1. Quantification of prototypes

As stated earlier a prototype can be a field soil sample or the central concept of a soil class. The quantification of a prototype under the SoLIM approach consists of 1) the derivation of a set of covariates to characterize the geographic configuration for the prototypes and 2) the property values of the prototype.

Field soil samples as prototypes: Clearly, the field soil samples are good sources for prototypes under the SoLIM approach. Each sample has soil attribute values observed. The environmental configuration of a sample may be characterized in the field directly but more than often it is done after the fact using geographic information processing techniques and remote sensing methods based on the (x,y) coordinate values of the sample location. In other words, the two elements of prototype can be very conveniently defined with the use of field sample points as prototypes. Thus, samples naturally fit the use of the Third Law of Geography well with the SoLIM approach.

Central concepts of soil classes as prototypes: For a soil classes we often have information about the typical values and the ranges of soil properties. In this case the property values of the prototype representing this class is not difficult to obtain. The characterization of environmental configuration for the prototype would present challenges due to the fact that the environmental configuration is often not complete, sometimes even not available at all in soil survey reports. However, local soil scientists, particularly local soil surveyors, would normally understand under what kind of environmental conditions (or configurations) the soils belonging to a particularly soil class would exist or develop. This information is very useful in defining the environmental configurations for soil classes. To obtain environmental configurations of soil classes from local soil surveyor, Zhu [18] developed a personal construct based approach for obtaining knowledge from local experts on environmental configuration. The approach employs fuzzy logic to express the environmental conditions where a soil type will develop fully (assigned a fuzzy membership of 1) and where the soil type does not develop at all (assigned a fuzzy membership of 0) and where the soil type develop at half (assigned a fuzzy membership value of 0.5). This approach of defining a prototype is available through the SoLIMSolutions software described later in this manuscript.

The other source of knowledge on the environmental configuration for a soil class is existing soil maps where the spatial distribution of soil classes is portrayed. This type of maps would implicitly contain the information needed to define and characterize the environmental configuration for a soil class. Qi and Zhu [19] developed an inductive learning (decision tree) approach to extract environmental configurations for soil classes from soil maps. Cheng et al. [20] furthered this effort to make a use of the knowledge captured at the individual polygon level. This capability is also provided in the SoLIM Solutions software.

Due to the fact these environmental configurations are extracted from human experts or from existing soil maps in the form of knowledge rules, soil prediction under the SoLIM approach using environmental configurations extracted in this way is referred to as “rule-based”. It must be noted that this “rule-based” is not the same as the relation-

ships used in the statistical approaches for the following two reasons. The first is that these "rules" are not expressed in any fixed quantitative form as those in the statistical approaches. Second, these "rules" are used to describe the environmental configuration, not to relate environmental covariates to soil property values directly.

3.2. Characterization of environmental configuration

Characterization of environmental configuration calls for 1) a comprehensive list of covariates that can effectively describe the geographic environment relevant to a soil property; 2) the hierarchy of these variables; 3) the spatial foot prints of the target soil properties. The current efforts environmental configuration characterization for the SoLIM approach has been focus on the development of comprehensive list of covariates with initial research underway on the other two.

In addition to conventional soil covariates used to describe the soil formative environment (climatic conditions, topographic conditions, geological conditions, vegetation conditions), the SoLIM effort has added two new environmental variables into the list. The first one is the fuzzy landscape positions (fuzzy slope components) characterizing slope positions (such as ridge top, shoulder slope, backslope, footslope and valley bottom) in the form of fuzzy membership value [21]. This way of characterizing slope positions allows the gradation from one slope position to another to be represented in the covariate dataset and makes the characterization more realistic than the Boolean slope partitioning.

The other covariate developed is referred to as the surface dynamic feedback patterns [22]. This variable describes how the land surface reflectance at a location changes over time. It is done by constructing a 3D surface describing the change of reflectance across spectral bands over time at a location using remote sensing techniques (Figure 2). It has been shown that the difference in reflectance surface between two points is related to the difference in soil conditions given that other environmental factors are the same [22]. Therefore, it has been effectively used to map spatial variation of soil particle composition over flat areas [23]. Recent developments in this area were able to relate reflectance to accumulative evaporation over time [24] and to relate to rainfall magnitude in an effort to correct the pattern for large area applications [25].

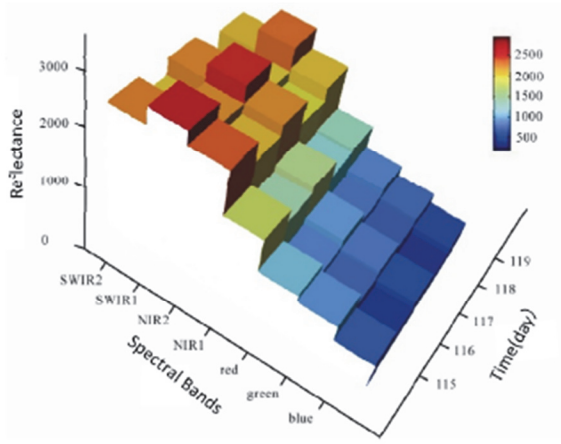

Fig. 2. Land surface dynamic feedback pattern of a location

4. SOFTWARE PLATFORMS AND OPERATIONS

The SoLIM approach has been made available to users through two distinct platforms. One is based on the desktop platform and the associated software is referred as SoLIMSolutions which is versioned by year. The other is based on the web platform which takes the advantages of the recent advancement in high performance computing and the cyber infrastructures. This platform is referred to as CyberSoLIM. Both of them are available through ***https://solim.geography.wisc.edu/software***. This section provides an operational overview of these two platforms by first presenting the overall design of the platform and then by outlining the steps for conducting digital soil mapping using SoLIM under different circumstances.

4.1. SoLIMSolutions

SoLIMSolutions comes in a zip file. No special installation procedure is needed to install except unzipping the zip file into a directory where you want SoLIMSolutions to reside. The package also contains the tutorial data in the directory named Tutorial_Data as well as the online help file (SoLIMSolutions_Help.chm). There are two sets of documents to assist users to use SoLIMSolutions for soil mapping. The first, refer to it as the "Functionality manual", is on the operation and functionality of the software which is contained in SoLIMSolutions_Help.chm and can be accessed through Help menu of the software. This manual is also available at the front webpage of SoLIMSolutions. The second document, referred to as the "Procedure manual", is on the detail procedures of DSM using SoLIMSolutions which is only available at the front page of SoLIMSolutions and it came with its own tutorial data sets. This document and the associated tutorial data were compiled for various workshops given on SoLIM. The entrance to SoLIMSolutions is SoLIMSolutions.exe which will lead to the interfaces shown in Figure 3. A comprehensive description of the functionality through the menus system shown in Figure 3 is given in the Help system. The steps to conduct DSM using SoLIMSolutions under major scenarios are described below.

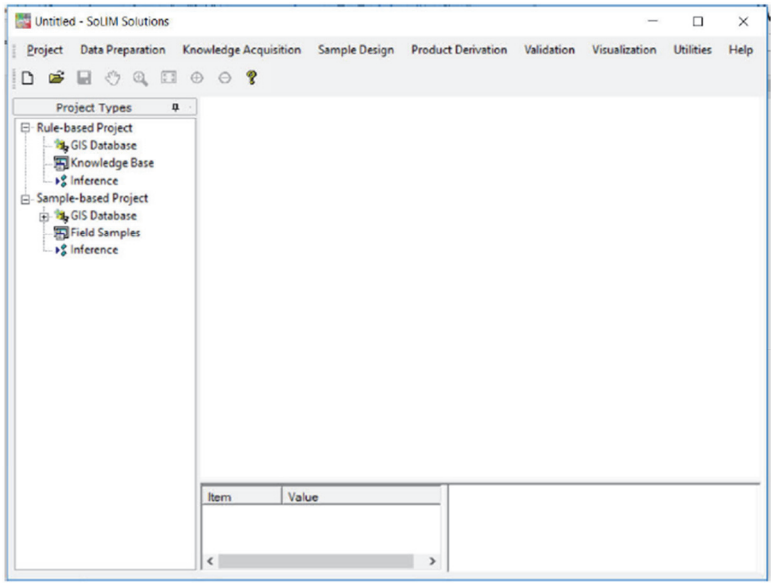

Fig. 3. Interface of SolIMSolution 2015

4.1.1. Digital soil mapping based on field samples

Under this scenario users will use field soil samples as prototypes. These samples may not be well distributed over the area and may be limited in number, which normally cannot be used with the DSM techniques based on the First Law of Geography or the statistical principle, but these samples can be used for soil mapping under the SoLIM approach due to the fact that SoLIM is based on the Third Law of Geography which does not require samples to be of certain size nor specific spatial distribution.

Step 1: Create a sample-based project

On the main menu of SoLIMSolutions, select "Project->New" to create a new project. Specify the project to be "sample_based".

Step 2: Add GIS data layers

Spatial data on environmental covariates are used to characterize the environmental configuration at each sample point. Therefore, spatial data on these set of covariates need to be loaded into SoLIMSolutions to characterize the configuration. In the left project panel, you will see five sub-nodes under the "GIS Database" node: "Climate Layers", "Parent Material Layers", "Topographic Layers", "Vegetation Layers" and "Other Layers". The environmental data layers can be loaded through these different sub-nodes. These sub-nodes are used to specify the hierarchy in the geographic configuration.

Step 3: Add the samples

Samples are the prototypes for digital soil mapping using the SoLIM approach. Each sample contains at least four pieces of information (Sample ID, X-coordinate, Y-Coordinate, Attribute). More than one attribute can be added for each point. The environmental configuration for each point does not need to be included in this sample point file because the environmental configuration can be easily defined once the location of the sample point is known and the spatial data on the covariates are loaded. The file containing the samples can be uploaded into SoLIMSolutions through the "Field Samples" node. It may be found that the panel on the right side will switch to a blank table correspondingly. Press the "Load Sample Point Table" button on the top to load the samples into this table.

Step 4: Run inference

Once both the spatial data on the covariates and the sample points are loaded, the environmental configuration for each sample as well as for any location in the study is constructed automatically in SoLIMSolutions. With the environmental configurations constructed, similarity in environmental configuration between each of the samples and any unsampled location can be computed. These similarities can then be used to predict the soil property value at the unsampled location by combining these similarities and the attribute values at the sample points involved using Equation 1. The "Inference" node will allow to perform the prediction.

Step 5: Result visualization

The results from the inference above can be viewed through the Visualization menu. The 2D tool is the in-house viewer in SoLIMSolutions but the 3D tool requires the installation of 3dMapper which is also available at the SoLIM software website.

Step 6: Validation

For validation, you need a set of independent validation samples which should be collected independently from the samples used as the prototypes. The validation samples are stored in a text file using the following format: SampleID, X-Coordinate, Y-Coordinate, SoilPropertyValue. The coordinate system used to define the locations of samples should be the same as that used for the spatial data as well as for the prototype samples. Validation is done through the "Property Validation" under the "Validation" menu.

4.1.2. Digital soil mapping based on knowledge from soil experts

The steps below describe the scenario when users only have local soil experts to provide the definition of the prototypes. The procedures given below are based on the assumption that users have obtained the knowledge from local experts on the prototypes. Figure 4 illustrates an example of such information. In this example, the knowledge on the prototypes of 4 soil classes is given. The environmental configuration for each of them was characterized by three environmental variables (Gradient, Elevation, and Profile curvature). The values for these environmental variables constitute the configuration. The soil *A*-horizon depth is the soil property.

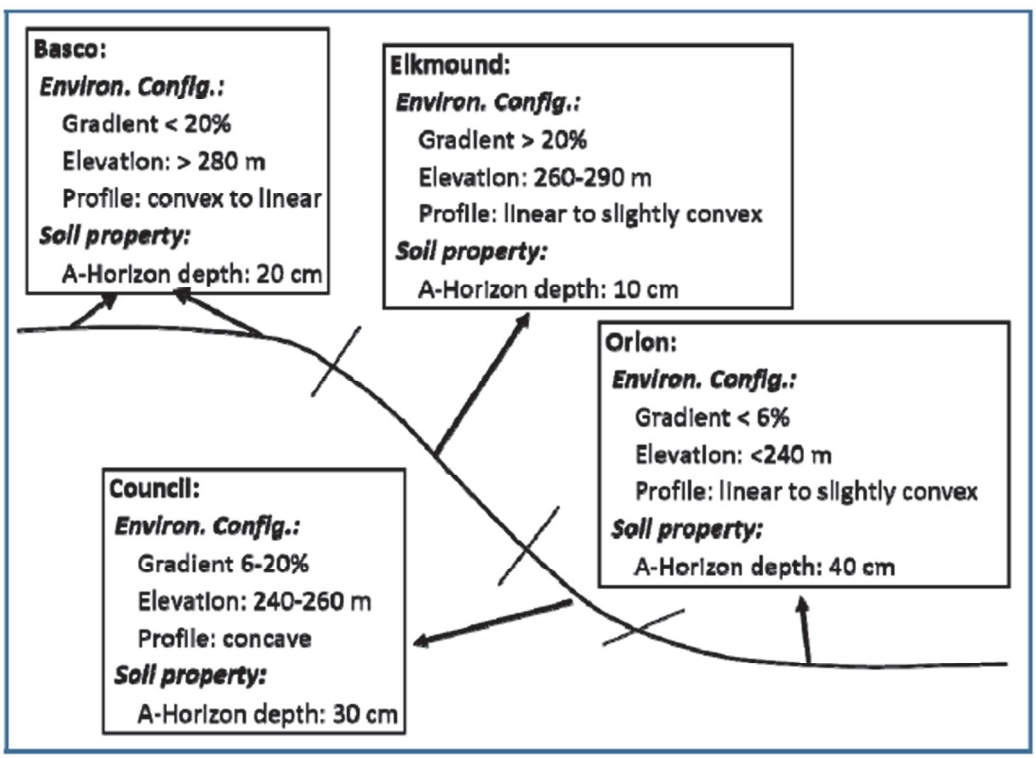

Fig. 4. Example of knowledge on the prototypes of soil classes

Below are the procedures for digital soil mapping based on this knowledge and the details of the steps are given in the Procedure manual.

Step 1: Create a rule-based project

To conduct DSM with SoLIMSolutions, you need to create a rule-based project due to the fact that the knowledge obtained on prototypes is described in the form of rules. Choose "Project -> new" on the main menu and specify the project type to be "rule-based".

Step 2: Create a GIS database for environmental configuration

Users now add the GIS data layers which are used to describe the environmental configurations. In this example, users need to add the GIS data layers on slope gradient, elevation, and profile curvature into the project. This is done by right clicking "GIS Database" under "Rule-based project" and select "Add Layer". Users can then add each of these GIS data layers into the project database.

Step 3: Define the prototypes for each soil class

For each class, users need to define the prototype representing this class using the knowledge extracted from local soil experts (such as these in Figure 4). This is done through **fuzzy membership curve** definition by right clicking on the "Knowledge Base" under "Rule based Project" and choose "Add Soil Type" and then for each soil type users define the typical environmental condition for this soil to develop under the given environmental variable, and the condition where the soil class will never develop and the value where the soil class will be halfway developed. Operational details of this task are provided in both the Functionality manual and the Procedure manual.

Step 4: Generate fuzzy membership maps of each soil class

Now users can compute the similarity of each location in the study area to the prototype of each soil class. The similarity is expressed as a fuzzy membership value. Fuzzy membership values to the prototype of a given soil class for all locations in the area make up a fuzzy membership map of that class. Generation of fuzzy membership maps is done through the "Inference" panel. Select the soil types to be inferred and specify where to save the result and the output format.

Step 5: Generate hardened soil map

If a soil class map is desired, users can achieve that through the "Hardened Map" hardening function under "Product Deviation" on the main menu. Add the fuzzy membership maps of the soil classes to be included in the soil class map and specify the output location. By hardening each location will be assigned a soil type to which the location has the maximum membership. Through this hardening process uncertainty maps associated with the creation of this hardened soil map are produced.

Step 6: Generate soil property map

Another product that can be derived from the fuzzy membership maps is soil property map. A look-up table that lists the typical soil property of each soil type should be prepared first. A weighted average approach as shown in Equation 1 is used to get the final soil property for each location. The "Property Map" function under the "Product Derivation" menu can be used to accomplish this task.

Step 7: Validation

Property map validation can be done using the step 6 in **4.1.1 Digital soil mapping based on field samples.** For validating the soil class map produced in Step 5 above, you also need a set of independent validation samples. The validation samples are stored in a text file in one of the predefined formats (see the Functionality Manual for details). Validation of soil class map is done through the "Type Validation" under the "Validation" menu.

4.1.3. Digital soil mapping based on knowledge from soil maps

Under this scenario, users are using knowledge from soil maps to define the prototypes for soil concepts (such as soil classes). The knowledge needed is characterized through a spatial data mining techniques [20]. Figure 5 illustrates the general process of mining knowledge for prototypes from existing soil maps. Due to the fact that the knowledge used to define prototypes are in the form of rules extracted from the soil maps, users need to set up the project as the "Rule-based Project" for this.

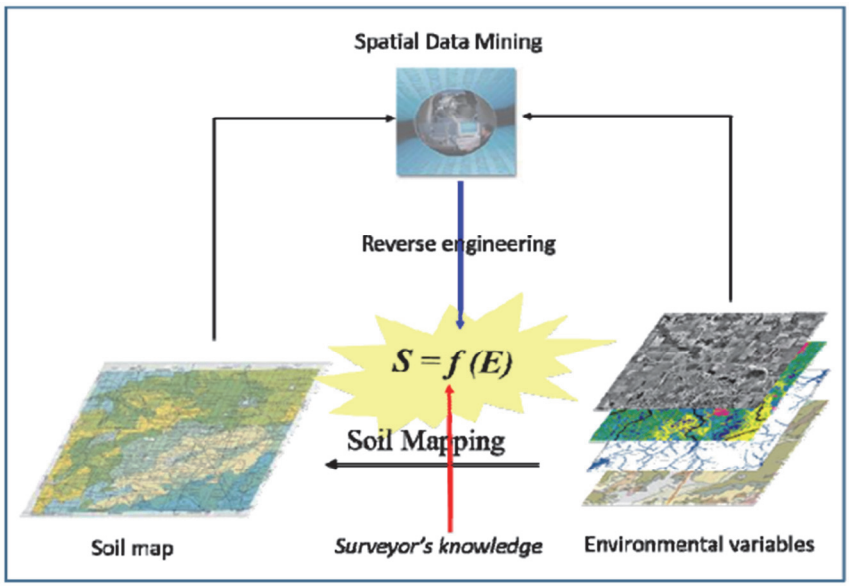

Fig. 5. Spatial data mining for knowledge defining the prototypes from existing soil maps

Step 1: Knowledge extraction from existing soil maps

The two elements needed for knowledge extraction from soil maps are: 1) a GIS database containing spatial data on environmental variables for defining the configuration; and 2) an existing soil map from which the knowledge on the prototypes of the mapped soil classes will be extracted. Once these are ready, select "Knowledge Acquisition->From Map" to start SoLIM-Knowledge Miner.

Step 2: Analysis of the extracted knowledge

The knowledge extracted from the existing maps may contain noise. The Knowledge-Miner in SoLIMSolutions allows the user(s) to increase the quality of knowledge by removing noises through knowledge analysis and editing. Knowledge analysis is

normally performed for every combination of map unit (soil class presented by polygon) and environmental data layer. Go to "Knowledge → Analyze ..." to start the knowledge analysis interface. When finished with editing, users can save the edits. Right click on the curve and choose "Save Knowledge Curve", the curve will be saved in a .txt file.

Step 3: Knowledge import into SoLIMSolutions for soil mapping

The generated curves from step 2 can be imported into SoLIMSolutions for soil mapping. The import can be accomplished during the definition of a new rule to associate a prototype with an environmental variable (**Step 3: Define the prototypes for each soil class** described in 4.1.2 **Digital soil mapping based on knowledge from soil experts**). In order to use the knowledge extracted in step 2 above, the type of the new rules needs to be "Freehand Rule". Click "Import From Data Mining Result" and specify the knowledge curve file (.txt file). The specified curve will be imported.

After all rules needed are added users can conduct inference soil type. The rest of steps are the same as in 4.1.2 (**Digital soil mapping based on knowledge from soil experts**).

4.2. CyberSoLIM

CyberSoLIM is another way to conduct DSM under the SoLIM framework and is a part of large framework, referred to as Easy Geographic Computing (EGC) contributed from the SoLIM group (Figure 6). It is a computing platform powered by intelligent geo-computing and high performance computing techniques. It provides a visual environment for easily constructing and executing DSM models for non-experts. The goal is to accomplish digital soil mapping tasks anywhere and anytime. CyberSoLIM provides a heuristically driven, visually assisted, high performance computing enabled cyber environment for digital soil mapping [26]. It exists in cyber space and can be accessed through the website stated above.

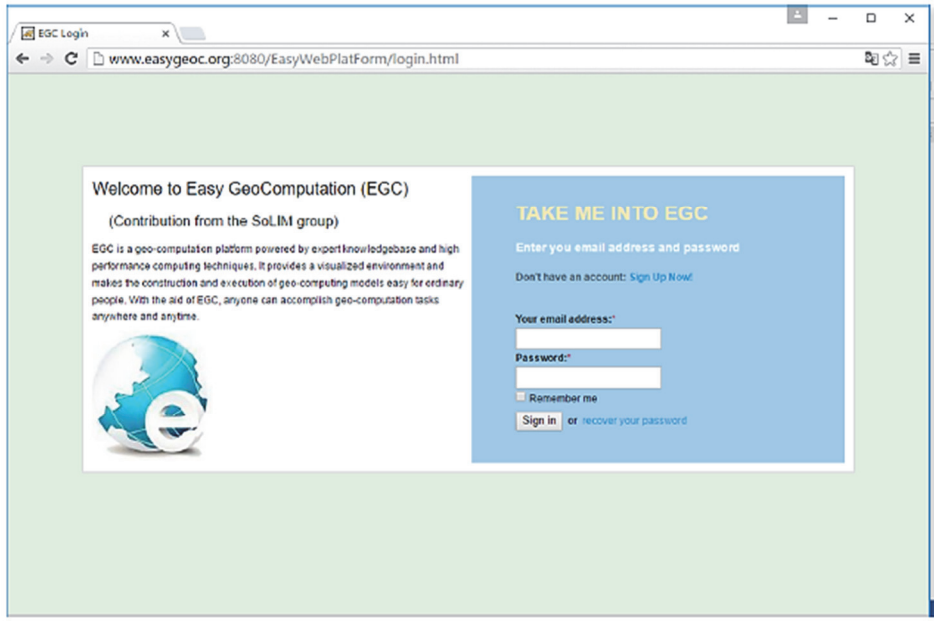

Fig. 6. CyberSoLIM through Easy Geographic Computing

At the time of this writing, CyberSoLIM is only capable of conducting DSM using the sample-based approach and is undergoing a major change in architecture and functionality. The description provided below is based on an earlier version of CyberSoLIM and includes data management, model construction, and model execution.

4.2.1. Data management

The current implementation of CyberSoLIM is for DSM under the sample-based approach using the SoLIM approach. Thus, we need both spatial data on environmental covariates and sample data. Due to the fact that it is based on cyber infrastructure, CyberSoLIM stores these data (environmental and samples) in cloud. The data management functionality of CyberSoLIM manages these data for users. Environmental data layers are required in "GeoTiff" format whose filename extension is .tif. The spatial reference (coordinate system) of all data should be consistent (the same). The samples locations should be in the same coordinate system as well and must be stored in .csv format. The easiest way to do this is to enter the field sample data into a spreadsheet and save it as a .csv file. In the table, there are at least three columns: X, Y and soil attributes and the file should contain a column heading so that it is clear which column is what.

The data a user uploaded to CyberSoLIM are under the control of the user through user account so the user can decide how the data are shared under CyberSoLIM. There are three basic modes for data sharing under CyberSoLIM. The most secure mode is that a user does not share any data with anyone. In this mode, the user is not able to access data shared by others except the data that are publically available. The next level is that a user shares the data with groups of the user's choice. In this case the user will be able to access the data these groups share within the group. The third level is that a user share data with anyone under CyberSoLIM. In this case any data that are shared by others publically will be available to this user. The level of sharing can be assigned to individual data set.

4.2.2. Model construction

One of the key striking features of CyberSoLIM is the intelligent and automatic model building of DSM work flow. With CyberSoLIM users are presented with a map of the world. A user can navigate to a study area of interest. Right clicking on the area for DSM will bring the user an interface similar to what shown in Figure 7. Once "Digital Soil Mapping" is selected, the user will be taken to the model construction view (Figure 8) where the basic soil mapping structure is presented through the connection of three ellipses and one rectangle. The ellipse labeled "Property Map" is for the user to define the output file for the resultant soil property map and the one labeled "Sample Data" is for the user to specify the file which contains the sample data set to be used.

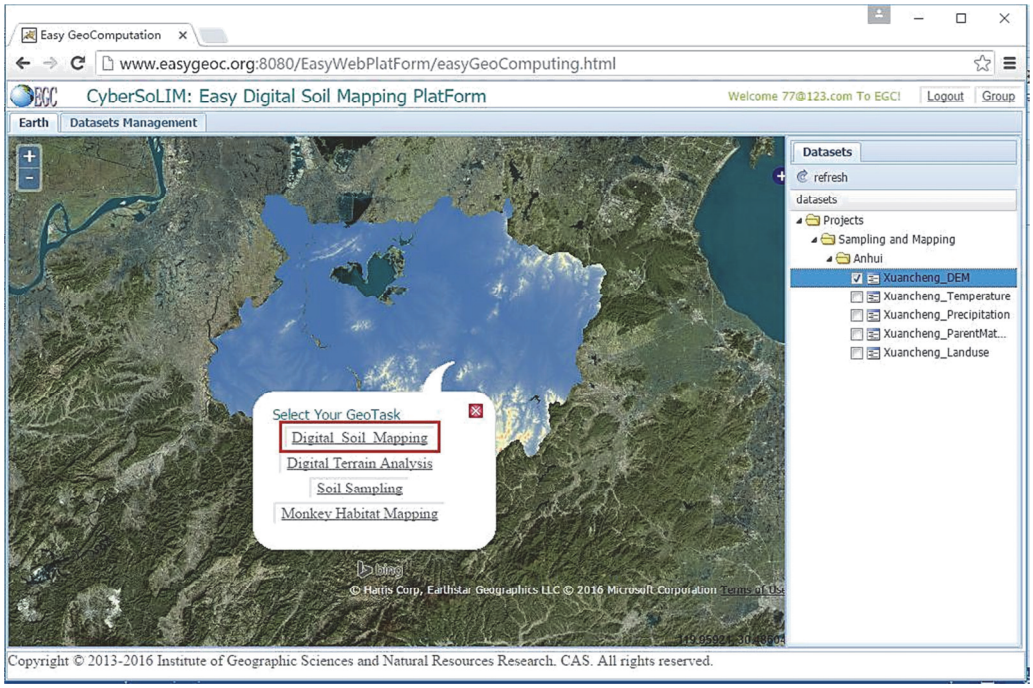

Fig. 7. Intelligent DSM model construction under CyberSoLIM

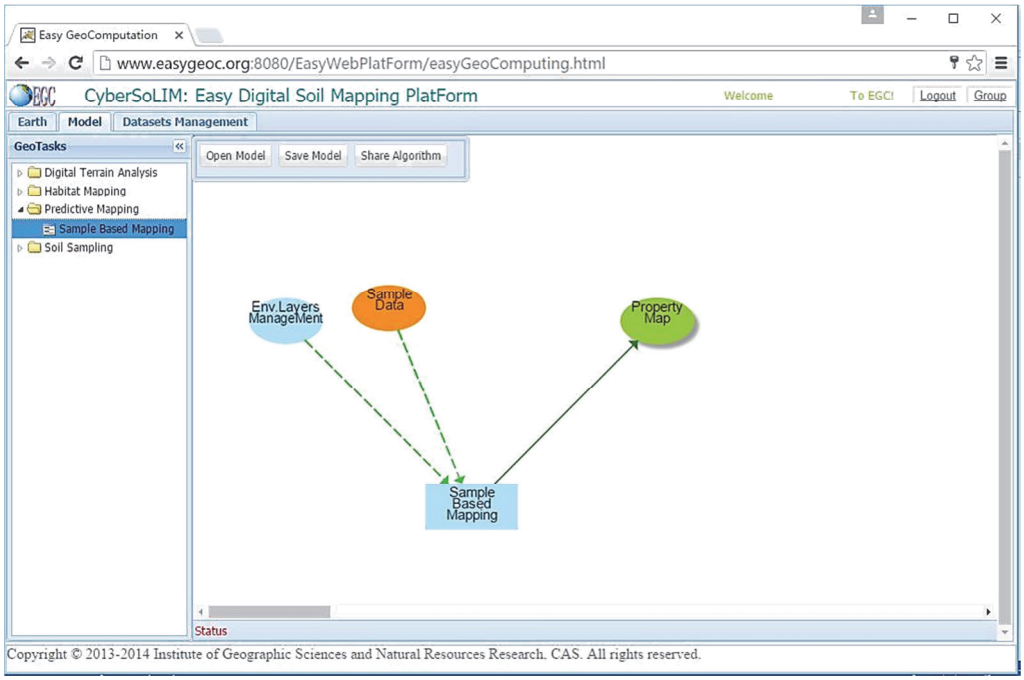

Fig. 8. The model construction view

The ellipse labeled with “Env. Layers Management” is for the user to define the environmental covariates to be used. Once the set of covariates is defined through this ellipse, the user will be presented with something as shown in Figure 9. Each of

the ellipses describes a covariate. The user can associate a data set to this covariate by right-clicking the ellipse, which will open a dialog box asking whether the user wants to provide a data set to it or the user wants CyberSoLIM to compute it. For variables such as TWI, slope gradient, profile curvature and planform curvature CyberSoLIM will be able to automatically compute them once the user specifies the gridded digital terrain model. For some of the covariates which use a common set of computing techniques, CyberSoLIM will automatically connect these techniques in a flow work (Figure 10). Once all of these covariates have been associated with a proper dataset, the model can be saved for later use.

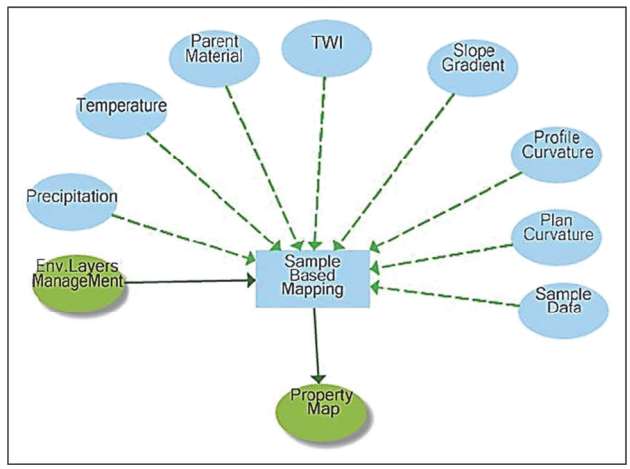

Fig. 9. Environmental covariates definition under CyberSoLIM (from [26])

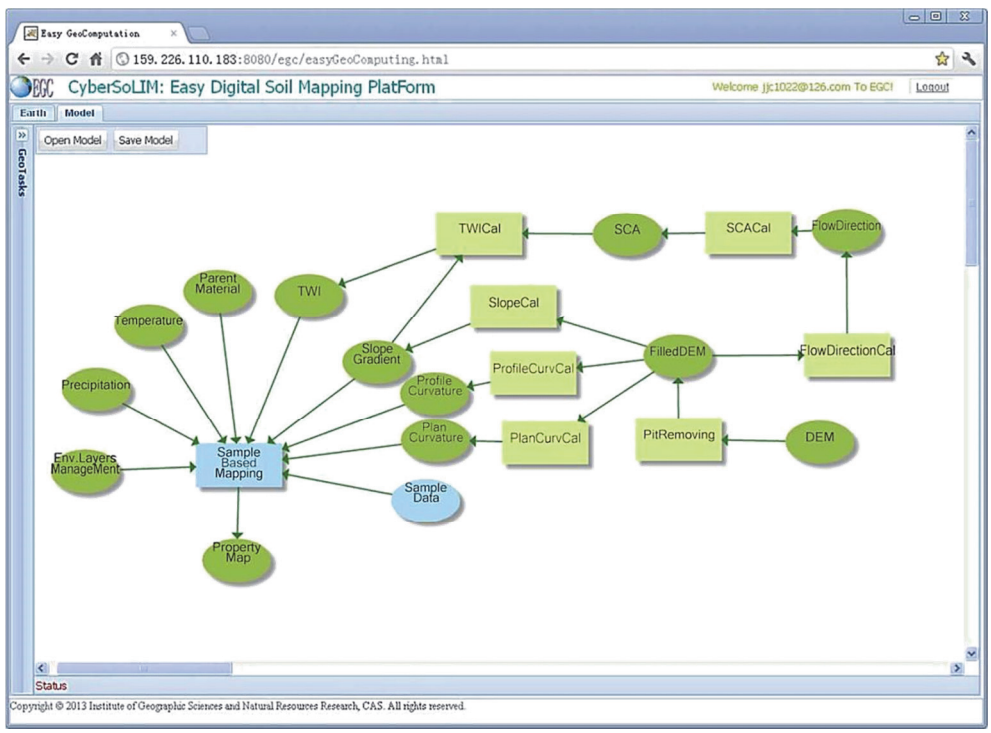

Fig. 10. A workflow model for DSM under CyberSoLIM

4.2.3. Model execution

Once all the environmental data and the soil sample data are set, the DSM model (workflow) has been constructed and is ready to be executed. The execution of the model is not done locally. In fact, the DSM model as captured in the work flow is sent to the high performance computer hosting CyberSoLIM and the work flow is then translated into executable web services and executed in the order specified in the work flow. This can be invoked by right-click on "Sample Based Mapping" and select run. The user can also click on "Operation Parameters" in "the Sample Based Mapping" box to adjust the parameters of the digital soil mapping model to customize this model. The result from the model will be presented through a web link once the execution is completed. It can be downloaded following this link and visualized in CyberSoLIM.

It is clear through this illustration, CyberSoLIM eliminates many tedious tasks for data preparation through intelligent model construction. GIS data preparation often becomes the bottle-neck for non-GIS specialists in their efforts in DSM. Under CyberSoLIM only a few source data layers (such as DEM, temperature, precipitation, remote sensing and geology) are needed, which dramatically reduce the burden on the users in data preparation. The other advantage is that computation is done using a high performance infrastructure which not only improve the speed of DSM but also removes the worry of maintaining computing hardware from users.

5. FUTURE RESEARCH ISSUES

The SoLIM approach is rather new, not only from the perspective of its methodological development but more importantly from the perspective of theoretical foundation (the application of the Third Law of Geography). Many research issues both in methodological and theoretical developments need further studying. We here highlight only a few which we think are key to the advancement of DSM under the Third Law of Geography and extend invitations to anyone who wants to collaborate to advance the research in these and other areas.

The first research issue is related to environmental configuration. As shown in Section 2.1, characterization of environmental configuration consists of three basic aspects. Aspect 1 is a comprehensive lists of environmental covariates which can effectively describe the environmental configuration for a given soil property. Although efforts are made in developing new variables [21, 24, 25], research efforts are needed in fuzzy landform characterization (such as plain, hills), dynamic vegetation growing conditions, creation of dynamic surface feedback patterns over large area.

Aspect 2 is the hierarchy of covariates for characterizing environmental configurations under the Third Law of Geography. There is little research on this. Research efforts are desired on questions such as following. What is the impact of hierarchy of covariates on environmental configuration characterization? How should the covariates be structured so that the characterization is more effective? Aspect 3 is the footprint for characterizing environmental configuration at a location for DSM [27]. Questions, such as: What is the footprint (neighborhood size) of environmental configuration for a given soil property? Is there a common footprint for all soil properties? — deserve more attention.

The second research issue is about sample verification. In SoLIM under the Third Law of Geography, the representativeness of a single sample is used. There is no doubt that this representativeness is extremely sensitive to the quality of this sample. The research question would be how to evaluate and increase the reliability of the soil samples under the Third Law of Geography and how this reliability impacts the quality of predicted soil map [28, 29].

The third research issue is knowledge extraction and integration for prototypes. Knowledge on environmental configurations about soil prototypes exist in various forms (paper maps, soil samples, survey reports etc.). Each of these forms has advantages and disadvantages as to the comprehensiveness and quality of the knowledge [30, 31]. Techniques are needed to extract knowledge from these various forms and integrate them into a holistic representation [30, 32].

The fourth research issue is the further development of CyberSoLIM. As it can be seen from above presentation, CyberSoLIM has two defining characteristics: the automatic construction of DSM work flow and the execution of the work flow using high performance computing. The automatic construction of work flow can drastically reduce the burden of users for knowledge on DSM work flow and the burden on conducting the analysis needed in the work flow. Sharing of analytical methods which can be used as web services are the bottleneck for systems like CyberSoLIM. The execution of DSM tasks on cyber platforms, particularly over platforms based on cloud infrastructure, demands new approaches to spatial data management and load management. Collaborative efforts in the deployment of cyber techniques in DSM are much needed.

6. CONCLUSIONS

This paper presents an overview of the SoLIM approach in light of the laws of geography and statistical principle used in DSM. The SoLIM approach was developed based on important geographic principle, now referred to as the Third Law of Geography. With SoLIM DSM does not require soil samples to be over certain size nor to be of specific spatial distribution. This dramatically reduces requirements for DSM.

The Third Law of Geography calls for the use of similarity in environmental (geographic) configuration between locations and uses this similarity for soil prediction. The SoLIM approach implemented the similarity of environmental configuration through the use of soil prototypes which can be defined by central concepts of soil classes or by field soil samples. This dramatically increases the sources available for defining soil prototypes, with the possibility of reducing the burden on collecting extensive new soil samples. The uncertainty derived based on similarities will also effectively target where the additional samples are needed to improve the quality of products efficiently.

The SoLIM approach are provided in two platforms: SoLIMSolutions and CyberSoLIM. SoLIMSolution is a desktop deployment and contains more comprehensive set of functionality for DSM while CyberSoLIM is a new effort to increase the availability as well as computational efficiency by using cyber infrastructures and intelligent computing techniques. All of these make SoLIM more applicable over large and complex geographic areas, easily available to people less savvy in geospatial analysis, and more efficient in the use of resources.

Acknowledgements

This paper would not be possible to be included as part of the special issue of *RUDN Journal of Agronomy and animal industries* without the kind supports from many individuals. Two of them I want to mention here. Professor Igor Savin first made me aware of the Autumn School on Smart Agriculture to be held in Moscow of Russia between September 24 and October 26, 2018. He then forwarded my interest in serving as a faculty lecturer at this School to the organization committee. Professor Timur Shiyapov, head of the office, Department for science and innovation policy of RUDN University, on behalf of steering committee of Smart Agriculture school invited me to participate. Due to my teaching commitments as well as the delay in getting a new passport I was unfortunately unable to participate to share and to learn at this Autumn School. Professor Timur Shiyapov was kind enough to invite me to write up the materials to be included in the special issue.

The work reported here was supported by grants from National Natural Science Foundation of China (Project No.: 41431177), National Basic Research Program of China (Project No.: 2015CB954102), PAPD, and Outstanding Innovation Team in Colleges and Universities in Jiangsu Province. Supports to A-Xing Zhu through the Vilas Associate Award, the Hammel Faculty Fellow Award, and the Manasse Chair Professorship from the University of Wisconsin-Madison are greatly appreciated. The materials presented here also greatly benefitted from the projects my colleagues and I worked on with the Natural Resources Conservation Services (NRCS), US Department of Agriculture. The early supports from NRCS are much appreciated.

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For citation:
Zhu AX, Qin CZ, Liang P, Du F. Digital soil mapping for smart agriculture: the SOLIM method and software platforms. *RUDN Journal of Agronomy and Animal Industries*, 2018, 13 (4), 317—335. doi: 10.22363/2312-797X-2018-13-4-317-335.

DOI: 10.22363/2312-797X-2018-13-4-317-335

ЦИФРОВОЕ КАРТИРОВАНИЕ ПОЧВ ДЛЯ ИННОВАЦИОННОГО СЕЛЬСКОГО ХОЗЯЙСТВА: SOLIM МЕТОД И ПЛАТФОРМЫ ПРОГРАММНОГО ОБЕСПЕЧЕНИЯ

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Ключевыми проблемами, с которыми сталкиваются многие из существующих подходов цифрового картографирования почв (DSM), являются жесткие требования к размеру проб почвы, необходимой для построения соответствующих взаимосвязей и обеспечения их устойчивости. Эти требования ограничивают применение методов DSM. В статье представлен обзор подхода SoLIM и специфика его использования через доступные программные платформы. SoLIM базируется на Третьем законе географии, который требует сравнения сходства в географическом (экологическом) положении прототипа и места без выборки, а использования этого сходства для прогнозирования значения свойства почвы в заданном месте. Реализация цифрового картографирования почв в рамках подхода SoLIM устраняет требование к размеру выборки и устойчивости взаимосвязей. Кроме того, неопределенность, рассчитанная на основе сходства, может быть использована для повышения эффективности усилий по уменьшению размера ошибок. Подход SoLIM реализован на двух платформах: SoLIM Solutions и CyberSoLIM. Теоретическое обоснование и наличие программных платформ для SoLIM открывают возможности для использования DSM в больших и сложных географических регионах.

Ключевые слова: цифровое почвенное картирование, DSM, SoLIM, Первый закон географии, Второй закон географии, Третий закон географии, пространственное прогнозирование

RUDN Journal of Agronomy and Animal Industries 2018 Vol. 13 No. 4 336—343
Вестник РУДН. Серия: АГРОНОМИЯ И ЖИВОТНОВОДСТВО http://journals.rudn.ru/agronomy

DOI: 10.22363/2312-797X-2018-13-4-336-343

ВОЗМОЖНОСТИ ИСПОЛЬЗОВАНИЯ СПУТНИКОВЫХ ДАННЫХ ПРИ СЕЛЬСКОХОЗЯЙСТВЕННОМ СТРАХОВАНИИ

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Проведен анализ возможности использования спутниковых данных для страхования посевов. Установлены особенности и основные направления использования спутниковых данных при сельскохозяйственном страховании. Показано, что спутниковые данные могут быть использованы для мониторинга состояния культур, оценки страховых рисков, оценки потерь урожая, а также для контроля состояния пастбищной растительности. Отмечена перспективность использования при страховании посевов наряду с космическими изображениями данных, получаемых с беспилотных летательных аппаратов.

Ключевые слова: спутниковые данные, мониторинг земель, состояние посевов, страхование урожая, БПЛА

Страховая индустрия представляет собой крупный рынок в размере 4,3 трлн долларов США, или 6,4 процента от мирового ВВП [1], в то время как на мировых рынках сельскохозяйственного страхования имеется коллективный страховой фонд в размере 12,5 млрд евро [2]. Из-за размера этих рынков страхование может стать важной областью применения для дистанционного зондирования.

Первые попытки использования данных дистанционного зондирования (ДДЗ) для целей страхования появились в 1975 году, когда Towery описал потенциал аэрофотосъемки и дистанционного зондирования в оценке ущерба для урожая от града [3, 4]. В литературе по дистанционному зондированию содержится множество других примеров, в которых демонстрируются возможности использования ДДЗ, например, при оценке ущерба от пожара [5], града [6] и засухи [7].

Но до сих пор ДДЗ так и не стали надежным орудием в сельскохозяйственном страховании. Одной из причин этого является опосредованность индикации ущерба по ДДЗ, а также отсутствие достаточно длинных временных рядов данных с высокой периодичностью съемки.

Как известно, в сельском хозяйстве существует два типа страхования: классическое, когда страхуется реальных ущерб, а также индексное, при котором страховой случай наступает при достижении выбранным показателем некой фиксированной величины (например, количества атмосферных осадков или критической температуры воздуха), независимо от реальных потерь. В обоих случаях на всех основных этапах страхования использование ДДЗ потенциально возможно.

Так, при определении страхового риска в большинстве случаев реальных данных недостаточно, поэтому использование ДДЗ потенциально должно являться достаточно эффективным [8]. Например, в регионах, где нет постоянного мониторинга засух, оценить их риск можно по данным многолетнего архива ДДЗ [9]. Но при этом существуют некоторые неопределенности с точностью информации, получаемой по ДДЗ [10], и, кроме того, оценки рисков, получаемые по ДДЗ, должны быть как-то пересчитаны на экономические потери. Несмотря на это, имеются публикации, демонстрирующие возможности использования ДДЗ для оценки рисков воздействия на посевы наводнений [11, 12].

Достаточно обширная научная литература существует в области использования ДДЗ для оценки непосредственно ущерба в результате гибели посевов от засухи, пожаров, града, морозов, болезней и вредителей. Аналогичным образом существует богатая литература, описывающая применение дистанционного зондирования для оценки ущерба, нанесенного строениям и инфраструктуре. Но действующей оперативной технологии так до сих пор и не разработано. Так, например, сообщаются противоположные выводы относительно возможности использования ДДЗ для поддержки страховой отрасли в оценке ущерба от града. Petersetal [13] показал пригодность многоспектральных изображений Landsat TM для оценки влияния искусственно вызванного повреждения града в кукурузе и сое. Напротив, более грубое разрешение спутниковых данных MERIS не дало надежной оценки площади повреждения урожая, основанного на снимках, полученных за несколько дней до и после града в Альберте, Канада [14].

Apan et al. [15] отмечают, что, несмотря на то, что ДДЗ обладает потенциалом для выделения участков с измененной фитомассой посевов, по-прежнему трудно отнести такие потери к ущербу, поскольку наблюдаемое изменение может быть вызвано и другими факторами, не связанными со страховым случаем. Однако в отдельных регионах подобные оценки вполне возможны (рис. 1).

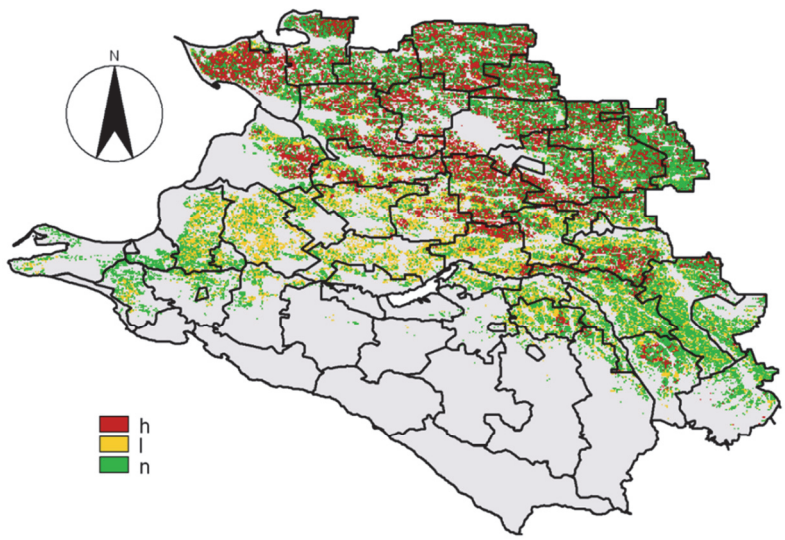

Рис. 1. Пример спутниковой оценки посевов, пострадавших от весенних заморозков в Краснодарском крае в 2009 году:

h — уничтоженные посевы, l — пострадавшие посевы, n — не пострадавшие посевы

Необходимо отметить, что успешность использования ДДЗ сильно зависит от типа используемых данных: их пространственного разрешения, периодичности съемки, типа съемки, а также от экранирующего влияния облачности. Так, без сомнения, чем выше пространственное разрешение изображений, тем точнее можно детектировать изменение состояния посевов. Но для оценки воздействия неблагоприятных факторов важно иметь изображения, полученные непосредственно до и после события (рис. 2).

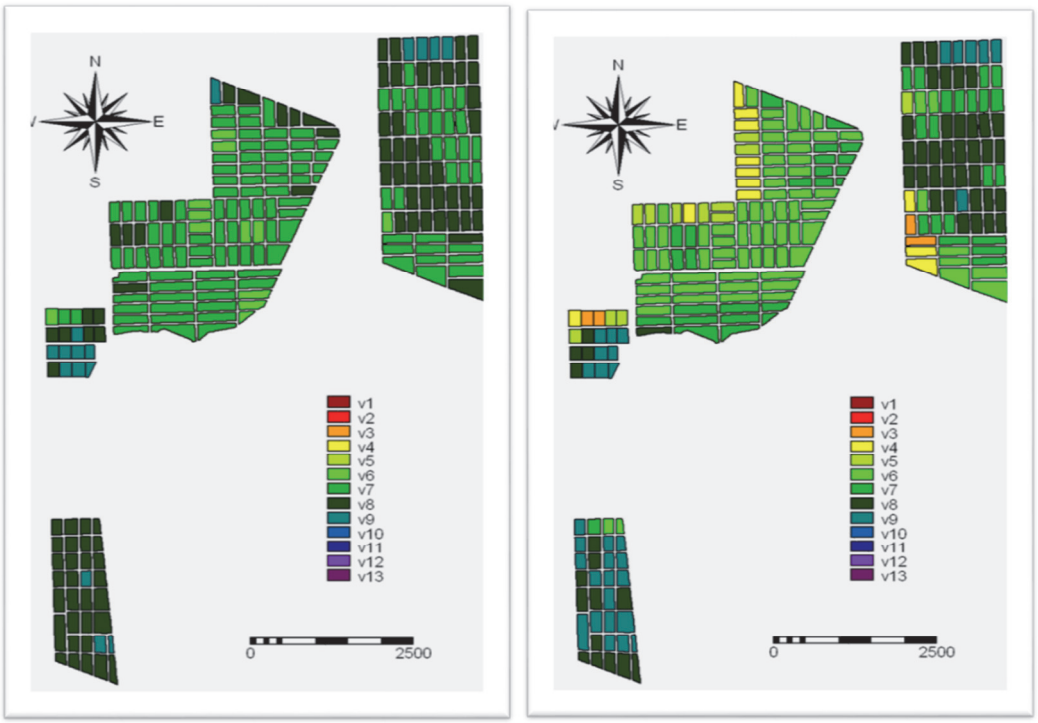

Рис. 2. Спутниковая оценка изменения фитомассы посевов риса до (слева) и после (справа) урагана в 2010 году (Краснодарский край) (чем выше число в легенда — тем больше надземная фитомасса)

И это накладывает очень большие ограничения. Так, наиболее доступные и распространенные снимки Landsat имеют периодичность съемки 15 дней, что делает их малопригодными для этих целей. Более пригодны изображения, получаемые со спутников RapidEyeAG (частота съемки — раз в 2—3 дня с пространственным разрешением около 3—5 метров) [16], WorldView-2 и Pleiades (многоканальные, высокого разрешения и такой же частотой съемки). Для оценки ущерба на отдельных полях лучше, чем спутниковые изображения, можно использовать данные, получаемые с беспилотных летательных аппаратов [17].

При индексном страховании ДДЗ позволяют получать оценки состояния растительности сельскохозяйственных угодий с различным пространственным разрешением и достаточно часто. Благодаря этим преимуществам неудивительно, что индексы дистанционного зондирования нашли свой путь в индексном страховании. Одним из примеров применения индексов дистанционного зондирования

является страхование, которое покрывает риск нехватки корма на пастбищных угодьях. Сильные корреляции NDVI и продуктивности были продемонстрированы для пастбищ Центральной Австралии [18], Северной Америки [19], Ближнего Востока [20] и Африки [21—23].

Агентство по управлению рисками Министерства сельского хозяйства США (RMA) предлагает страхование пастбищ и пастбищных угодий, основанное на временных рядах осадков и NDVI [24]. Страхование предназначено для защиты фермеров от сокращения кормов. Выплаты возмещения определяются на основе отклонения от нормы комбинированного показателя NDVI и количества осадков.

В Кении частными страховыми компаниями предлагается схема страхования для животноводов, основанная на специальных индексах IBLI, которые основаны на сезонных и пространственно-агрегированных данных NDVI, полученных со спутника MODIS [25]. Страхование предлагается два раза в год, за месяц до наступления сезона дождей (март—май) и во время кратковременных дождей (октябрь—декабрь). Выплата производится, когда совокупный индекс NDVI падает ниже порогового значения, соответствующего прогнозируемой смертности домашнего скота в 15%.

До сих пор не создано ни одной системы индексного спутникового страхования для посевов сельскохозяйственных культур, но разработки в этом направлении активно ведутся в нескольких странах (в Индии [26], в Казахстане [27], в Сирии [28]). Pantakar et al. [29] описали пилотное исследование в штатах Чхаттисгарх и Андхра-Прадеш в Индии, где был разработан и предложен фермерам комплексный индекс страхового продукта на основе NDVI и осадков.

Анализ связи между индексом осадков и индексом NDVI с данными по урожайности кукурузы и хлопчатника из девяти районов Зимбабве [30] позволил сделать вывод о том, что NDVI был лучшим показателем, поскольку он выявил более высокие корреляции с урожаем, чем показатель осадков. C.G. Turvey и M.K. McLaurin [31] исследовали потенциал NDVI как показателя для индексного страхования урожая. Они сообщили, что взаимосвязь между NDVI, осадками, экстремальным теплом и урожайностью очень варьирует и зависит от местоположения, и сделали вывод о том, что применение NDVI для индексации конструкции страховой продукции требует калибровки по месту.

Кроме использования спутникового индекса NDVI для индексного страхования, исследователи отмечают большой потенциал спутниковых индексов осадков [32]. L. Johnson [33] описывает, что автоматизированные метеорологические станции, составляющие основу страхования погодных индексов в Кении и Уганде, слишком дорогостоящие для содержания, и именно по этой причине были начаты исследования по расследованию возможности оценки количества атмосферных осадков со спутника. Поскольку осадки имеют высокую пространственную изменчивость, особенно для коротких временных масштабов, для создания эффективного пространственного представления дождя необходимы достаточно плотные сети метеостанций. Когда такие сети не существуют или не обеспечивают надежную доставку данных, спутниковые данные могут частично восполнить этот пробел. Они состоят из тепловизионных наблюдений, в основном

с геостационарных спутников, а также активных и пассивных СВЧ наблюдений с полярно-орбитальных спутников. Существует много спутниковых продуктов, которые объединяют комбинирование данных об осадках из различных ДДЗ [34].

Все вышеупомянутые примеры индексационного страхования с ДДЗ предоставляют продукты микрострахования, ориентированные на конкретные домохозяйства. В последнее время растет интерес к страховым продуктам на мезо- и макроуровне. Эти продукты не нацелены на отдельных фермеров. Они нацелены на агентства по оказанию помощи и региональные и национальные правительства, которые заинтересованы в финансовой поддержке фермеров, пострадавших от стихийных бедствий. Например, в Аргентине и Уругвае были проведены технико-экономические исследования мезострахования, основанные на показателях отслеживания индексов NDVI для пастбищ [35, 36], предназначенных для страхования потери домашнего скота. Это мезострахование, если оно будет реализовано, позволит правительству своевременно выплачивать средства на поддержку владельцев скота для поддержания их стад в случае сильной засухи.

Потенциально индексное спутниковое страхование может также применяться и к многолетним культурам. Например, Lou et al. [37] описывают применение теплового дистанционного зондирования при контроле за воздействием заморозков на плантации чая.

Несмотря на большие перспективы спутникового индексного страхования, существует ряд технических проблем.

Первая — это непрерывность данных нужного пространственного разрешения и временной периодичности получения, что обеспечить в настоящее время достаточно затратно.

Вторая проблема связана с качеством данных, их сильной зашумленностью, влиянием облачности и состоянием атмосферы. Эта проблема в настоящее время достаточно успешно решается [38].

Еще одна проблема связана с выбором индекса, который бы показывал высокую корреляцию с реальными потерями, понесенными землевладельцами.

Спутниковые данные имеют большой потенциал для использования в сельскохозяйственном страховании. Они позволяют оценивать риски воздействия того или иного неблагоприятного фактора, оценивать потери урожая, а также осуществлять оперативный мониторинг состояния посевов. Для небольших участков более эффективным является использование данных, получаемых с беспилотных летательных аппаратов.

Наиболее эффективным является использование спутниковых данных при индексном страховании посевов и пастбищной растительности.

Практическая реализация имеющегося потенциала затруднена как техническими возможностями получения данных в нужное время и нужного качества, так и их все еще высокой стоимостью.

Перспективным направлением дальнейших исследований является поиск новых спутниковых индексов, которые наиболее полно бы отражали состояние посевов и пастбищ.

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DOI: 10.22363/2312-797X-2018-13-4-336-343

POSSIBILITIES OF SATELLITE DATA USAGE IN AGRICLUTURAL INSURANCES

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Abstract. Analysis of usage of satellite data in agricultural insurance was conducted. Main peculiarities and ways of potential usage were listed. It was highlighted that satellite data can be successfully used for crop monitoring, risks and damages assessment, as well as for pastures monitoring. The perspectives of usage of UAV images instead of satellite data for small areas were noted.

Keywords: satellite data, land monitoring, crop status monitoring, crop insurance, UAV

RUDN Journal of Agronomy and Animal Industries 2018 Vol. 13 No. 4 344—352
Вестник РУДН. Серия: АГРОНОМИЯ И ЖИВОТНОВОДСТВО http://journals.rudn.ru/agronomy

DOI: 10.22363/2312-797X-2018-13-4-344-352

IMPLEMENTATION OF MUNICIPAL LAND CONTROL IN VOLGOGRAD

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Abstract. Identifying the main land legislation violations in Volgograd is a prerequisite for the preservation and rational use of the territory. The measures system development aimed at improving the effectiveness and efficiency of the municipal land control implementation will contribute to the city district main wealth preservation. The article presents the activities of specially authorized organizations and bodies for the land legislation observance. The average workload per 1 specialist in municipal land control carrying out amounted to 1.4 of inspections carried out for legal entities. The sequence of actions during the scheduled and out-of-scheduled inspections is given here. The report on the municipal land control carrying out in Volgograd for 2016—2017 is submitted. According to the land control results in 2016, from the total number of carried out inspections, 16.5% of them were revealed with violations, and in 2017 — 25.7%. Amount of the prescribed fines in 2017 increased by more than twice in relation to 2016. Violations of land legislation in Volgograd occurred on the unauthorized occupation of land plots and their parts, the failure to comply with legal regulations on the violations elimination, the use of land not in accordance with its permitted use. Municipal land control was carried out in compliance with the general principles, duties, restrictions and prohibitions in carrying out control measures, as well as the requirements for processing the results of inspections. On the basis of the material reviewed, suggestions were made to improve the effectiveness and efficiency of the municipal and state land control implementation.

Key words: municipal land control, scheduled inspections, out-of-scheduled inspections, inspector, violations of land legislation

INTRODUCTION

In Russia, ensuring land protection and its rational use is the main goal in the field of land transformations. A necessary tool for performing tasks is land control over the protection and use of land at various structural levels. For violations in the field of land relations, a special role is assigned to administrative responsibility, which is an indicator of the state's reaction to the land legislation violators' actions. The essence of this responsibility lies in the application of administrative measures established and enshrined in the land legislation, to the guilty subject in violation of legal norms regarding the protection and land use, as well as the land users, landholders and landowners rights.

The department of municipal land control of the Volgograd administration municipal property department carries out municipal land control in Volgograd.

There are 8 officials (inspectors) in this department who are responsible for carrying out inspections in the Volgograd administrative districts. Accordingly, each inspector is assigned a specific area for the land control implementation at the municipal level [1].

The goal of the research is to consider and analyze the process of municipal land control implementation in Volgograd.

MATERIALS AND METHODS

The land control implementation is divided into several stages, which are the administrative procedures presented in Figure 1.

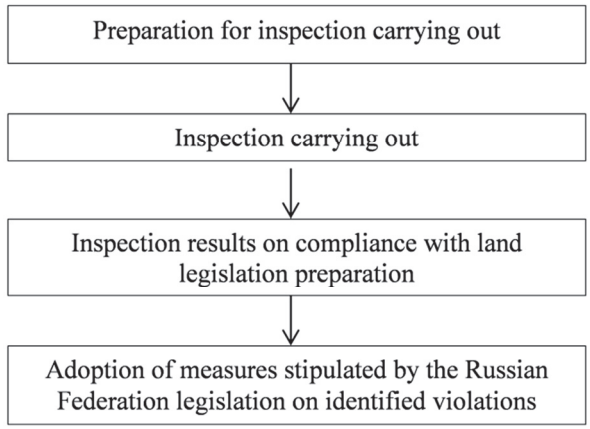

Fig. 1. Scheme of administrative procedures

In order to implement land control, scheduled and out-of-scheduled inspections are carried out [2]. In Figure 2 we consider in details the organization and rules for carrying out scheduled inspections.

When carrying out planned inspections, they observe the land legislation in respect of legal entities and individual entrepreneurs and carry them out no more often than once every three years.

Scheduled inspections are carried out in accordance with the annual inspection plan.

The regular scheduled inspections inclusion in the annual plan for inspections of legal entities and individual entrepreneurs is carried out in connection with the expiration of 3 years from the day of:

1) the legal entity or an individual entrepreneur state registration, or the start of a legal activity;

2) the end of the last scheduled inspection.

In due time, the Federal Service for State Registration, Cadaster and Cartography and its territorial bodies send annual plans drafts to the prosecutor's office. The prosecutor's office considers and makes proposals to eliminate the detected comments, and also makes additional proposals for carrying out joint inspections with the land control authorities.

The approved inspection plan is communicated to concerned parties by posting it on official websites or by other available means.

The total duration of the inspection (from the date of commencement to the date of drawing up the inspection report) may not exceed 20 working days. In relation to one small business entity, the total duration of a scheduled inspection cannot exceed 50 hours for a small enterprise and 15 hours for a micro-enterprise per year.

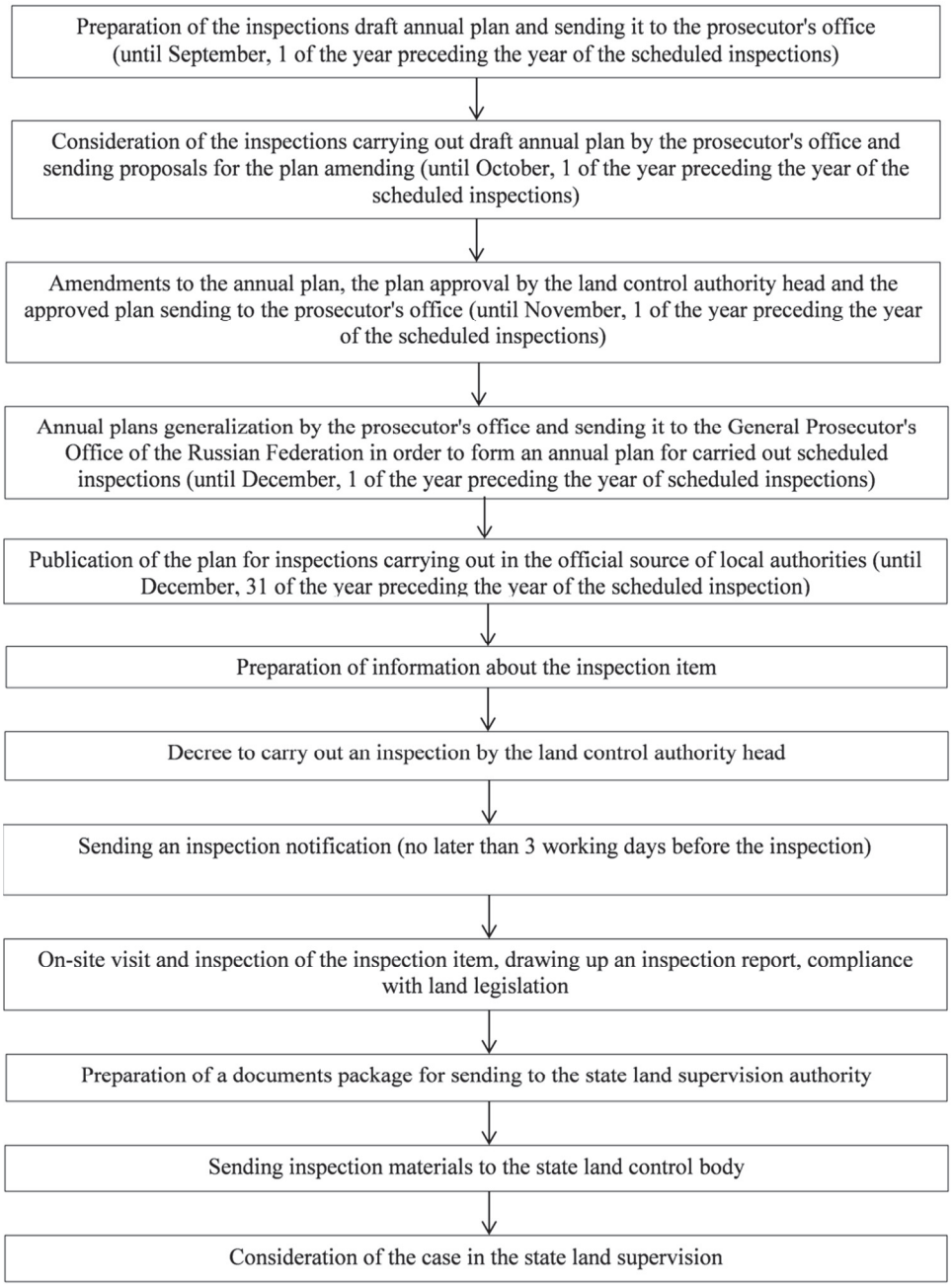

Fig. 2. The sequence of administrative procedures at implementation municipal land control when carrying out planned inspection

In exceptional cases, related to the need for a complex or lengthy research, testing, special investigations and examinations on the basis of proposals from the Federal Service for State Registration, Cadaster and Cartography officials carrying out a scheduled inspection, the period of an on-site scheduled inspection can be extended by the Chief Inspector, but not more than 20 working days for a small business, and not more than 15 hours for a micro-enterprise.

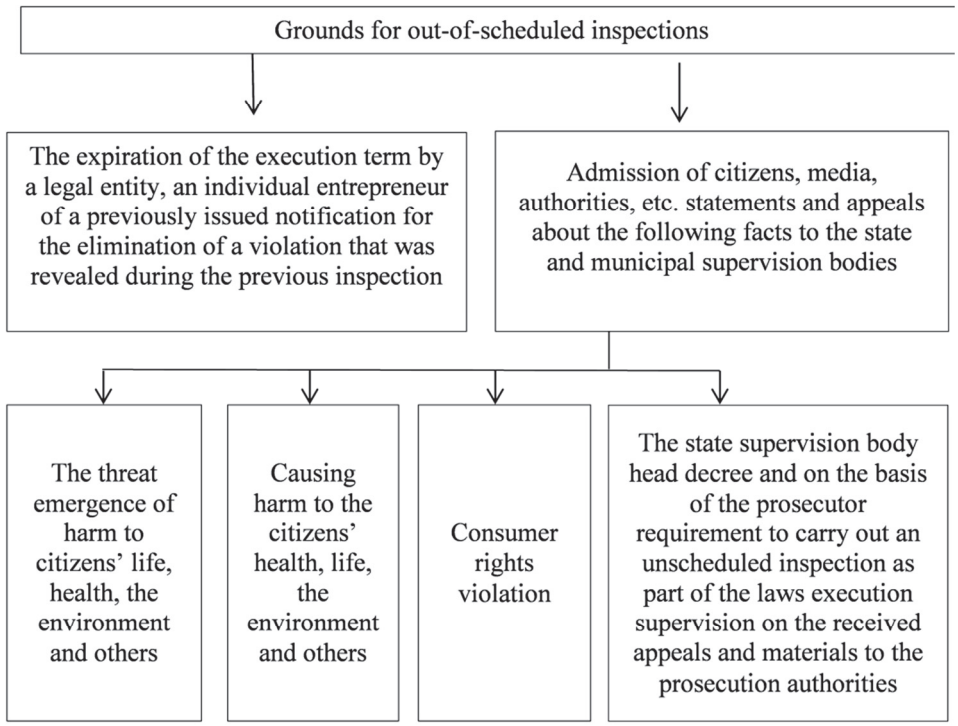

Fig. 3. Grounds for out-of-scheduled inspections carrying out

The inspected person is informed about the extension of the inspection period in writing, as well as by telephone or facsimile, e-mail no later than the day following the date of signing.

Let us turn to the organization of an out-of-scheduled inspection in Figure 3.

The subject of an out-of-scheduled inspection is compliance with the mandatory requirements in the activities implementation, compliance with the requirements of Federal Service for State Registration, Cadaster and Cartography, measures to prevent harm to citizens' life and health, the environment, etc.

Applications and appeals that do not allow to identify a person who has applied to the body of state or municipal supervision, as well as appeals and statements that do not contain information about the facts, cannot serve as the basis for an out-of-scheduled inspection carrying out.

An out-of-scheduled inspection is carried out in the form of a documentary inspection or an on-site inspection.

The procedure for the approval of the state and municipal supervision with the prosecutor's office of an out-of-scheduled on-site inspection is established by the Russian Federation Prosecutor General order.

In Figure 4, we consider a phased procedure for out-of-scheduled inspections carrying out and documents preparation.

On the day of prescription, the decree to carry out an out-of-scheduled on-site inspection is sent by registered mail with a statement of receipt or in the form of an electronic document signed by an enhanced qualified electronic signature on the application for an out-of-scheduled on-site inspection approval.

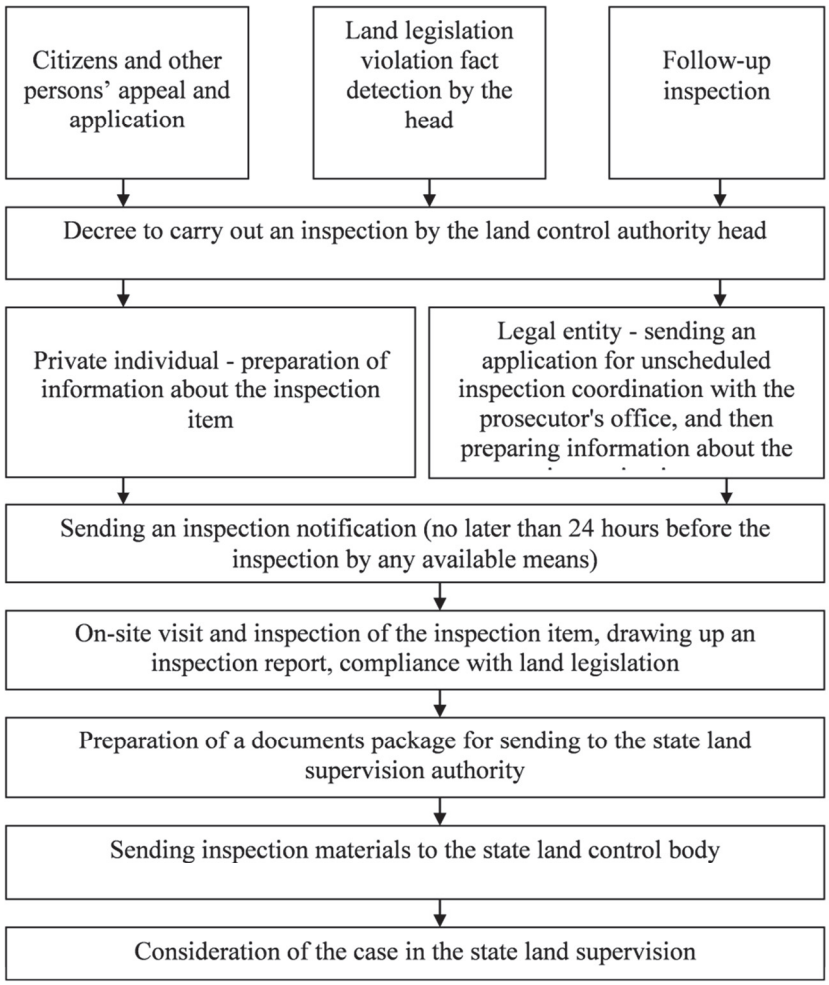

Fig. 4. The sequence of administrative procedures at municipal land control implementation when out-of-scheduled inspection carrying out

Now let us consider the bodies and organizations that exercise control over the land use.

Specialists and officials carry out state land control in accordance with the prescribed procedure in the resolution approving the on state land supervision provision [4]. Specially authorized bodies are the Federal Service for State Registration, Cadaster and Cartography, the Federal Service for the Supervision of Natural Resources and the Federal Service for Veterinary and Phytosanitary Supervision.

Officials and specialists in accordance with the legislation of the Russian Federation and the adopted regulatory and legal acts of local self-government bodies carry out municipal land control.

The specially authorized body exercising municipal land control in Volgograd is the Department of Municipal Property of the Administration of Volgograd. The main regulations for this control type implementation in Volgograd is the decision of the city administration on the administrative regulations for the implementation of municipal land control [5].

The average workload per 1 specialist in municipal land control carrying out amounted to 1.4 of inspections carried out for legal entities, individual entrepreneurs per year (including: in the first half of the year — 0.8 of inspections for a specialist, in the second half-year — 0.6 of inspections for 1 specialist).

In 2016, in Volgograd, municipal land control was carried out in respect of 1 legal entity and 96 citizens. It was not possible to carry out scheduled inspections of 46 citizens for reasons of the ownership change, lack of attendance and the impossibility of notifying the inspected ones about the forthcoming inspection (Table 1). In 2017, the department specialists carried out: 88 scheduled inspections, out-of-scheduled inspections in relation to individuals; participated in 39 inspections carried out in relation to economic entities; 4911 surveys, land surveys for their actual use.

Table 1

Report on municipal land control carrying out

	Inspections (all on-site inspections)			Inspections' results					Failed	Imposed fines, units/rub.
	total	scheduled	out-of-scheduled	without violations	With violations (AOC RF)					
					total	art. 7.1	part 1 art. 8.8	part 1 art. 19.5	part 1 art. 19.4.1	
2016										
Legal entities	1	—	1	1	—	—	—	—	—	—
Citizens	96	67	29	74	22	13	3	6	6	18/76 700
Total	97	67	30	75	16	13	3	6	6	18/76 700
2017										
Legal entities	13	12	1	10	3	2	—	1	—	1/100 000
Citizens	88	64	24	65	23	17	1	5	6	22/77 900
Total	101	76	25	75	26	19	1	6	6	23/177 900

According to the inspections plan for 2017, 76 on-site inspections were approved, of which 12 are against legal entities and 64 are against citizens. In addition, the Volgograd municipal land control department specialists carried out 24 out-of-scheduled inspections of citizens and 1 out-of-scheduled inspection of a legal entity, the data are presented in table 1.

In 2016, following the results of the inspections included in the plan for this year, 22 violations of land legislation by citizens were revealed, most of which unauthorized occupied the land plot or its part. In 2016, 13 citizens were brought to administrative responsibility, and fines totaling 76700 rubles were imposed.

In 2017, following the results of the municipal land control in 2017, violations were revealed in relation to 3 legal entities and 23 citizens, 177 900 rubles were imposed for the violation of the land legislation.

Violations of land legislation in Volgograd occurred on the unauthorized occupation of land plots and their parts, the failure to comply with legal regulations on the elimination of violations, the use of land not in accordance with its permitted use.

CONCLUSIONS

Municipal land control is carried out in compliance with the general principles, duties, restrictions and prohibitions in carrying out control measures, as well as the requirements for processing the results of inspections.

In the period from 2016 to 2018, when carrying out the state control (supervision) and municipal control, no scheduled inspections were carried out with respect to legal entities and individual entrepreneurs related to small businesses.

The following can contribute to improve the effectiveness and efficiency of the municipal land control implementation:

1) regular implementation of practical seminars covering issues of interaction between municipal control bodies and state supervisory authorities;

2) increasing the size of fines imposed for land legislation administrative violation of.

In order to increase the efficiency of municipal control carrying out, it is necessary to transfer the authority to draw up protocols on land legislation administrative offenses to the level of municipalities.

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For citation:

Saushkina NV. Implementation of municipal land control in Volgograd. *RUDN Journal of Agronomy and Animal Industries*, 2018, 13 (4), 344—352. doi: 10.22363/2312-797X-2018-13-4-344-352.

DOI: 10.22363/2312-797X-2018-13-4-344-352

ОСУЩЕСТВЛЕНИЕ МУНИЦИПАЛЬНОГО ЗЕМЕЛЬНОГО КОНТРОЛЯ НА ТЕРРИТОРИИ ГОРОДА ВОЛГОГРАДА

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Выявление основных нарушений земельного законодательства на территории города Волгограда является необходимым условием для сохранения и рационального использования территории. Разработка системы мероприятий, направленных на повышение результативности и эффективности осуществления муниципального земельного контроля, будет способствовать сохранению главного богатства городского округа. В статье представлена деятельность специально уполномоченных организаций и органов за соблюдением земельного законодательства. Средняя нагрузка на 1 специалиста по проведению муниципального земельного контроля составила 1,4 проверки. Приведена последовательность действий при проведении плановых и внеплановых проверок. Представлен отчет о проведении муниципального земельного контроля на территории города Волгограда за 2016—2017 годы. По результатам земельного контроля в 2016 году из общего числа проведенных проверок с нарушениями выявлено 16,5%, а в 2017 году — 25,7%. Сумма предписанных штрафов в 2017 году выросла больше чем в два раза по сравнению с 2016 годом. Нарушения земельного законодательства на территории города Волгоград приходились на самовольное занятие земельных участков и их частей, невыполнение законных предписаний об устранении нарушений, использование земельного участка не в соответствии с его разрешенным использованием. Муниципальный земельный контроль осуществляется с соблюдением общих принципов, обязанностей, ограничений и запретов при проведении мероприятий по контролю, а также требований к оформлению результатов проведения проверок. На основании рассмотренного материала приведены предложения для повышения результативности и эффективности осуществления муниципального и государственного земельного контроля.

Ключевые слова: муниципальный земельный контроль, плановые проверки, внеплановые проверки, инспектор, нарушения земельного законодательства

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Для цитирования:
Саушкина Н.В. Осуществление муниципального земельного контроля на территории города Волгограда // Вестник Российского университета дружбы народов. Серия: Агрономия и животноводство. 2018. Т. 13. № 4. С. 344—352. doi: 10.22363/2312-797X-2018-13-4-344-352.

RUDN Journal of Agronomy and Animal Industries 2018 Vol. 13 No. 4 353—359
Вестник РУДН. Серия: АГРОНОМИЯ И ЖИВОТНОВОДСТВО http://journals.rudn.ru/agronomy

DOI: 10.22363/2312-797X-2018-13-4-353-359

EFFECTIVE CULTIVATION OF EXTRA-EARLY SOYBEAN CULTIVAR CV. 'VNIIOZ 86' UNDER IRRIGATION

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Abstract. The influence of sowing methods and irrigation regimes on productivity of extra-early soybean cultivar VNIIOZ 86 has been studied at Russian Research Institute of Irrigated Agriculture.

The use of differential irrigation regimes (70—80—70% and 80—80—70% of FMC) resulted in 2.42 ... 2.51 t/ha yield and mid-level profitability of grain production (80.6%), which was 0.25 ... 0.34 t/ha more compared to the control (80—80—80% of FMC), as synchronization of vegetative growth and reproductive development increased grain amount in total biomass up to 36.2%. Differential irrigation regimes restrained an increase in crude protein level in the seeds (at the level of 35.4 ... 41.2%) compared with the control (36.5 ... 41%). However, they increased fat content in the seeds (18.1 ... 21.4%) compared to the control variant (16.1 ... 18.6%). Optimization of irrigation regime increased protein (up to 0.68 ... 0.94 t/ha) and fat (0.37 ... 0.46 t/ha) compared to the control (0.64 ... 0.83 t/ha and 0.29 ... 0.35 t/ha, respectively). The smallest water consumption coefficient was observed in sites with differential irrigation regimes — 1,174 ... 1,524 m^3/t, when in the control site it was 1,651 ... 1,977 m^3/t.

Extra-early VNIIOZ 86 plants require 8 ... 14 irrigations at a rate of 190 ... 230 m^3/ha. It is enough to perform 8 irrigations in relatively favorable years (2013), and up to 14 irrigations in dry years (2014—2015). To maintain a differential irrigation regime, it is necessary to perform 8 ... 10 irrigations in wet and up to 10 ... 13 irrigations in dry years.

The highly profitable cultivation of early soybeans (107.9%) was achieved using drilled sowing (0.30 × 0.042 m) that resulted in significant yield increase (up to 3.02 t/ha) which was 0.41 t/ha higher compared to wide-space sowing technique (0.70 × 0.024 m).

Key words: soybeans, cultivars, irrigation regimes, yield, growth stages, protein and fat content in seeds

INTRODUCTION

Increase in protein and vegetable oil consumption, as well as produce of pharmaceutical and other industries, defines the relevance of the research aimed at optimizing reclamation and other agricultural methods of soybean cultivation, ensuring stable grain yields under limited resource consumption conditions.

Soybean is a light requiring crop with a positive response to irrigation. Its world cultivation is concentrated in southern countries such as the USA, Brazil, Argentina, China, and India. Here, natural soil moisture reaches up to 400 mm and growing season is long enough (up to 180 days) which make it possible to cultivate medium-late

maturing high-yielding cultivars (up to 3.0 ... 3.5 t/ha) while world yield level averages 2.6 t/ha.

Despite the rapid increase in soybean plantings (2.6 million hectares) in the Russian Federation, average soybean yields remain low (1.5 t/ha). The insufficiently favorable natural hydro-thermal conditions for highly profitable soybean cultivation in the main agricultural regions of Russia impel to use early soybean cultivars characterized by low yield level (on average 2 t/ha) without irrigation. Hard weather events that periodically occur during growing season do not contribute to increasing in the seed protein content, which reaches 30 ... 35% of dry matter while US standard is over 35%).

Soybean has a high positive response to irrigation, resulting in yield increase from 2.5 to 4.5 t/ha and increase in crude protein content up to 35 ... 45% in seeds depending on the cultivar, agricultural practices [1—3]. However, cultivation process of early soybean cultivars under various sprinkling irrigation regimes and sowing methods is not described enough [4—6].

MATERIALS AND METHODS

The experiments were conducted in Russian Research Institute of Irrigated Agriculture in 2013—2017. Seeds of early soybean cultivar VNIIOZ 86 (Volgograd selection) included in State Register of breeding achievements approved for cultivating in the Lower Volga region were used [7]. Irrigation regimes (2013—2015) applied were a permanent one with 80—80—80% of FMC (control) and changing regimes depending on basic soybean growth stages: 70—80—70% of FMC (emergence — flowering at 70%, flowering — seed formation at 80%, maturity at 70% of FMC), and 80—80—70% of FMC (emergence — flowering at 80%, flowering — seed formation at 80%, maturity at 70% of FMC). We used two sowing methods: wide-space (0.30 × 0.024 m) as a control and drilled sowing (0.30 × 0.042 m).

RESULTS AND DISCUSSION

The experiments showed that extra-early soybean cv. VNIIOZ 86 requires 2,135 ^{o}C of active temperatures during emergence-maturity (92 days) under irrigation to form yield. Soybean plants need most heat (26.1%) during seed formation-maturity, and the smallest heat (9.4%) is required at emergence. In emergence-flowering period the mean temperature is 22.1 °C, and in flowering — beginning maturity period the mean temperature approaches 24 °C, therefore, it is important to coordinate improvement of irrigation regimes with heat availability in crops during different growth and development stages of particular cultivar, smoothing out temperature extremes.

Analysis of the photosynthetic activity of VNIIOZ 86 plants showed low formation level of the maximum leaf surface area (25.7 ... 31.3 thousand m^2/ha), which is characteristic of early cultivars. The photosynthetic potential (FP) was 1,480 ... 1,880 thousand m^2 days/ha, the average daily increase in organic matter was 84.6 ... 95.5 kg/ha, dry biomass was 6.92 ... 7.83 t/ha, and yield was 2.17 ... 2.51 t/ha.

The use of differential irrigation regimes resulted in significant increase in VNIIOZ 86 grain productivity (Table 1).

Table 1

Influence of irrigation regime on cv. VNIIOZ 86 productivity

Irrigation regime, % of FMC	Yield, t/ha				Deviation from the control	
	2013	2014	2015	average	t/ha	%
70—80—70	2.74	2.5	2.25	2.51	0.34	15.7
80—80—70	2.61	2.46	2.19	2.42	0.25	11.5
80—80—80	2.35	2.13	2.04	2.17	—	—
LSD_{05} (factor A)	0.16	0.03	0.05	0.05		
LSD_{05} (factor B)	0.16	0.04	0.06	0.06		
LSD_{05} (factor AB)	0.23	0.04	0.06	0.06		

The yield increase reached 0.25 ... 0.34 t/ha or 11.5 ... 15.7% compared to the control irrigation regime (80—80—80% of FMC). High VNIIOZ 86 yields under changing irrigation regimes were formed in 2013 and amounted 2.61 ... 2.74 t/ha, which was significantly higher than in the control variant (2.35 t/ha).

Analysis of the plant productivity structure showed that irrigation regimes coordinated with the main stages of organogenesis led to increase in plant number — up to 27 plants/m^2, grain mass — up to 9.6 g/plant, grain size — up to 149.8 g/1000 seeds and grain part in the total biomass — up to 36.2% which contributed to yield increase.

Differential irrigation did not increased protein content in seeds (35.4 ... 41.2%) compared to permanent irrigation (36.5 ... 41%), since process of crude protein accumulation in seeds is negatively correlated with crop yield, even under irrigation conditions ($r = –0.7$) [8]. Considering the negative correlation between protein and fat content in seeds ($r = –0.5$), we established that the differential irrigation regime increased fat concentration in the seeds (18.1 ... 21.4%) compared to the permanent one (16.1 ... 18.6%).

Analysis of grain quality characteristics in irrigated soybean showed that it is important to count protein and fat amount per unit of area harvested. Optimization of irrigation regime led to increase in gross yield of crude protein up to 0.68 ... 0.94 t/ha compared to the control (0.64 ... 0.83 t/ha); and fat (0.37 ... 0.46 t/ha) compared to the permanent irrigation regime (0.29 ... 0.35 t/ha). Therefore, it is important to apply differential irrigation regimes concerning growth stages of certain cultivar in order to obtain significant protein and fat levels.

The water balance of VNIIOZ 86 soybean plants is composed of irrigation rate (62.9 ... 68.2%), precipitation (24.5 ... 29.2%) and soil moisture (7.2 ... 7.9%). When analyzing dependence of number of irrigations on irrigation regime, weather conditions and water consumption at different VNIIOZ 86 growth stages, it was established (Table 2) that soybean plants required 8 ... 14 irrigations with rates 190 ... 230 m^3/ha. In 2013, which was more favorable, plants required 8 irrigations with rate of almost 190 m^3/ha. To ensure differential irrigation regimes with irrigation water, it is necessary to perform 8 ... 10 irrigations in wet years (2013) and up to 10 ... 13 irrigations in dry years (2014, 2015).

Table 2

Dependence of irrigation regime on weather conditions and growth stages of soybean cv. VNIIOZ 86

Growth stage	Irrigation regime % of FMC	Year					
		2013		2014		2015	
		1	2	1	2	1	2
Emergence — shoot development	70—80—70	20.7	0	26.6	1	21.8	1
	80—80—70	32.3	1	35.6	2	30.9	2
	80—80—80	30.5	1	35.6	2	31.1	2
Shoot development — flowering	70—80—70	27.8	0	33.1	1	29.1	0
	80—80—70	40.4	1	43.8	2	40.6	1
	80—80—80	40.7	1	47.8	2	40.4	1
Flowering — pod formation	70—80—70	46.5	2	48.9	2	42.1	2
	80—80—70	46.4	2	45.4	2	41.9	2
	80—80—80	46.8	2	48.8	2	42.0	2
Pod formation — seed formation	70—80—70	48.5	2	54.1	3	64.2	3
	80—80—70	48.8	2	54.3	3	64.4	3
	80—80—80	48.9	2	59.2	3	64.0	3
Seed formation — beginning maturity	70—80—70	33.2	3	34.8	3	40.0	3
	80—80—70	33.3	3	36.3	3	42.6	3
	80—80—80	35.6	3	38.3	3	44.0	3
Beginning maturity — full maturity	70—80—70	19.5	1	17.5	1	16.8	1
	80—80—70	19.7	1	18.2	1	16.5	1
	80—80—80	34.5	2	34.2	2	33.8	2
Total (emergence — full maturity)	70—80—70	196.2	8	215.0	11	214.0	9
	80—80—70	220.9	10	233.0	13	236.9	12
	80—80—80	237.0	11	263.9	14	255.3	13

1 — average daily water consumption, m^3/ha; 2 — number of irrigations.

Permanent water regime was maintained by conducting 11 irrigations in wet and 14 irrigations in dry years.

An important characteristic of cultivar in agricultural production is ability to use moisture sparingly when forming yield. Studies have shown that the lowest water consumption — 1,174 ... 1,524 m^3/t — was observed in crops grown under differential irrigation regimes, while under permanent irrigation regime it was 1,651 ... 1,977 m^3/t.

Irrigation is a fairly expensive item in calculation of all production costs (27.8 ... 29.7 thousand rub./ha), which is 29.9 ... 33.3%. Hence, cultivars forming yields at the level of 2.5 t/ha are characterized by medium profitability (80.6%) and cost value (11.1 thousand rub./t) ensuring low margins. Therefore, it is also important to optimize agricultural practices for early cultivars. For light requiring soybeans these are sowing methods.

Cultivar is an important factor in the discussed question about advantages of drilled sowing compared to wide-space sowing under irrigation [8—10]. The early soybean cv. VNIIOZ 86 is characterized by low branchiness and low leaf formation. When crop density is higher (up to 500 thousand plants/ha) beans are formed higher on plants (0.16 m), grain losses during harvesting are smaller (by 0.25 ... 0.45 t/ha as compared to low crop density in wide-space plantings (to 400 thousand plants/ha). Therefore, the research showed that drilled sowing with more uniform placement of plants in rows (0.30 × 0.042 m) resulted in 3.02 t/ha, exceeding the yield of wide-space sowed crops (0.70 × 0.024 m) by 0.41 t/ha. Cost value of grain produced amounted to 10 thousand rub./t, profitability was 107.9%.

CONCLUSIONS

Under irrigation conditions efficiency of cultivation of early soybean cultivars (VNIIOZ 86) is enhanced by optimizing the irrigation regime and plant growing space. Differential irrigation regimes (70—80—70% of FMC and 80—80—70% of FMC) resulted in yield increase up to 2.51 t/ha, protein and fat content increased up to 0.94 t/ha and 0.46 t/ha, respectively. Drilled sowing of early soybean cultivars led to significant yield increase — up to 3.02 t/ha and profitability of grain production rose up to 107.9%.

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For citation:
Tolokonnikov VV, Chamurliev GO, Kantser GP, Koshkarova TS, Kozhukhov IV. Effective cultivation of extra-early soybean cultivar VNIIOZ 86 under irrigation. *RUDN Journal of Agronomy and Animal Industries*, 2018, 13 (4), 207—215. doi:

DOI: 10.22363/2312-797X-2018-13-4-353-359

АГРОМЕЛИОРАТИВНЫЕ ПРИЕМЫ РЕНТАБЕЛЬНОГО ВОЗДЕЛЫВАНИЯ РАННЕГО СОРТА СОИ ВНИИОЗ 86 В УСЛОВИЯХ ОРОШЕНИЯ

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Во Всероссийском научно-исследовательском институте орошаемого земледелия изучено влияние режимов орошения и способов посева на продукционный процесс ультраскороспелого сорта сои ВНИИОЗ 86.

Применение дифференцированных режимов орошения (70—80—70% НВ и 80—80—70% НВ) способствовало формированию 2,42—2,51 т/га и достижению среднего уровня рентабельности (80,6%) производства зерна, что существенно (на 0,25—0,34 т/га) превысило урожайность на контроле. (80—80—80% НВ) за счет достижения синхронности вегетативного роста и репродуктивного развития и таким образом — повышения доли зерна в общей биомассе — до 36,2%. Дробные режимы орошения сдерживали рост содержания сырого протеина в семенах (на уровне 35,4—41,2%) по сравнению с контролем (36,5—41%), однако приводили к большему увеличению концентрации жира в семенах (18,1—21,4%), чем на контрольном варианте (16,1—18,6%), Оптимизация режима орошения увеличивала выход белка (до 0,68—0,94 т/га) и жира (0,37—0,46 т/га) относительно контроля (соответственно 0,64—0,83 т/га и 0,29—0,35 т/га). Наименьшим коэффициентом водопотребления характеризовались посевы с дифференцированными режимами орошения — 1174—1524 м^3/т, по сравнению с контролем — 1651—1977 м^3/т.

Посевы очень скороспелого сорта ВНИИОЗ 86 нуждаются в проведении 8—14 поливов нормами 190—230 м^3/га. В относительно благоприятные годы (2013) достаточно осуществить 8 поливов, в засушливые (2014—2015) — до 14. Для поддержания дифференцированного режима орошения необходимо дать 8—10 поливов во влажные и до 10—13 — в засушливые годы.

Высокорентабельное возделывание ранней сои (107,9%) достигалось применением рядового посева (0,30 × 0,042 м), способствующего значительному росту (до 3,02 т/га) урожайности (на 0,41 т/га) относительно широкорядного агроценоза (0,70 × 0,024 м).

Ключевые слова: соя, сорта, режимы орошения, урожайность, фазы роста и развития, содержание и сбор белка и жира в семенах

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Для цитирования:
Толоконников В.В., Чамурлиев Г.О., Канцер Г.П., Кошкарова Т.С., Кожухов И.В. Агромелиоративные приемы рентабельного возделывания раннего сорта сои ВНИИОЗ 86 в условиях орошения // Вестник Российского университета дружбы народов. Серия: Агрономия и животноводство. 2018. Т. 13. № 4. С. 353—359. doi: 10.22363/2312-797X-2018-13-4-353-359

RUDN Journal of Agronomy and Animal Industries 2018 Vol. 13 No. 4 360—365
Вестник РУДН. Серия: АГРОНОМИЯ И ЖИВОТНОВОДСТВО http://journals.rudn.ru/agronomy

УДК 635.132: 631.559:
DOI: 10.22363/2312-797X-2018-13-4-360-365

IMPROVED TECHNOLOGY OF CARROT CULTIVATION UNDER DRIP IRRIGATION

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Abstract. The article considers technologies of carrot cultivation of Shantene Korolevskaya hybrid under irrigation conditions on light chestnut soils of the Lower Volga region. Against the background of various methods of tillage, modern methods of using water-soluble fertilizers have been studied. It was found that deep chisel plowing with 0.18—0.20 m soil overturning results in 1.25 fold increase in productivity of edible carrot compared to moldboard plowing and blade cultivation. Using water-soluble fertilizer NS 30:7 through fertigation increases productivity by 7% compared with ammonium nitrate. Measurements of soil density showed that carrot yield averaged 1.25—1.32 t/m^3 over 2015—2017. In variants after deep chisel plowing rate of water infiltration during all three research years was the highest and averaged 4.2 mm/min. The smallest water infiltration was observed in variants after blade cultivation. Using ammonia nitrate during fertigation in 1—4 applications and NS 30:7 fertilizer in 5—8 applications, and NS 30:7 fertilizer during all 8 applications increased carrot yield of hybrid Shantene Korolevskaya by 4.7—5.9 and 0.5—2.4 t/ha compared to control. In addition, combination of deep chisel plowing with ammonium nitrate fertigation in 1—4 applications and NS 30:7 fertilizer in 5—8 applications resulted in the highest carrot yield in 2015—2017 and averaged 90.6 t/ha. The lowest carrot yield over 2015—2017 was observed in the variant after blade cultivation with ammonium nitrate fertigation and amounted to 77.5 t/ha.

Key words: carrots, drip irrigation, tillage, fertigation

INTRODUCTION

Growing vegetables, both in protected and open ground, is rising to a new quality level now. Hence, agricultural science has to develop modern technologies based on the use of high-productive equipment and progressive irrigation methods, such as combined, fine and sprinkler [1, 2].

Carrots, like other vegetables, respond well to the use of high-quality seeds — first generation hybrids, innovative irrigation techniques, water-soluble complex fertilizers and effective methods of deep tillage [3—6].

The high aridity of the territory with light chestnut soils requires obligatory watering of vegetable crops during the entire growing season [7].

The main reason for fertigation popularity is the effective absorption of nutrients from irrigation water. The introduction of fertigation, as an innovative way to use water-soluble fertilizers, can significantly reduce the production costs of their placing. Moreover, synchronizing water and nutrients supply to the roots of vegetable crops leads to

a significant increase in the efficiency of fertilizer use. Despite the overall positive result, fertigation requires the use of modern complex water-soluble fertilizers, which can increase potential productivity of vegetables and significantly improve economic indicators of their production [8].

Therefore, the use of complex water-soluble fertilizers through fertigation in carrot cultivation leads to a significant cost reduction and formation of high yields [9, 10].

MATERIALS AND METHODS

According to the results of previous field experiments we found optimum irrigation regime for carrot, which was adapted to soil and weather conditions of the light-brown soils subzone. The regime had 80% of FMC differentiated pre-irrigation soil moisture threshold in 0.2 m soil layer during period 'seeding — beginning of root formation', 90% of FMC moisture threshold in 0.4 m soil layer during period 'beginning of root formation — technical maturity' and 75% of FMC during period 'technical maturity — full maturity'.

Two-factor experiments including variants of deep tillage and variants of fertigation were conducted in accordance with generally accepted methods and included in factor A three variants of deep tillage: moldboard plowing at 0.25 ... 0.27 m, blade cultivation at 0.25 ... 0.27 m and chisel plowing at 0.38 ... 0.40 m with soil overturning at 0.18 ... 0.20 m. Factor B involved three variants of water-soluble fertilizers applied: ammonium nitrate; ammonium nitrate at 1—4 applications and NS 30:7 fertilizer in 5—8 applications; NS 30:7 fertilizer nutrient solution.

Doses of fertilizers were calculated based on projected yield of 80 t/ha and amounted to: nitrogen — 210 kg/ha, phosphorus — 130 kg/ha and potassium — 110 kg/ha. Fertilizers were added to soil in the following way: 50% of nitrogen, 100% of phosphorus and 100% of potassium were added during deep tillage, and 50% of nitrogen was added as additional fertilizing during irrigation.

The polyfactorial experiment had split-plot design with four replications. Size of first order plots was 396 m^2 (8.4 × 45 m), sown area of second order plots was 126 m^2 (8.4 × 15 m), registration area of second order plots was 64 m^2 (6.4 × 10 m).

Determination of soil density was performed by the method of cutting rings on horizons 0 ... 10; 10 ... 20; and 20 ... 30 cm. Infiltration losses of soil moisture were measused by lysimetric method. Weed infestation was determined by the quantitative-weighing method.

The experiments were carried out at the territory of Y.Y. Lemyakin farm in the subzone of light chestnut soils. Depending on composition soils can be characterized as heavy loamy, humus content in 0 ... 0.3 m horizon is on average 1.98%.

Carrots were cultivated according to regional recommendations.

Shantene Korolevskaya edible carrot hybrid was cultivated after winter rye grown as green manure crop. Seeds were sown at the rate recommended by seed producers — 1 million seeds/ha. We used drip irrigation system of Israeli company Netafim. Venturi injection pump was used for fertigation.

RESULTS AND DISCUSSION

In spring soil density was in the range of 1.25 ... 1.32 t/m^3 over research years from 2015 to 2017.

During the phase of root formation soil density in plots after chisel plowing was 0.03 and 0.06 t/m^3 smaller than in plots after other cultivation methods of deep tillage. Before harvesting this tendency remained, and topsoil density in plots after chisel plowing was smaller by 0.03 t/m^3 compared to moldboard plowing, and 0.07 t/m^3 smaller compared to blade cultivation.

Table 1

Topsoil density depending on soil cultivation (average for 2015—2017), t/m^3

Variant	Before seeding	Root formation	Before harvesting
Moldboard plowing at 0.25—0.27 m	1.28	1.34	1.37
Blade cultivation at 0.25—0.27 m	1.32	1.37	1.41
Chisel plowing at 0.38—0.40 m with 0.18—0.20 m soil overturning	1.25	1.31	1.34
LSD_{05} 2015	0.02	0.02	0.01
LSD_{05} 2016	0.02	0.01	0.01
LSD_{05} 2017	0.02	0.02	0.02

Rate of water infiltration in variants with deep chisel plowing turned out to be the highest over the research years and was within 4.2 mm/min. The lowest water infiltration was observed in variants with blade cultivation and averaged 3.7 mm/min.

Irrigated plots under prolonged vegetable cultivation are characterized by higher weed infestation, which does not completely eliminate herbicide applications. This circumstance requires a thorough review of tillage system when growing vegetable crops, as tillage is one of the effective ways to control weeds in vegetable crop rotations.

In the experiment Stump herbicide was applied to soil before planting carrots. Deep tillage also influenced significantly weed infestation. Weed number on average for 2015—2017 in the phase of root formation ranged from 0.4 plants/m^2 after chisel plowing to 2.6 plants/m^2 after blade cultivation.

The total number of weeds increased before harvesting, and the advantage of tillage with soil overturning became more obvious. Weed vegetation in variants with blade cultivation was 2.6 ... 3.3 fold higher compared to chisel plowing with soil overturning.

Table 2

Effect of tillage on weed infestation during root formation of carrot, plants/m^2

Variant	2015	2016	2017	Average
Moldboard plowing at 0.25—0.27 m	0.4	0.6	0.7	0.6
Blade cultivation at 0.25—0.27 m	1.0	1.4	2.6	1.3
Chisel plowing at 0.38—0.40 m with 0.18—0.20 m soil overturning	0.5	0.7	0.8	0.7
LSD_{05}	0.08	0.06	0.08	

During research years carrot yields depending on fertigation averaged 84.7 ... 90.6 t/ha after deep chisel plowing, 81.2 ... 85.9 and 77.5 ... 82.4 t/ha after moldboard plowing and blade cultivation that is 3.5 ... 4.7 and 7.2—8.2 t/ha lower compared to chisel plowing.

Table 3

Impact of tillage and fertigation methods on carrot yield, t/ha

Tillage	Fertigation	2015	2016	2017	Average
Moldboard plowing at 0.25 ... 0.27 m	NH_4NO_3	79.7	80.5	83.4	81.2
	NH_4NO_3 + NS 30:7	84.5	84.9	88.3	85.9
	NS 30:7	83.2	84.0	85.1	84.1
Blade cultivation at 0.25 ... 0.27 m	NH_4NO_3	75.4	77.1	80.0	77.5
	NH_4NO_3 + NS 30:7	79.8	81.7	85.7	82.4
	NS 30:7	79.4	81.2	85.1	81.9
Chisel plowing at 0.38 ... 0.40 m with 0.18 ... 0.20 m soil overturning	NH_4NO_3	83.1	84.3	86.7	84.7
	NH_4NO_3 + NS 30:7	89.0	89.8	93.0	90.6
	NS 30:7	86.3	87.9	90.4	88.2
	LSD_{05}AB	0.06	0.08	0.08	

Application of ammonia nitrate (through fertigation) in 1—4 additional fertilizations and NS 30:7 fertilizer in 5—8 additional fertilizations increased carrot yield (Shantene Korolevskaya hybrid) by 4.7 ... 5.9 t/ha. Application of NS 30:7 in 8 additional fertilizations resulted in 0.5 ... 2.4 t/ha yield increase compared to ammonia nitrate fertigation in 2015—2017.

The highest carrot yield was observed when combined chisel plowing and ammonium nitrate fertigation in 1—4 applications and NS 30:7 fertilizing in further 5—8 applications amounted to 90.6 t/ha in 2015—2017.

The lowest carrot yield was 77.5 t/ha after blade cultivation and ammonium nitrate fertigation on average for 2015—2017.

CONCLUSIONS

Regarding tillage, chisel plowing at 0.38 ... 0.40 m with 0.18 ... 0.20 m soil overturning results in the highest carrot yields on light chestnut soils of Volga—Don interfluve under drip irrigation; the most effective fertigation variant was 1—4 ammonium nitrate applications and following 5—8 NS 30:7 fertilizer applications.

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For citation:
Pleskachev YN, Chamurliev OG, Gubina LV. Improving technology of carrot cultivation under drip irrigation. *RUDN Journal of Agronomy and Animal Industries*, 2018, 13 (4), 360—365. doi: 10.22363/2312-797X-2018-13-4-360-365.

УДК 635.132: 631.559:
DOI: 10.22363/2312-797X-2018-13-4-360-365

СОВЕРШЕНСТВОВАНИЕ ТЕХНОЛОГИИ ВОЗДЕЛЫВАНИЯ МОРКОВИ НА КАПЕЛЬНОМ ОРОШЕНИИ

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Рассматриваются приемы возделывания моркови столовой гибрида «Шантенэ Королевская» в условиях орошения на светло-каштановых почвах Нижнего Поволжья. На фоне различных способов обработки почвы изучались современные методы использования водорастворимых удобрений. Было установлено, что глубокое чизелевание с оборотом пласта на 0,18—0,20 м увеличивает в 1,25 раза продуктивность моркови столовой в сравнении с менее глубокими отвальными и плоскорезными обработками. Использование водорастворимого удобрения NS 30:7 в виде фертигации повышает продуктивность в сравнении с аммиачной селитрой на 7%. Измерения плотности почвы

показали, что в среднем за 3 года, с 2015 по 2017 гг., весной она находилась в пределах 1,25—1,32 т/м^3. На вариантах глубокого чизельного рыхления скорость инфильтрации воды все три года исследований оказывалась наибольшей и в среднем находилась в пределах 4,2 мм/мин. Наименьшая инфильтрация воды отмечалась на вариантах плоскорезной обработки. Применение во время фертигации моркови аммиачной селитры в первые 4 подкормки и удобрения NS 30:7 в 5—8 подкормки и удобрения NS 30:7 во время всех 8 подкормок по сравнению со стандартной фертигацией аммиачной селитрой повышало урожайность моркови столовой гибрида «Шантенэ Королевская» в среднем на 4,7—5,9 и 0,5—2,4 т/га. Также отмечено, что при сочетании глубокого чизельного рыхления с использованием во время фертигации аммиачной селитры в первые 4 подкормки и удобрения NS 30:7 в 5—8 подкормки в среднем за 2015—2017 гг. наблюдалась наибольшая урожайность моркови столовой и равнялась 90,6 т/га. Наименьшая же урожайность моркови столовой в среднем за 2015—2017 гг. наблюдалась на варианте плоскорезной обработки с применением во время фертигации питательного раствора с аммиачной селитрой и составляла 77,5 т/га.

Ключевые слова: морковь столовая, капельное орошение, обработка почвы, фертигация

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Для цитирования:
Плескачёв Ю.Н., Чамурлиев О.Г., Губина Л.В. Совершенствование технологии возделывания моркови на капельном орошении // Вестник Российского университета дружбы народов. Серия: Агрономия и животноводство. 2018. Т. 13. № 4. С. 360—365. doi: 10.22363/2312-797X-2018-13-4-360-365.

RUDN Journal of Agronomy and Animal Industries 2018 Vol. 13 No. 4 366—372
Вестник РУДН. Серия: АГРОНОМИЯ И ЖИВОТНОВОДСТВО http://journals.rudn.ru/agronomy

UDC 633.18:632.165
DOI: 10.22363/2312-797X-2018-13-4-366-372

RICE LODGING RESISTANCE

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Abstract. The main direction of breeding grain crops is to increase yield and improve grain quality. For maximum crop yield, it is necessary to develop cultivars with high productivity and quality potential, resistant to biotic and abiotic environmental factors. One of the main characteristics is lodging. Resistance to lodging is determined by well-defined morphological traits: plant height, diameter of the lower internode, panicle length, angle of leaf plate, etc. Short plants are more resistant to lodging than tall ones. Short straw trait can be used when evaluating cultivars for lodging resistance. The ratio of plant height to the diameter of the lower internode is an objectively reliable indicator of rice lodging resistance. The value of this ratio in non-lodging cultivars is less in comparison with lodging ones. The article presents the results of studying morphological traits of different rice cultivars, determining resistance of rice plant to lodging. In the experiment, cultivars differing in height was grouped into three groups (I — tall; II — medium and III — short). Short stem forms were established to be more resistant to lodging. Accents in breeding high-yielding rice cultivars with the lowest risk of yield loss resulting from lodging were identified. The correlation analysis revealed a positive relationship (r = 0.877; 0.945 and 0.500, respectively, in groups) between grain mass per plant and diameter of the lower internode. This indicates a close genetic relationship of the traits studied.

Key words: lodging, morphological traits, characterizing resistance trait, productivity

INTRODUCTION

Lodging is a complex process that expresses the response of a plant to the effects of biotic and abiotic environmental factors. It leads to significant, often to irreversible losses and may occur due to reasons of an objective and subjective nature. The latter include cultivation technology violations (even insignificant; unbalanced mineral nutrition (excess nitrogen supply, leading to intensive vegetative growth, tissue softening, stem thinning; poor checks layout, untimely flooding and discharge of irrigation water, high contamination, high plant density, wrong herbicide treatment, etc.). Weather conditions (rain, wind, hail, extreme temperatures) during the growing season can also lead to lodging.

The main of the reasons should be considered a hereditary genetic trait of the cultivar, due to the level of endogenous gibberellins (GA). GAs are specific phytohormones of rice capable of being incorporated into rice metabolism and causing a wide range of responses. In particular, the increased content of GAs in the plant initiates internode lengthening, plant height, thinning of the stem diameter, and susceptibility to diseases and pests, which causes lodging due to pathogens and insects injuries of lower internode [1]. A prerequisite for the successful development of lodging resistant cultivars is knowledge of distinctive traits that characterize non-lodging and lodging cultivars. Lodging resistance of cultivars depends on plant architectonics, in particular, the angle

of leaf deviation from the stem: the smaller this angle is, the more the cultivar is resistant to lodging [2—4]. Lodging occurs because the plant cannot balance its growth under certain conditions [5]. Lodging should be considered as a plant reaction affecting certain aspects of the metabolism, in particular, substances that make up mechanical tissues of the stem [6]. Structural polysaccharides protect tissue cells and organisms, give them shape and support it. Resistance to lodging is caused by the content and distribution of mineral elements, silica, in particular, and cellulose in various plant organs. Mineral silicon in rice tissues is present mainly in the form of silica gel, filling aperture in cellulose micelle of cell walls, forming a silicon-cuticular double layer. The layers of silica gel are bound to the layers of cellulose in cell walls, mainly in external and mechanical tissues. A close correlation is noted in epidermis, sclerenchyma, vascular bundles, straw internodes and leaf sheaths [7].

As lodging resistance is a complex trait, including the qualitative and quantitative characteristics of plants, morphological characteristics were studied. For this, the plants selected after full ripeness were used to determine plant height, diameter of the lower internode, head length, ratio of head length to diameter of the lower internode, number of filled and non-filled grains, mass of 1000 grains, and yield. Semi-dwarf (medium growth) cultivars have the most favorable combination of these characteristics [8]. The direct dependence of plant height and lodging was revealed. Depending on straw length rice forms are divided into dwarf (up to 50 cm), short (51—80 cm), medium (81—110 cm), tall (111—140 cm), very tall (more than 140 cm). Floating rice plants can have a straw up to 500 cm long. Such a difference in straw length leads to a different location in space [9].

The stem of rice straw is formed by sheaths of leaves, reaching different lengths. The straw is divided by stem nodes into internodes with a thickness of 0.4—1.5 cm. A single layer of epidermis is on straw surface. The outer wall of epidermal cells is thickened and enriched with silica. Layer of intercellular parenchyma has ring of sclerenchyma fibers with thickening around the circumference where collateral closed conductive bundles occur. Cavity formed after death of parenchyma cells is in the center of the straw.

Short stem forms are more resistant to lodging than tall stem plants. The trait of a short straw can serve as an indicator (in combination with others) for selection for plant resistance to lodging. The lower internode is shorter in lodging resistant plants than that of the lodging ones [10].

All the above stated made it possible to formulate the purpose of the study — to study the morphobiological traits of various rice genotypes that contribute to lodging resistance.

MATERIALS AND METHODS

Rice cultivars of Russian Rice Research Institute collection were studied. They were previously grouped by plant height:

1) tall (111—140 cm) — Aromir, Fisht and Nafant;
2) medium (81—110 cm) — Privolny-4, Kumir and Favorit;
3) short (51—80 см) — Ryzhik, Mavr and Sonet.

The experiment had four replications. Each plot size was 7.2 m^2 (5.19 × 1.4 m) having eight rows, distance between the rows was 15 cm, distance between the plots was 40 and 50 cm. Mineral fertilizers were applied in soil before sowing — $N_{120}P_{60}K_{40}$ and in tillering stage — N_{100}. Soybean was a precursor crop. The research work was carried out in accordance with GOST 15.101.80 'The order of research work' and the methods developed in Russian Rice Research Institute [11] and the methodology of experimental work on breeding [12].

The experimental part of the research was carried out in field conditions in rice irrigation system of experimental production department of Russian Rice Research Institute. Sowing dates were end of April to beginning of May. Samples (10 plants from each replecation) were taken during full ripeness to determine morphological characteristics. The main anatomical and morphological, and lodging traits were measured: plant height, diameter of the first internode, grain mass per plant, productive tillering. Ratio of stem height to diameter of the lower internode was calculated, and correlation of the described traits was determined [12, 13].

RESULTS AND DISCUSSION

Phenological observations and biometric analysis showed that the strength of rice straws is limited by plant height, diameter of the lower internode and mass of generative organs (grain formed). The experiment was optimal in combination of these traits in cultivars with different plant height (Table 1).

Table 1

Anatomical, morphological and biometric traits of different rice cultivars

Group	Cultivar	Plant height, cm, (h)	Productive tillering, stems	Grain mass per plant, g	Diameter of the lower internode, cm, (d)	Ratio of the stem height to the diameter of lower internode, (h/d)
I	Aromir	118	1.8	4.8	0.54	218.5
	Fisht	122	1.6	5.7	0.60	203.3
	Nafant	117	1.5	5.5	0.56	209.0
II	Privolny-4	90	2.1	4.4	0.50	180.0
	Kumir	87	2.0	4.7	0.52	167.3
	Favorit	92	2.0	4.6	0.52	177.0
III	Ryzhik	70	3.1	4.5	0.48	145.8
	Mavr	73	3.3	4.1	0.47	155.3
	Sonet	80	2.8	4.4	0.51	156.9
LSD_{05}		5.7		0.81	0.08	

The data presented show that lodging resistance is different for the cultivars studied. Non-lodging cultivars had a smaller value of this indicator in comparison with lodging ones. The cultivars studied are resistant to lodging, however, the results obtained demonstrate their susceptibility to lodging in stressful environmental conditions. So, cultivars of the I-th group (Aromir, Fisht and Nafant) are potentially lodging, since the value of the resistance index exceeds 200. Cultivars of the second group (Privolny-4, Kumir and Favorit) are less susceptible to lodging and cultivars of the third group (Ryzhik, Mavr and Sonet) have the maximum resistance to lodging.

cv. Aromir cv. Fisht cv. Nafant

Fig. 1. Lodging of tall rice cultivars, 2018

Table 2

Correlation of traits of rice plants of different varieties

Group	Trait	Mean value	Dispersion	Plant height, cm (h)	Productive tillering, stems	Grain mass per plant, g	Diameter of the lower internode, cm, (d)
I	Plant height, cm, (h)	119.0	2.646				
	Productive tillering, stems	1.6	0.153	0.000			
	Grain mass per plant, g	5.33	0.473	0.520	–0.854		
	Diameter of the lower internode, cm, (d)	0.57	0.031	0.866	–0.500	0.877	
	Ratio of stem height to diameter of lower internode, (h/d)	210.3	7.679	–0.655	0.756	–0.986	–0.945
II	Plant height, cm, (h)	89.7	2.517				
	Productive tillering, stems	2.0	0.058	0.115			
	Grain mass per plant, g	4.57	0.153	–0.434	–0.945		
	Diameter of the lower internode, cm, (d)	0.51	0.012	–0.115	–0.999	0.945	
	Ratio of stem height to diameter of lower internode, (h/d)	174.8	6.638	0.804	0.683	–0.884	–0.683
III	Plant height, cm, (h)	74.3	5.132				
	Productive tillering, stems	3.1	0.252	–0.761			
	Grain mass per plant, g	4.33	0.208	–0.016	–0.636		
	Diameter of the lower internode, cm, (d)	0.49	0.021	0.858	–0.986	0.500	
	Ratio of stem height to diameter of lower internode, (h/d)	152.7	6.000	0.816	–0.246	–0.591	0.403

Cultivars with index exceeding 200 are characterized by relatively moderate resistance to lodging, however, they may be susceptible under adverse environmental conditions or non-compliance with agricultural technology (high doses of nitrogen fertilizers, wrong irrigation regime, high plant density, etc.) (Figure 1).

Nature and closeness of correlation of economically valuable traits in the studied rice cultivars indicates specificity of plant metabolism in each group, due to the genotype (Table 2).

The resulted obtained show that diameter of the lower internode positively correlates with height (r = 0.866 and 0.858) in cultivars of I and III groups, respectively. It indicates an increase in the values of one trait with a similar change of the other one. In plants

of group II, this correlation is mediated and insignificant (r = –0.115).The negative correlation of straw diameter with productive tillering of the groups presented indicates the biological regularity of assimilates distribution and the growth of vegetative organs, i.e. the diameter of each stem decreases with an increase in the stem number.

It was found that grain mass per plant correlates positively with straw diameter in all groups. Cultivars of I and II group had close correlation of these characteristics (r = 0.877 and 0.945, respectively) that indicates increasing in straw diameter with an increase in productivity at genetic level. And there is an average correlation of these characteristics (r = 0.500) in short rice cultivars. This suggests the potential for increasing grain mass per plant with a constant straw diameter or — that these traits are indirectly related.

In conclusion, it should be noted that breeding of new rice cultivars should be aimed not only at increasing yields and technological characteristics of rice grain quality, but also at keeping formed yield when adverse environmental conditions occur (rains, gusty wind, etc.) during harvesting.

CONCLUSIONS

The quantitative values of the studied traits in cultivars and their role in lodging resistance were presented.

It was established that cultivars of I and II group were in the zone of rice yield loss. In this connection, the breeder in his work should select parental forms not only with high productivity and quality of grain and milled rice, but also with a complex of morphological traits: with thickened internodes, a large number of internodes, high silicon content, etc., which give additional mechanical strength to the stem.

Short stem forms (group III) were more resistant to lodging, which was due to morphological traits and the ability to not only form a high yield, but also to keep it. The results obtained allow breeders to work on increasing yield of short stem cultivars without any lodging risk.

A positive correlation between grain mass per plant and diameter of the lower internode was established; it indicates a close genetic correlation between these traits.

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For citation:
Avakyan ER, Dzhamirze RR. Rice lodging resistance. *RUDN Journal of Agronomy and Animal Industries*, 2018. Т. 13. № 4. С. 366—372. doi: 10.22363/2312-797X-2018-13-4-366-372.

УДК 633.18:632.165
DOI: 10.22363/2312-797X-2018-13-4-366-372

УСТОЙЧИВОСТЬ РАСТЕНИЙ РИСА К ПОЛЕГАНИЮ

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Главным направлением селекции зерновых культур является увеличение урожайности и улучшение качественных показателей зерна. Для формирования растениями максимального урожая необходимо создание сортов с высоким потенциалом продуктивности и качества, устойчивостью к биотическим и абиотическим факторам окружающей среды. Одной из основных характеристик, играющих важную роль, является полегание. Устойчивость к полеганию обусловливается вполне определенными морфологическими признаками: высотой растения, диаметром нижнего междоузлия, длиной метелки, углом расположения листовой пластины к стеблю и т. д. Низкорослые формы более устойчивы к полеганию, нежели высокорослые. Признак короткой соломины может быть использован при оценке сортообразцов на устойчивость к полеганию. Отношение высоты растения к диаметру нижнего междоузлия — объективно надежный показатель устойчивости растения риса к полеганию. Величина этого отношения у неполегающих сортов меньше в сравнении с полегающими. В данной статье представлены результаты изучения морфологических признаков разных сортов риса и степень их взаимосвязи, определяющая в целом устойчивость растения риса к полеганию. Для проведения эксперимента сорта, различающиеся по высоте растения сгруппированы

в три группы (I — высокорослые; II — среднерослые и III — низкорослые). Установлено, что короткостебельные формы более устойчивы к полеганию и имеют потенциальную возможность сформировать и удержать более увесистую (продуктивную) метелку. Определены акценты в селекции высокоурожайных сортов риса с наименьшим риском потери урожая в результате полегания растений. Методом корреляционного анализа выявлена положительная связь (r = 0,877; 0,945 и 0,500 соответственно по группам) между массой зерна с растения и диаметром нижнего междоузлия. Это свидетельствует о тесной генетической связи изученных признаков.

Ключевые слова: полегание, морфологические признаки, отличительный признак устойчивости, продуктивность

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Для цитирования:
Авакян Э.Р., Джамирзе Р.Р. Устойчивость растений риса к полеганию // Вестник Российского университета дружбы народов. Серия: Агрономия и животноводство. 2018. Т. 13. № 4. С. 366—372. doi: 10.22363/2312-797X-2018-13-4-366-372.

RUDN Journal of Agronomy and Animal Industries 2018 Vol. 13 No. 4 373—382
Вестник РУДН. Серия: АГРОНОМИЯ И ЖИВОТНОВОДСТВО http://journals.rudn.ru/agronomy

DOI: 10.22363/2312-797X-2018-13-4-373-382

THE EUROPEAN UNION'S COMMON AGRICULTURAL POLICY AND DEVELOPMENT OF AGRO-FOOD SECTOR IN GREECE

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Abstract. The article analyzes the Common Agricultural Policy of the European Union (EU), reforms and consequences in agricultural sector of Greece. Since its inception, the Common Agricultural Policy has been the subject of many reforms that are being developed to meet the changing needs of society, market and competition of the European Union's (EU) rural economy with other alliances around the world. Of particular interest is the development of agricultural sector in Greece in the last 36 years, following its entry into the European Union. The contribution of agriculture (including agriculture, forestry and fisheries) in the Greek economy over the last 15 years has been significantly reduced. Data show a small trend in concentration of production and an increase in agricultural crop yield. There is an imbalance, which is reflected in large fluctuations in types of agricultural products. This applies to the volume of basic food production, which is governed by the economic policy of the European Union, expressed in product quotas and their production volumes. This leads to gradual decline in production, which is reflected in the need to meet domestic demand for plant and livestock foodstuffs, such as common wheat, legumes, sugar, lemons, more animal products and mainly meat. At the same time, imports of similar products from EU Member States are increasing, exacerbating the negative trade balance between the EU and the Republic of Greece. It is noted that the volume of subsidies in the EU remains at the same level, additional subsidies for farmers are gradually decreasing and the system of linking subsidies with production volume has been partially eliminated. The most effective support mechanisms for farmers will minimize the negative impact on the reduction of the overall financing in agricultural sector. The money saved as a result of reduction in subsidies will be spent on development of rural areas and improving effectiveness of structural policies. Also, comparative data are presented for other states of the European Union.

Key words: Greece, EU common agricultural policy, agro-food sector, European Agricultural Fund for rural development

INTRODUCTION

Creation of the Common Agricultural Policy (CAP) in the European Union (EU) — Basic Principles and Objectives: The creation of the Common Agricultural Policy (CAP) is reflected in the Treaty of Rome, signed in 1957 by the six founding members of the European Economic Union (EEU)) (France, Germany, Holland, Belgium, Luxembourg, Italy). In particular, Article 33 of the Treaty of the European Community (formerly Article 39 of the Treaty of the EEU), which includes the main objectives (CAP):

a) increasing agricultural productivity by developing technological progress, ensuring the rational development of agricultural production and the optimal use of resources, in particular labor force;

b) ensuring a fair standard of living for rural population, in particular by increasing the individual earnings of agricultural workers;

c) stabilization of markets. Ensuring supplies;

d) ensuring reasonable prices in provision of goods to consumers.

The same article indicates that during CAP implementation it is necessary to consider:

a) the specific nature of agricultural activity as a result of the social structure of agriculture and structural and natural differences between different agricultural areas;

b) the need for gradual implementation of appropriate adjustments;

c) the fact that in the member states agriculture is a sector that is closely related to the entire economy [1—5].

Article 34 of the European Union (EU) Treaty provides that achievement of the above objectives requires creation of a Single Market Organization (SMO). Depending on SMO products, it can take the form of general rules of competition, or the form of mandatory coordination of various national market organizations or the form of organization of the European market. Each SMO includes the whole range of measures necessary to achieve the objectives, in particular, price regulation, production support, marketing and other storage mechanisms and general mechanism for stabilizing imports and exports [2, 3].

The agro-food sector in the European Union affects the economy and, consequently, the society of its countries. The population increases with aging and decline of the rural population, due to the migration of rural youth into the cities where there is a strong urbanization phenomenon, with the result that many areas remain unused. The reasons for this phenomenon are low returns for farmers, lack of resources to cover the costs associated with agricultural production, quantity and quality of products. However, production has increased due to improved environments and technologies. Until 1992, agricultural spending represented 49% of the EU budget [2, 3, 5, 6].

In addition, the accession of new countries to the Union has a serious impact on the adopted policy. EU enlargement in 2004 augmented the number of farmers from 7 to 11 million, increased rural land by 30% and production by 10—20%. New countries that joined in 2004 immediately got access to price support measures for export financing and intervention purchases. New countries have access to a rural development fund with a budget of 5 billion euros. The meaning of the Common Agricultural Policy (CAP) is to use market mechanisms in conjunction with government regulation [5]. The policy was based on the following principles: organization of agricultural market, establishment of uniform prices for most types of agricultural products, high degree of protection of internal agricultural market and price regulation, providing workers employed in the agricultural sector with a decent standard of living comparable to other economy sectors [2, 7].

A number of factors predetermined successful functioning of the CAP until the end of the 70s. The most important result is that the Community has achieved self-sufficiency in agricultural products. The CAP largely reflects the EU's leading role in global food production and trade in agricultural products. Today, the EU accounts for 17% of world food exports; The EU is ranked second as a global exporter of dairy products and pork

and third place as an exporter of poultry and grain. According to the FAO, in 2013 the European Union exported agricultural products worth 151.2 trillion dollars [4].

The original goals of the European Community have already been achieved. A single domestic market for agricultural products has been created, self-sufficiency has been achieved. However, agrarian policy had significant drawbacks. If at first the CAP was based on fairly rigid mechanisms, then in the new conditions these mechanisms and especially amount of financial resources and their distribution needed to be revised [1, 7—9]. Thus, in order to improve the unified agrarian policy, a series of reforms were carried out: the first reform — 1984, the second — 1992, the third — the end of the 90s, the fourth in 2003 and the last reform of the CAP— in 2013. Within its framework, the following tasks were set: Implementing single payments to farmers, regardless of production, but observing environmental standards and ensuring food safety, strengthening policies for rural development, reducing direct payments to large farms and creating the European Agricultural Fund for Rural Development (EAFRD) and the European Agricultural Guarantee Fund (EAGF) in accordance with the regulations on financing of the CAP on June 21, 2005 [10]. It was proposed to change the principle of issuing subsidies, which made it possible to optimize the pan-European agro-budget in connection with entry of new countries into the EU. Creating a common domestic market without any trade barriers should provide new opportunities for rural producers. In general, the reform is aimed at organizing agriculture in the European space that would satisfy market and consumers requirements [2].

The gradual redirection of funds from subsidies to environmental protection measures should lead to elimination of crisis of overproduction of agricultural products and the excessive use of natural resources due to the use of chemicals and fertilizers [2]. The idea of reforming the CAP is also to move from a "productive" agrarian policy model to a more environmentally friendly and based on qualitative criteria. Therefore, a system of single direct payments is introduced, depending on environment preservation and safety of products, humane attitude to animals, and increase in employment. It was planned that volume of subsidies in the EU will remain at 42 billion euros, and their annual growth should not exceed 1%, offsetting the effect of inflation. Additional payments for farmers have gradually been reduced (by 3% in 2005, 4% — 2006, and further by 5% per year) [7].

The European Union pursues two goals: first, to reduce the budget expenditures on the CAP and increase the costs of other areas (in particular, scientific research); secondly, because of agriculture, negotiations in the WTO, which deal with a much wider range of problems, do not complicate.

Thus, the main conclusions in the evaluation of the EU agrarian policy are: the multiplicity and ambiguity of agricultural programs carried out within framework of the EU agrarian policy, the complicated process of obtaining subsidized funds leads to the development of bureaucracy and does not contribute to the growth of competitiveness among agricultural producers. The CAP has already come a long way of becoming in the 20th century, and now it has the opportunity to truly become the European agricultural model of the 21st century [7]. It is worth emphasizing that since 2014, the EU has been implementing a new phase of the common agrarian policy, the main priorities

of which are: improving the competitiveness of agriculture: sustainable management of natural resources, as well as combating climate change; achieving a balanced territorial development of the rural economy, creating and maintaining employment. The specified priorities of general agrarian policy for the program period 2014—2020 reflect the continuity of previously identified priorities.

In essence, the MFS illustrates EU policy priorities in various areas of funding. The costs will be directed to financing Pillar 1 (CAP) for the period 2014—2020 and amount to current prices of 317.2 billion euros, while Pillar II will require a total of 101.2 billion euros. About 17.1 billion euros will be added to these year expenditures, not provided in the MFS, which will increase the total budget for agriculture 2014—2020 to 435.6 billion euros. The resources that the CAP ultimately absorbs account for 37.7% of the total EU budget, which is reduced in comparison with the period 2007—2013, which was 41.7%. The multi-year financial structure (MFS) defines the resources for financing European policy, including the CAP, as a rule, for the next seven years. Therefore, the MFS illustrates the EU's policy priorities in various areas of funding. It is noted that the development of the European Union (EU) economy will begin to progress but will remain below 2% per year. After several years of decline in euro price, its currency ratio is considered to be formed at the level of 1.37 dollars per euro in 2024 [10].

The Common Agricultural Policy (CAP) from its inception to date has been the subject of many ongoing reforms, developed to meet changing needs of society, market and competition of the rural economy of the European Union (EU) with other alliances around the world. More radical reforms are considered to be Ray Mac Sharry in 1992 and CAP in 2003, which established direct payments to producers established independently of production and type of agricultural products [1, 7].

The general trend shows that CAP development and reforms have a protective character and interventionism, which characterizes it, gives way to a more liberal model. It should be noted that these goals have not been replaced and not changed by the successive reforms of the original Treaty and are an important and integral part of the Treaties to date.

For Greece, we should not follow the example of rich European countries that can afford to keep inefficient agriculture through market price support measures. Financial resources must be invested in know-how, modern technology, management skills and infrastructure that Greek agriculture needs in order to become competitive. The greatest attention should be paid to consulting services, village infrastructure, as well as measures aimed at preserving the environment [1, 2, 7, 8].

MATERIALS AND METHODS

The main indicators of agricultural sector of the Republic of Greece: Agricultural land in Greece accounts for 31% of the total area of the country, which is 517.8 thousand hectares [11—14]. Approximately 71% of this area (367.4 thousand hectares) is located in mountainous or foothill area, and its size has remained stable for many years with a slight upward trend [13].

According to the Ministry of Rural Development and Products, 54.6% of the total agricultural land was used for tilled crops, 2.8% for melon and vegetable crops, 32% for

permanent garden fruit plantations and only 10.6% remain unprocessed in 2014 [14, 15]. The average land size of one farmer in the country still remains very small. According to statistics, in 2014 it amounted to 4.77 hectares per farm, which is slightly higher compared to the beginning of the 90s. Studying data from other EU countries we see that corresponding average land size of a farmer is 22.0 hectares. On average, one Greek farmer accounts for an area that is four times smaller than the area occupied by farmers from small EU countries. It is characteristic that the average size of agricultural farm in Greece remained practically unchanged from 1990 to 2014, whereas in European countries with high agricultural production and employment of a significant part of population in the agricultural sector (Portugal, Italy and Bulgaria) the area per farmer during this period increased significantly. It should be noted that Greece is one of the few EU countries with a high percentage of fruit trees from the total arable land. In total agricultural production 19% are vegetables, followed by fruit 18.5%, animal husbandry 14.5%, animal products, milk, butter — 14% grains and olive oil — 8% [13]. The total value of agricultural products in Greece is 9.7 billion US dollars. Of this amount, 69% is accounted for by the production of plant products [15]. Therefore, production of plant products occupies a dominant position in the rural economy of the country.

In terms of value, agricultural production in Greece has not changed from 2010 to date. However, in comparison with the EU statistics, it decreased from 2.91 to 2.5%.

According to 2007 data the number of agricultural farms is about 854.1 thousand [12]. The number of farms decreased by 100 thousand in the period 1987—1990, which was a consequence of the economic policy of the European Union by subsidizing large farms. A significant part of small farms could not receive subsidies, since their products were intended primarily for personal consumption and did not create a surplus in the sales market being an unofficial income for citizens living in urban and semi-urban areas. A high number of farms are associated with the division of property and possession between members of the same family in order to avoid taxation or to receive subsidies. Despite the survival of a very small number of agricultural farms (up to 2 hectares in area), there is a steady trend of land concentration in larger farms. It is expressed for categories of farms with agricultural land within an area of 30 to 100 hectares, and there is also a slight increase in the percentage of farms with a total area of up to 20 hectares. Thus, the number of farms up to 20 hectares in 1990 accounted for 97.43% of all farms and occupied 76.30% of the total land area, and in 2014 — 95.66% of the total number of farms, which amounted to 64.35% of the total land area in the country. Of particular interest is the development of farms based on the form of ownership. The state maintains a form of transfer of agricultural land for hire to third parties. This trend is obvious, since in 2014 it accounted for almost 32% of the total amount of land used for agriculture, which is 10% higher than the 1990 figures [12, 13].

The main statistical indicator for studying changes in the structure of farms is the annual standard gross margin. It should be clarified that this indicator is not an indicator of the profitability of the farm, as it includes work of farmer and his family members, someone else's labor employed by the farmer (variable capital), but also part of the fixed capital [13].

Thus, the annual standard gross margin as an indicator allows to evaluate some general trends, since it is not comparable between different types of farms. For example, with regard to the cultivation of cotton, a farm of 10 hectares gives about 45,000 euros and a net income of 5,000 euros, while growing wheat on the area of 40 hectares, the same 45,000 euros, and a net profit of 12,000 euros. According to the above, in the first case the net profit amounted to 500 euros, and in the second only 300 euros per hectare. It has been established that a farm with an annual standard gross margin of less than 48,000 euros cannot even ensure its simple sustenance [12].

As for livestock, more than 43% of the livestock (in 2007 total number of farms amounted to 860.2 thousand) belongs to 371.2 thousand farms. Livestock remains with minor fluctuations at sustainable levels, which indicates its lack of development and relative stabilization. There is a significant reduction in the number of livestock farms, as evidenced by data confirming a decrease in the number of farms from 475.6 thousand livestock farms in 1990 to 371.2 in 2007, which is 23% less.

Based on the available data on the state of livestock sector, the following conclusions can be made:

1) presence of a highly time-stable number of small livestock farms, which account for at least 10% of the total livestock;

2) strengthening trends in the concentration of livestock in larger farms, where 2.4 thousand livestock farms control 22% of the total livestock population;

3) products used as animal feed and cost of its production in Greece are among the highest in the EU with an increase from 63.6% in 2010 to 76.7% in 2014 [12, 13];

4) gross domestic meat production decreased from 572.0 thousand tons in 1981 to 491.0 tons in 2010. In the same period, there was an increase in meat consumption from 70.0 kg in 1981 to 83.0 kg in 2010. This growth was fully covered by imports, so the degree of self-sufficiency in meat declined from 84% to 50% over the same period [12, 16]. There is a very limited degree of self-sufficiency in beef, 57% in 1981 and 24% in 2010 and relatively high availability of lamb meat — 87% and poultry meat — 79% in 2010 [3, 6, 8].

Data on employment, total employment of hired labor and self-employment in rural farms show a clear downward trend in employment in agricultural production as a whole [11—13].

The participation of the agricultural sector (which includes agriculture, forestry and fisheries) in the Greek economy has declined significantly over the past 15 years. The contribution of gross value added (GVA) of agrarian sector to the total GVA of the country in 2014 was 4.3% from 6.1% in 2000 and 8.8% in 1995. The above figures should also include contribution of processing industry of food, beverages and tobacco, which accounts for almost 3.0% of the total gross value added (GVA), while it accounts for 31% of the total processing industry [10].

The agro-food sector (agricultural sector and food, beverage and tobacco processing industry) contributed 7.2% of the total gross value added (GVA) in 2014, compared with 9.1% in 2000 and 11.8% in 1995. Moreover, it accounts for 15% of the total employment in Greece [10]. In addition, over the past decade, agricultural income in Greece has decreased on average by 0.4% per year compared with a growth of 1.6% in the Eurozone countries.

Since 2007 (EAP) has invested more than 19.53 billion Euros in agriculture and rural areas of Greece in total costs for direct payments, market and development measures for rural areas. Most of the funds sent to Greece for the period 2008—2013 from the CAP, particularly direct payments (77.7%), were higher by 10% on average in the EU (68%). By 2013, share of the CAP should have been (32%). On the contrary, the share of expenditures on the Regional Policy in 1988 amounted to 17% of the EU budget, 46.7% of the EU budget was spent on it, 49.8 billion euros in 2006 (more than 48.5 billion euros in 2005), and it was planned to increase to 36% in 2013.

In particular, 12.5% of total employment and 2.7% in food, beverage and tobacco processing industry work in the agricultural sector. Between 2000 and 2014, the number of people employed in the agricultural sector decreased by 29 %, while the number of people working in the food, beverage and tobacco processing sector increased by 8%. The trade balance of Greek goods in agro-food sector is negative [16]. Almost 69% of exports went to the countries of the European Union (EU), and 80% of imports of this category of goods — from EU countries [10]. The largest category of exports of agricultural products in 2012—2014 for fruit and vegetables amounted 34%, followed by fish — 10.8%, dairy products — 7.9%, vegetable fats and oils — 7.8%, tobacco — 7.6%, and cotton — 7.1% [10]. The most important products that are of high quality (high competitiveness at high prices) are: olive oil, olives, pickles, raisins, canned vegetables, tobacco, tomato paste, yogurt, rice, sheep and goat skins, cotton seed oil and bakery products. Lower prices are the main comparative advantage of such highly competitive products as fruit, cotton, sheep cheese, wine and so on. In the case of imports of this category, the largest percentage relates to livestock products — 17%, followed by dairy products — 13%, fruit and vegetables — 10%, grain and feed concentrates 9% [16]. Agricultural products are the third largest category of exported products, accounting for 5.2 billion euros in 2014, or 19% of total exports, representing 14% of imports [10]. Vegetables and horticultural products account for the largest share of exports. The trade balance of agro-food products is negative. The decline in imports that began in the last 5 years due to the economic crisis, combined with good export performance of agricultural products, led to a reduction in the deficit, which in 2014 was limited to 1.36 billion euros. Most individual product categories have a deficit balance, with the exception of fruit and vegetables, fish, tobacco, cotton, vegetable oils and fats, which have a positive balance.

RESULTS AND DISCUSSION

If we consider contribution of agriculture to the EU and Greece gross value added of the agricultural sector in Greece amounted to 3.3% of GDP in 2014, compared with 1.4% in the EU. For the period 2012—2014 Greece brought 3.0% of gross value added in the EU agricultural sector and the countries with the highest participation were Italy — 16.0%, France — 15.9%, Spain — 12.2%, Germany — 10.4%, United Kingdom — 6.0%, the Netherlands — 5.5%. The value added obtained in agricultural production lags behind key competitors. From the distribution of value added along the agro-food chain, it seems that for every 1 euro, the value of primary products in processing sector of food and beverages adds value of 0.4 euros in our country, when in Spain and Italy this amount is 1.5 euros.

In the period 2004—2014 agricultural income in Greece decreased by an average of 0.4% per year, while in the EU it increased by 3.4% and in the Eurozone countries by 1.6%. Gross fixed investment in the agricultural sector in Greece as a percentage of the gross value added of the agricultural sector is more than 10% lower than the EU average, reflecting a reduction in investment in the agricultural sector and consequently, inability to modernize farming management methods.

In 2014—2020 the Common Agricultural Policy of the European Union will invest in Greece more than 19.5 billion euros in agriculture and rural areas. The budget for direct payments in Greece is about 15.4 billion euros. Thirty percent of direct payments will be associated with three environmentally friendly farming methods: crop diversification, continuous pasture conservation, and 5 % will be channeled towards environmental interest preservation or measures that are thought to have equivalent environmental benefits. Greece will allocate more than 4.2 billion euros to measures in favor of rural areas in accordance with the priorities outlined in the rural development program [10, 15].

CONCLUSIONS

In the development process of agricultural sector, its structure, applied technology, level of farmer experience, and classical factors of production, such as science, politics, financing, market, etc., play a decisive role.

In this process, the key point is the accession of Greece in 1957 to the European Economic Union. The current structure and organization of Greek agriculture and the composition of its primary production reflect the influence of both the country's national policy and the Common Agricultural Policy of the European Union (EU) before and after Greece joined the EU. The total area of agricultural land remains stable compared to the 1980s. The average size of farms in Greece remains very small. Many small farms remain without economic activity in order to receive subsidies. The number of farms with small areas of agricultural land (up to 2.0 ha) is increasing. There is a steady trend of land concentration in larger farms. The number of farms decreased in the period 1987 to 1990, which was a consequence of the economic policy of the European Union subsidizing large farms. 12.5% of the total employment are in agricultural sector and 2.7% in processing industry of food, beverages and tobacco. Between 2000 and 2014, the number of people employed in the agricultural sector decreased by 29%, while the number of people working in the food, beverage and tobacco processing sector increased by 8%. In the total agricultural output, vegetables make up the largest part and followed by fruits and livestock, animal products, milk, butter, cereals, and olive oil. The total value of agricultural products in Greece is 9.7 billion US dollars. In terms of value, agricultural production in Greece has not changed from 2010 to date. However, comparing the percentage with that of the EU, it has decreased from 2.91 to 2.5%. 69% of this amount is for production of plant products, which occupies a dominant position in the rural economy of the country. In 2014, the gross value added of the agricultural sector in Greece amounted to 3.3% of GDP, compared with 1.4% in the EU. The agro-food sector in 2014 amounted to 7.2% of the total gross value added (GVA), compared with 11.8% in 1995. For the period 2012—2014 Greece brought 3.0% of gross

value added in the EU agricultural sector. The trade balance of Greek agro-food products is negative and is the third largest category of exported products, accounting for 19% of total exports and 14% of imports. Almost 69 % of exports are directed to the countries of the European Union (EU), and 80% of imports of this category of goods are from EU countries. Gross investment in fixed assets in the agricultural sector of Greece as a percentage of the gross value added of the agricultural sector is just above 10%, lower than the EU average, reflecting a reduction in investment in the agricultural sector and consequently, inability to modernize farm management practices. The figures given in this study show that impact of the Common Agricultural Policy of the European Union on economy of agricultural sector in Greece was insignificant.

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For citation:
Paskhalidis C, Petropoulos D, Sotiropoulos S, Papakonstantinou L. The European Union's common agricultural policy and development of agro-food sector of Greece. *RUDN Journal of Agronomy and Animal Industries*, 2018, 13 (4), 373—382. doi: 10.22363/2312-797X-2018-13-4-373-382.

DOI: 10.22363/2312-797X-2018-13-4-373-382

ЕДИНАЯ АГРАРНАЯ ПОЛИТИКА ЕВРОПЕЙСКОГО СОЮЗА И РАЗВИТИЕ АГРОПРОДОВОЛЬСТВЕННОЙ ОТРАСЛИ ГРЕЦИИ

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Анализируется единая аграрная политика Европейского Союза (ЕС), реформы и ее последствия для агропродовольственного сектора Греции. Единая аграрная политика (ЕАП) с момента ее создания до сегодняшнего дня была предметом многих проводимых реформ, развивалась для удовлетворения меняющихся потребностей общества, рынка и конкуренции сельской экономики Европейского Союза (ЕС) с другими альянсами по всему миру. Особый интерес представляет развитие агропродовольственного сектора в Греции за последние 36 лет, после ее вступления в Европейский Союз Участие сельского хозяйства (включая сельское, лесное хозяйство и рыболовство) в греческой экономике за последние 15 лет значительно сократилось. Данные свидетельствуют о небольшой тенденции по концентрации производства и увеличении урожайности сельхоз культур. Отмечен дисбаланс, который выражается в больших колебаниях по видам сельскохозяйственной продукции. Это касается объема производства основных продуктов питания, который регулируется экономической политикой Европейского Союза, выраженной в квотировании видов продукции и объемов их производства. Это постепенно приводит к снижению объемов производства, что отражается в необходимости обеспечения внутреннего спроса на продукты питания растениеводства и животноводства, такие как мягкая пшеница, бобовые, сахар, лимоны, большинство продуктов животноводства и особенно мяса. В то же время растет импорт аналогичных видов продукции из стран — членов ЕС, что усиливает отрицательное сальдо торгового баланса между ЕС и Республикой Грецией.

Отмечается, что объемы субсидий в ЕС сохраняются на прежнем уровне, доплаты для фермеров постепенно сокращаются, частично ликвидирована система привязки субсидий к объемам производства. Более эффективные механизмы поддержки фермеров позволят минимизировать негативные последствия для сокращения общего финансирования аграрного сектора. Сэкономленные в результате сокращения субсидий деньги пойдут на развитие сельских территорий и повышение эффективности структурной политики. Также приведены сравнительные данные по другим государствам Европейского Союза.

Ключевые слова: Греция, Единая аграрная политика ЕС, агропродовольственный сектор, европейский сельскохозяйственный фонд для развития села

RUDN Journal of Agronomy and Animal Industries 2018 Vol. 13 No. 4 383—395
Вестник РУДН. Серия: АГРОНОМИЯ И ЖИВОТНОВОДСТВО http://journals.rudn.ru/agronomy

DOI: 10.22363/2312-797X-2018-13-4-383-395

PHYSICAL METHODS OF PRE-PLANTING AND POSTHARVEST TREATMENT OF POTATO: A REVIEW

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Abstract. Potato is an important staple food crop. Potato tubers require proper treatment before planting and after harvest to produce high yields and avoid storage losses. Among different techniques of potato treatment physical methods are of special interest: thermal treatment using hot water and steam, ultraviolet (including continuous-wave UV using pulsed Xe-lamps) and gamma-irradiation, treatment with magnetic and electromagnetic fields (including microwaves). The majority of physical methods is environmentally friendly and can be applied without special registration and in the developing countries. In the present paper, for the first time, the scientific papers on physical methods of potato treatment for the last 35 years are comprehensively reviewed. The review demonstrates that such an approach is perspective both for pre-planting and postharvest treatment of potato. Physical treatment affects biochemical, cellular and physiological status of potato. Methods of physical treatment enable to control phytopathogens, and some methods (ultraviolet and gamma-radiation) even are capable of improving immunity of plants. The main traits of potato tubers that can be influenced by physical treatment are sprouting (stimulation or inhibition), susceptibility to rot and black leg diseases, and starch, reducing sugars and ascorbic acid contents. The tuber response to physical treatment depends on dosage and date of treatment, duration and temperature of storage, agricultural technology and cultivar. Low doses of treatment may be inefficient while too high dosage may result in cell deterioration or death and poor immunity, and eventually to disease development. Too early treatment may damage a tuber since it should pass through suberization (wound healing) after harvest; too late treatment requires higher doses. The proper adjustment of treatment is necessary for cultivar and individual storage conditions.

Key words: potato, physical treatment, hydrothermal treatment, steam, ultraviolet, xenon lamp, gamma-irradiation, electromagnetic microwaves, phytopathogen

INTRODUCTION

Potato tubers require proper treatment before planting, after harvesting and at storage to produce high yields and avoid storage losses. Different methods are used to reduce infection, promote sprouting or keep dormancy, maintain required physiological and biochemical status of a tuber.

There are different methods of potato tuber treatment, such as chemical, biological, breeding, and physical; application of them is determined by the aim and the cost

of technique. Physical treatment does not require complicated technologies such as chemical synthesis or development of new potato varieties or new bacteria strains and can be afforded by developing economies. In addition, it is ecological friendly and does not affect consumer health. Then, some physical methods may be competitive to genetic and chemical modifications as they do not require registration or special permission and may be more effective in certain aspects. On the other hand, such practices as hot dry, steaming, UV and gamma radiation are far from introduction at industrial scale as technologies and are still perspective and promising approaches. Moreover, each physical method if applied at improper dosage can damage or even kill a tuber so it is crucial to find a proper time and dose in each case depending on the aim of treatment and cultivar.

The aim of this paper is to review the achievements in physical treatment methods of potato.

TEMPERATURE TREATMENT

Hot water treatment of potato tubers significantly reduces rot bacteria contamination [1]. Mackay and Shipton [2] did not detect *Pectobacterium atrosepticum* and *P. carotovorum* subsp. *carotovorum* in tuber peel after dipping naturally infected potato tubers for 10 min in water at 55 °C as well as no blackleg symptoms were observed in plants grown from the treated tubers. Similarly, Wale and Robinson [3] and Shirsat et al. [4] showed that periderm and lenticels contamination can be significantly reduced by incubation in water at 44 °C for 30 min or at 56 °C for 5 min. However, if insufficiently dried out, the tubers may rot due to multiplication of survived bacteria. Drying tubers under forced ventilation with air knives may help to overcome this problem not only removing water from tuber surface but also keeping heat [5]. Hydrothermal treatment helps to control several fungal pathogens causing gangrene, skin spot, silver and black scab [6]. Abbas et al. [7] showed that treatment of tubers with hot water at 37 °C for 2—3 hours reduces Potato Leaf Roll Virus in plants grown from them by up to 37%. However, hydrothermal treatment may lead to delay in sprouting or even death of tuber depending on the physiological state and eventually to yield losses [1].

Steam can be used as an alternative to hot water to control fungi and bacteria especially *P. atrosepticum* and *P. carotovorum* present in tuber periderm. Steam treatment reduced periderm contamination from 26 ... 59% to 1 ... 3% [8]. Bartz and Kelman [9] observed that external population can be eliminated by hot air dry at 50 °C. Hot air dries tubers and stimulates wound healing without affecting subsequent sprouting. However, longer incubation time required for steam treatment compared to hot water can adversely affect a tuber [1].

Lavrova et al. [10] studied the effect of pre-planting cool treatment on seed tubers artificially infected with nematodes: sprouted tubers were kept at +5 °C for 2 hours during 6 days. As a result, yield quantity and quality (starch and ascorbic acid content) were improved when potato was grown on infested soils. Eremeev et al. [11] showed that pre-planting heat shock at 30 °C for 2 days with subsequent keeping at +12 ... +15 °C for 5 days in the light make it possible to get more tubers 5—10 days earlier than in the control that may be explained by increasing physiological age of the seed tubers.

SHORT-WAVELENGTH ULTRAVIOLET IRRADIATION

Short-wavelength ultraviolet light (UV-C) has strong antimicrobial effect. It provokes single and double-strand breaks in DNA, producing thymine dimmers and 4,6-photoproduct as well as reactive oxygen species. The resulted DNA lesions interfere with replication and transcription, eventually disabling or killing microorganisms [12—15]. Additionally, the UV-C light results in tryptophan, tyrosine, phenylalanine and cysteine oxidation with subsequent degradation and DNA-protein bonds that inhibit cell survival and proliferation [16].

The UV-C rays cover the wavelength range 100—280 nm, in practice mercury-vapor lamps (Hg-lamps) with a strong emission line at 254 nm are widely used for disinfection purposes. The UV-C energy is unable to penetrate tuber tissue to reach the vascular pathogens and therefore can be used for surface microorganisms mainly [1].

The UV-C irradiation at 10 kJ m^{-2} reduces incidence and severity of common scab by 28% and 62%, respectively, while silver scab is inhibited at 15 kJ m^{-2} by 22% and 36%, respectively [17]. Ranganna et al. [18] demonstrated that UV-C irradiation at 15 kJ m^{-2} inhibits soft and dry rot in the tubers inoculated with *Fusarium solani* and *P. carotovorum* at +8 °C for 3 months. However, the inhibition efficiency of UV-C dropped at longer inoculation of the tubers. Rocha et al. [19] observed that the UV-C treatment at 34.5 kJ m^{-2} significantly reduced postharvest soft rot incidence by 60% in potato seed tubers stored at +25 °C for 9 days after inoculation with *P. carotovorum* subsp. *carotovorum*. However, the disease was completely inhibited both in untreated and UV-C-treated tubers when they were stored under fluorescent light (280 ... 400 nm, UV-A + UV-B; 1.6 μmol m^{-2} s^{-1}). The disease inhibition may be associated with the accumulation of α-solanine and α-chocanine glycoalkaloids in periderm and flesh in UV-C- and fluorescent light treated tubers. Thus, the UV-C irradiation affects not only surface microorganisms but also tuber periderm layer changing its biochemical status, thus enhancing its immunity.

Besides disinfection of tubers the UV-C treatment can be used for potato tuber dormancy regulation at storage. Conventionally, low positive temperatures and ethylene are used to inhibit sprouting, but these techniques can give rise to high sugar concentrations reducing the processing quality of potatoes [20—22]. Cools et al. [23] demonstrated that sprouting inhibition in response to the UV-C treatment is cultivar specific. Tuber sprouting of the most sensitive to UV-C irradiation cultivars was inhibited at 10 kJ m^{-2} and associated with increase in ascorbic acid level; the strongest effect appeared at 10% eye movement. However, even at such high dose as 30 kJ m^{-2} authors did not find sprouting stimulation as it was observed in other experiments [9, 24, 25]. The UV-C treatment at 3.4 ... 13.6 kJ m^{-2} significantly reduced number of spouts in the irradiated tubers if stored at +20 °C for up to 20 days [26].

The UV-C treatment can affect biochemical parameters of tubers. Hg-lamp irradiation at 15 kJ m^{-2} changed neither texture nor color of a tuber as well as did not damage starch granules [25]. Cools et al. [23] did not found DNA degradation or cyclobutane pyrimidins in periderm of the tubers irradiated at 5 ... 30 kJ m^{-2}. The total reducing sugar content in the UV-C-treated tubers was 1.65 ... 2.02 fold lower compared to the

untreated control after storage at +4 °C for 28 days. Lin et al. [27] suggest that the UV-C treatment possibly regulates the gene cascade in potato tuber (sucrose phosphate synthase, invertase inhibitor 1/3, and invertase 1) that reduced accumulation of reducing sugars and prevented oxidative injury in potato cells.

CONTINUOUS-WAVE ULTRAVIOLET RADIATION

Mercury-free pulsed xenon lamp (Xe-lamps) can be used as an alternative to Hg-lamp. Xe-lamp is characterized by continuous emission spectrum (190 ... 400 nm, i.e. UV-A, UV-B and UV-C) that provides bactericide effect to the irradiated objects independent from the absorption spectrum at a shorter exposition time compared to Hg-lamp. The efficiency of the continuous-wave pulsed UV-light was proved for water disinfection [28, 29]. The advantages of Xe-lamps allow them to be widely used for disinfection purposes in different spheres: food production and storage [30, 31], disinfection of surfaces [32], and packaging [33].

To estimate the Xe-lamp irradiation effect on the integrity of DNA of *Dickeya solani*, pathogen causing potato blackleg, Kroupin et al. [34] performed quantitative polymerase chain reaction (qPCR). It was shown that each genomic 100 bp is damaged by ~10 J m^{-2}. The comparison of bactericide effects of Hg- and Xe-lamps demonstrated that Xe-lamp damages 4.9% of *D. solani* genome at 10 kJ m^{-2} while Hg-lamp only 1.5% at the same dose; unlike Hg-lamp, Xe-lamp irradiation resulted in protein degradation and aggregation [35]. The experiments on potato slices showed that Xe-lamp irradiation at 12 kJ m^{-2} almost totally inhibited the rot development while Hg-lamp irradiation even at 36 kJ m^{-2} resulted in the rot spot diameter three fold larger than that in the Xe-lamp variant [36, 37]. Further study of Xe-lamp irradiation may show its perspective in controlling pathogens as well as its effect on physiological and biochemical status of potato tubers.

Both UV-C and continuous-wave UV-irradiation can be used not only for tuber treatment but also for hygienic measures such as disinfection of irrigation water, machines and tools, storage and package.

GAMMA IRRADIATION

High-energy ionizing radiation affects enzyme activity, protein synthesis, metabolic processes in the cell, cell division, differentiation, hormone balance and gas exchange. As a result, considerable changes can occur in the tuber dormancy, starch, sugar and ascorbic acid content and, eventually, technological and custom properties of the tuber. Depending on the dose gamma irradiation may either induce or inhibit metabolic processes in the tuber, having some antimicrobial effect.

Stimulation effect of low gamma irradiation doses

Low doses of gamma radiation can be used for potato *in vitro* propagation to stimulate tuber formation. Al-Safadi et al. [38] found that irradiation at 2.5 Gy simulates microtuber production *in vitro* by up to 38% in comparison to untreated plantlets without fear of genetic changes in the used cultivars. Li et al. [39] demonstrated that irradiation

of the potato plantlets *in vitro* significantly stimulated microtuberization by 116.7% and 34.5% in the studied cultivars compared to the non-irradiated control and increase in fresh mass. Low doses (2 ... 3 Gy) increased starch content; medium doses (4 ... 6 Gy) increased protein content; higher doses (6 ... 8 Gy) increased ascorbic acid and reducing sugars in microtubers.

Moderate doses can be applied to stimulate sprouting of tubers with low germination ability, e.g. in maintenance of genetic stocks. Salomon et al. [40] demonstrated the stimulation effect of 20 Gy on germination of botanical seeds while Tikhonov et al. [41] observed the stimulation effect of 50 Gy on seed tubers.

Inhibiting effect of high gamma irradiation doses

The main goal of storage is to keep dormancy as close to postharvest level as it is possible. Second task is to keep starch content at the initial level. The sprout of tuber buds enhances respiration and induces enzymatic hydrolysis of the starch. Low temperature inhibits sprouting but results in accumulation of reducing sugars ('cold-induced sweetening'), which induce discoloration of potato chips or French fries [42]. Ionizing irradiation inhibits sprouting without using low temperatures at storage. It is especially critical for south regions with warm postharvest seasons. The third task is to keep tubers free from rot during storage period. Moderate gamma-irradiation doses may promote immunity response in the tuber, while too high doses may damage tissues and result in tuber rot.

The recommended gamma irradiation dose for sprout suppression varies from 40 ... 50 Gy to 120 ... 150 Gy [43—47]. The dose sufficient for sprout inhibition is apparently cultivar specific. For example, the total inhibition in cv. Lorkh and cv. Lyubava tubers was observed at dose higher than 18 Gy and 21 Gy, respectively [48].

Moderate gamma irradiation inhibits rot and scab severity. Tikhonov et al. [41] observed inhibition of silver scab after gamma irradiation at 100 Gy, whereas dose increase up to 150 Gy resulted in the development of common scab, silver scab and ring rot that may be explained by the inhibition of the tuber cell immunity at higher doses. Afify et al. [49] demonstrated that 50 Gy inhibits sprouting without causing rot that may be associated with high antioxidant enzyme activities (peroxidase, polyphenol oxidase, glutathione-S-transferase, superoxide dismutase, and catalase) and the lowest level of lipid peroxidaiton. Mahto et al. [46] suggests that the rot suppression at 150 Gy may be due to higher polygalacturonase-inhibiting protein activity in non-sprouted tubers compared to the sprouted tubers.

At higher gamma irradiation doses the cell wall and membrane are disrupted; rot development, water loss, skin and flesh darkening, bud blackening and tuber softening are observed. In most studies the destructive processes are shown at doses higher than 1 kGy [44, 50, 51]. Apparently, 500 Gy is the highest acceptable (threshold) dose and depends on the cultivar and tuber condition [46, 47]. Since bud tissues in eyes are meristematic and are least tolerant to gamma irradiation, their blackening occurs at 500 Gy [46]. Tuber lots with high rot potential may not be suitable for gamma irradiation treatment at all [43].

The date of tuber treatment (days after harvest, DAH) is critical: periderm is very susceptible to high irradiation doses immediately after harvesting and tuber should pass through suberization. The day of treatment influences the accumulation of sugars in tuber. Dhali et al. [47] showed that 150 Gy 15 DAH resulted in less sugar accumulation during 2 months of storage rather than treatment 30 DAH. Rezaee et al. [45] demonstrated that the later the treatment (10, 30 and 50 DAH were compared), the more sugar is accumulated after 5 months of storage both at +8 °C and +16 °C. After 120 days of storage tubers irradiated 5 DAH contained less reducing sugars than that 30 DAH [50].

The day of treatment determines the dose of gamma irradiation: the latter the treatment the higher dose is required. Treatment at low dose (40 Gy) on 6 DAH inhibits sprouting stronger and does not accompanied with shriveling compared to treatment on day 30 after harvest; treatment at both 80 and 120 Gy 5 and 30 DAH showed good tuber appearance [50]. Rezaee et al. [45] showed that treatment 10 DAH totally inhibits sprouting at 100 Gy; the irradiation dose for treatment 30 and 50 DAH should be raised up to 150 Gy. Dhali et al. [47] observed that weight loss of tubers irradiated 15 DAH is less than 30 DAH (150 Gy, 2 month of storage).

Temperature of storage is crucial since it is gamma irradiation make it possible to store potato tubers at moderate-to-high temperatures without accumulation of reducing sugars as it happens at low temperature storage. The higher the temperature of storage, the less sugar is accumulated in tubers. Dhali et al. [47] showed that tubers exposed to 150 Gy and subsequently stored at +6 °C for 2 months contained more sugars compared to +15 °C. Rezaee et al. [45] found that tubers irradiated at 100 Gy and subsequently stored at +8 °C accumulated more sugars than that at +16 °C. Ezekiel et al. [52] demonstrated the response to the storage temperature was cultivar specific: cv. Kufri Jyoti contained less reducing sugars at +16 °C than at +8 °C by 34%, whereas cv. Kufri Chandramukhi by 15% (150 Gy, 90 days of storage). Rezaee et al. [45] suggests that less sugar content at higher storage temperature may be due to higher respiration rates. In addition, temperature influences weight loss: weight loss was higher if tubers were stored at +16 °C than at +8 °C (100 Gy, 5 months of storage); these losses can be compensated by dose increase to 150 Gy.

Gamma irradiation may affect the integrity of starch granules and amylose and amylopectin molecules and the response of the cell may be cultivar specific [50, 52]. Ezekiel et al. [52] showed that the degradation of starch and, as a consequence, changes in sugar accumulation and amylose content after irradiation at 500 Gy was specific for each of three cultivars studied. Mahto et al. [46] found that cv. Kufri Sindhuri is characterized by increase in the textural parameters after irradiation at 500 Gy and storage at +12 °C for 120 days compared to cv. Kufri Jyoti. Cell wall remained rigid, accounting for higher textural values registered after treatment at 120 Gy and storage at +22 °C for 4 months; exposition to 1 kGy induced damage and resulted in more collapsed cells with less rigid cell walls [50].

The dose of gamma-irradiation affects the ascorbic acid content in tubers. The increase in irradiation dose resulted in drop in ascorbic acid in tubers when stored at +15 °C and +16 °C, respectively [45, 47].

Gamma irradiation leads to rise in amylose content [52, 53]. Lu et al. [53] suggests that it may be associated with the effect of gamma-rays on the branched molecules of amylopectin susceptible to low radiation dosage; the newly de-branched amylopectin chains contributed to the amylose content. At extreme dosage both amylose and amylopectin molecules are depolymerized and thus the starch shows the decreased amylose content [54, 55]. Tubers exposed to 150 Gy and stored at +24 °C for 35 days showed the starch content at the level of the untreated control [51]. Lu et al. [53] showed that starch content significantly decreased in two studied cultivars irradiated at 100 Gy and stored at +8 °C for 5 months.

Irradiation dose affects sugar content in tubers: the higher radiation, the higher sugar content. Apparently, sugar molecules are a product of starch glycoside bonds breakage by gamma-rays [54]. Frazier et al. [43] demonstrated that a dramatic raise in sugar content occurs after irradiation at 20 ... 40 Gy and storage at +7.2 °C; then the sugar content is normalized to the untreated control level in 210 days if stored at +7.2 °C or in a month if stored at +15 °C. The content of reducing sugars in tubers exposed to 1 kGy was 1.3 fold lower than at 80 Gy when stored at +22 °C for 120 days [50]; glucose content in tubers at 2 kGy was 1.8 fold higher than at 150 Gy when stored at +24 °C for 35 days. The glucose content dynamics through storage is cultivar specific: raise in glucose content in tubers of cv. F03031 and F03028 exposed to 100 Gy and stored at +8 °C was 1.5 and 2 fold, respectively; cv. Shepody did not show significant changes in glucose content [53]. Tubers of cv. Kufri Jyoti and cv. Kufri Chandramukhi treated at 500 Gy and stored at +16 °C contained 20% and 5% reducing sugars more than at 100 Gy, respectively.

Thus, the gamma irradiation treatment of potato tubers at moderate dosage (40 ... 150 Gy) should be thoroughly optimized. The main advantages of gamma irradiation are the inhibition of sprouting, the opportunity to store treated tubers at moderate (15 ... 22 °C) temperature and rot and scab control. On the other hand, the increase in dose results in raise in reducing sugar and amylose content, loss in starch and ascorbic acid content, drop in weight mass and specific gravity, rot development due to cell structure damage. The dosage, date of treatment (day after harvest), and temperature should be properly adjusted depending on a particular cultivar and storage duration.

MAGNETIC AND ELECTROMAGNETIC TREATMENT

Magnetic field can be used for tuber disinfection to promote sprouting of seed tubers and for postharvest treatment.

Pre-planting electromagnetic microwave irradiation of seed tubers at 10 kJ m^{-2} decreased significantly the incidence of round rot in the yield by 35%; irradiation at 1 kJ m^{-2} inhibited strongly common and silver scab incidences by 30% and 28% and severity by 40% and 11%, respectively. Postharvest treatment at 15 kJ m^{-2} reduced the incidence of round rot by 80% whereas 6 kJ m^{-2} did not affect the disease incidence [17].

Marks et al. [56] observed stimulation of length and number of stems, foliage and stem mass, and tuber sprouting index by canopy treatment with variable magnetic field.

Tikhonov et al. [17] found that small dosage of electromagnetic microwaves stimulated growth of sprouts that became more competitive to parasitic mycelium. Vasiliev et al. [57] showed the effect of electromagnetic microwave field on yield and starch content in tubers; the effect depended on the agricultural techniques used in the field experiments. The dosage of variable electromagnetic field affects the losses of tuber weight during storage at +25 °C for 16 days: the losses at 6 ... 8 mTs did not exceed 20% whereas at higher doses they raised to 75% compared to 39% in the untreated control [58].

OTHER PHYSICAL TREATMENTS

There are other perspective methods of potato tuber treatment studied in papers: helium plasma [59], treatment with modulated low-frequency electric field [60] and others.

CONCLUSIONS

In conclusion, the following physical treatment approaches have been studied for the last 35 years: steam and hot water, ultraviolet (UV-C and continuous-wave UV) and gamma irradiation, magnetic field. High-temperature treatment with hot water or steam can be used mainly to control blackleg. Treatment with high frequency waves (microwaves, gamma and ultraviolet irradiation) is characterized by more complex effect on tuber: disinfection (especially blackleg, scab and rot), immunity enhancing/suppression, sprouting stimulation/suppression, changes/maintenance of biochemical compounds content. The crucial parameters for physical field treatment are the proper adjustment of the dosage, date and storage conditions. Too low dosage may be insufficient to suppress pathogens, while too high dosage may result in cell wall damage and starch degradation that leads to rots and losses. A special attention should be paid to the influence of treatment on tuber dormancy: sprout stimulation is required for pre-planting treatment while postharvest treatment should inhibit sprouting. Finally, since UV and gamma irradiation are cultivar specific, the treatment technology should be finely adjusted for each cultivar depending on the conditions of growing and storage.

ACKNOWLEDGMENT

The work is financially supported in the frames of State Task of Federal Agency of Scientific Organization of Russia № 0574-2018-006.

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For citation:
Kroupin PY, Semenov OG. Physical methods of pre-planting and postharvest treatment of potato: a review. *RUDN Journal of Agronomy and Animal Industries*, 2018, 13 (4), 383—395. doi: 10.22363/2312-797X-2018-13-4-383-395.

DOI: 10.22363/2312-797X-2018-13-4-383-395

ФИЗИЧЕСКИЕ МЕТОДЫ ПРЕДПОСАДОЧНОЙ И ПОСЛЕУБОРОЧНОЙ ОБРАБОТКИ КАРТОФЕЛЯ: ОБЗОР

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Картофель является важной продовольственной культурой. Правильная предпосадочная и послеуборочная обработка клубней позволяет получить высокий урожай и избежать потерь при хранении. Среди различных методов обработки картофеля особое положение занимают физические методы: тепловая обработка горячей водой и паром, ультрафиолетовое (в том числе широкополосное) и гамма-облучение, обработка магнитными и электромагнитными полями (в том числе сверхвысокочастотными). Большинство физических методов относительно безвредны для окружающей среды и могут быть использованы без специальной регистрации и развивающимися странами. В статье впервые проведен обзор научных статей за последние 35 лет, посвященных физическим методам обработки картофеля (включая патенты на изобретения). Обзор научных статей показал перспективность данного направления как для предпосадочной, так и послеуборочной обработки картофеля. Физическая обработка оказывает воздействие на биохимический, клеточный и физиологический статус картофеля. Методы физической обработки позволяют контролировать фитопатогены, а отдельные методы (ультрафиолетовая, гамма-радиация) даже способны повышать иммунные свойства. Основные параметры клубней картофеля, на которые влияют методы физической обработки, — это прорастание глазков (стимуляция или ингибирование), поражение гнилью и черной ножкой, содержание крахмала, редуцирующих сахаров и аскорбиновой кислоты. Реакция клубней картофеля на методы физической обработки зависит от дозы, даты обработки, сроков и температуры хранения, агротехники и сорта. Низкие дозы обработки могут оказаться неэффективными, а слишком высокие могут привести к повреждению или гибели клеток и снижению иммунитета, а в конечном счете к развитию заболеваний. Слишком ранняя обработка может повредить клубень, так как после уборки ему необходимо пройти процесс суберинизации (заживления); при слишком поздней требуется повышение доз. При выборе метода физической обработки необходимо тщательно оптимизировать указанные параметры для конкретных условий хранения и сорта.

Ключевые слова: картофель, физическая обработка, гидротермическая обработка, пар, ультрафиолет, ксеноновая лампа, гамма-облучение, электромагнитные микроволны, фитопатоген

Для цитирования:
Крупин П.Ю, Семёнов О.Г. Физические методы предпосадочной и послеуборочной обработки картофеля: обзор // Вестник Российского университета дружбы народов. Серия: Агрономия и животноводство. 2018. Т. 13. № 4. С. 383—395. doi: 10.22363/2312-797X-2018-13-4-383-395.

RUDN Journal of Agronomy and Animal Industries 2018 Vol. 13 No. 4 396—404
Вестник РУДН. Серия: АГРОНОМИЯ И ЖИВОТНОВОДСТВО http://journals.rudn.ru/agronomy

DOI: 10.22363/2312-797X-2018-13-4-396-404

THE INTEREST OF HYPEROSMOLAR EXTENDERS IN ROOSTER SPERM CRYOPRESERVATION

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Abstract. The aim of the present study was to investigate thepotential benefit of hyperosmolar extender during the freezing-thawing process in rooster sperm. The goal was to minimize the amount of intracellular water and reduce the impact of intracellular icecrystal during the freezing process. A total of five 45-week old Hubbard commercial broilers were subjected to bi-weekly semen collections. Collected sperm was pooled and divided in three aliquots. The control aliquot was diluted with Tris-extender without further supplementation at 300 mOsm (Control). The two (2) other aliquots were diluted with Tris-extender at 300 mOsm containing vitamin E (Vit E) or with Tris-extender without Vitamin E but at osmolarity of 450 mOsm (Hyper). After incubation at 22 °C for 15 min, all aliquots were cooled and then frozen in liquid nitrogen. A Computer Aided Semen Analysis (CASA) was used to investigate the impact on different motility parameters. After thawing, Hyperosmolar (Hyper) and vitamin E extenders showed the highest values in terms of sperm motility preservation. Hyperosmolar extender (Hyper) showed particularly the highest values in terms of VSL velocities and progressive motile spermatozoa, known as indicators of sperm quality. In conclusion, the present results revealed that a significant impact was observed when using extenders at osmolarity of 450 mOsmin rooster sperm, in the same manner and even better then when using vitamin E. The positive impact is probably related to the reduction of intracellular ice formation.

Key words: Poultry, sperm, cryopreservation, hyperosmolar extender, oxidative stress, cold chock, spermatozoa, intracellular water

INTRODUCTION

It is well-known in rooster that artificial insemination (AI) is routinely carried out after short term storage at 4°C and it evidenced that fertilizing capacity of freshly collected avian semen is dramatically lost after half an hour [1] compromising consequently fertility outputs. In addition, fertility success of frozen poultry semen is far lower than any of the domesticated mammalian species. It has been estimated that post-thawing chicken semen retains only 1.6% of the fertilizing capacity of fresh semen [2].

Therefore, development of semen extenders, including investigation of different active compounds, was considered worldwide by research groups to enhance hatchability results and to protect post-thawed sperm motility.The strategies are essentially focused to protect cell membrane against cold shock and oxidative stress [3, 4].

Oxidative stress, targeting cell membranes, has been identified as the main factor damaging phospholipids in different animal species including chicken [5, 6] and turkey [7]. Particularly, semen preservation for extended period causes several time-dependent structural and biochemical damages in avian [8, 9] and other mammalian species [10]. In avian, related to the high proportion of PUFA [11—13], cells membranes are more subjected to oxidative stress damages [14, 15].

There is also evidence that during cryopreservation, membrane damage caused by intracellular ice formation in spermatozoa varies to a great extent [16, 17] with the membrane damage related to cold shock and oxidative stress being [18, 19]. The main damage caused by cold shock is observed during the cooling process, when membrane lipids undergo a phase transition from a liquid to a gel state with subsequent disturbance of the membrane structure [20, 21].

During the cryopreservation phase when there is ice crystal formation, there is simultaneously excessive generation of reactive oxygen species (ROS) with a minimal sperm antioxidant capacity existing in the gametes [22]. The first targets of ROS are membrane lipids, particularly polyunsaturated fatty acids (PUFA), inducing the peroxidation and alteration in membrane fluidity and permeability [23—25].

The current study aimed to reduce intracellular ice formation by subjecting rooster spermatozoa to hyperosmolarity (450 mOsm) to increase a driving force for water efflux from the cell. We assume that reducing intracellular ice formation will reduce sperm cells injuries and consequently will protect sperm motility, known as the major factor conditioning fertility outputs.

MATERIALS AND METHODS

All experiments were conducted in accordance with the legislation governing the ethical treatment of animals. A total of five 45-weeks old Hubbard commercial broiler reproductive cocks were used during the experiment. The animals were housed in conventional individual cages under 14 hours of daily illumination, and fed with a standard commercial food at the rate of 155 g/day/animal.

The roosters were subjected to bi-weekly semen collections by dorso-abdominal massage as described by Burros and Quinn [26]. The collected ejaculates were then pooled and analysed. In order to minimize animal stress, the collection was carried out by the same operator and under the same conditions. Necessary precautions were taken during collection to avoid contamination by cloaca fluids. Sperm motility was calculated using a computer-assisted sperm analyser (Sperm class analyser, SCA Microptic, S.L., Version 3.2.0, Barcelona, Spain).

Motility was assessed using a Computer Assisted Sperm Analyzer (CASA; Sperm class analyzer, SCA Microptic, S.L., Version3.2.0, Barcelona, Spain). To facilitate the image capture, the samples were diluted (10—20 · 10^6 Spz/ml) using Tris-extender. Subsequently, 5 μl of each sample was placed onto a warmed (37 °C) 20 μm Leja® 3 chamber slide (Leja Products B.V., Nieuw-Vennep, The Netherlands). Leja® slide was placed under a phase-contrast microscope (Nikon E200®-LED microscope) on a warmed stage (37 °C) and images were captured using a video camera (Caméra Digital Basler A312 fc Germany) at magnification ×10. Four sequences were scanned and

at least 200 spermatozoa were analyzed. The standard settings were set at 25 frames/s, 20—90 μm^2 for head area and VCL > 10 μm/s to classify a spermatozoa as motile. Kinetic variables that were assessed were: total motility (TM%), progressive motility (PM%), movement linearity (LIN%); straightness (STR%); wobble (WOB%); curvilinear velocity (VCL μm/s); straight linear velocity (VSL μm/s); average path velocity (VAP μm/s); amplitude of lateral movement of the head (ALH μm); beat cross frequency (BCF Hertz), % rapid gametes (fraction of cells moving with VCL > 75 μm/s), % moderately motile gametes (fraction of cells moving with 45 < VCL < 75 μm/s) and % slow gametes (fraction of cells moving with 10 < VCL < 45 μm/s). Total motility (TM) was defined as the percentage of spermatozoa with VCL > 10 μm/s, and progressive motility (PM) was defined as the percentage of spermatozoa with VCL > 25 μm/s and STR > 80%.

All samples had a minimum of 70% motile sperm. The sperm samples were then pooled and divided into three aliquots. The control aliquot was diluted with Tris-egg yolk extender without further supplementation at 300 mOsm(Control). The two (2) other aliquots were diluted with Tris-egg yolk extenders at 300 mOsm, containing vitamin E (Vit E) or with Tris-egg yolk extenders without Vitamin E but at osmolarity of 450 mOsm (Hyper). Tris-extender was composed of 300 mM tris[hydroxymethyl]aminomethane, 95 mM citric acid monohydrate, 28 mM D-(+)-Glucose.

All samples were equilibrated for 2 h at 4 °C and then packaged into 0.25 ml straws and frozen in liquid nitrogen vapor using a programmable freezer (CryoMed, Thermo Fisher Scientific, Waltham, MA, USA). Frozen straws were then thawed in a water bath at 5 °C for 3 minutes and sperm motility analyzed using a Computer Aided Sperm Analyzer (CASA).

Calculation of means, standard error of the mean (SEM), and statistical analysis were performed using Statview 4.02 software (Abacus Concepts Inc., Berkeley, CA, USA). Values for each variable were expressed as the mean ± SEM. Variables used for comparison purposes were the three sperm extenders (control, Hyper, Vit E). Differences between treatments were assessed using one-way ANOVA, followed by *posthoc* Fisher's test. Values were considered significant when $P < 0.05$.

RESULTS AND DISCUSSION

Percentages of total motility and progressive motility

The data regarding the effect of different sperm extenders are presented in figure 1. Compared to the control, the results indicated that Hyperosmolar and vit E extenders protected all CASA motility variables.

Concerning TM, a significant difference ($P < 0.05$) was observed between the control and the two others tested extender (Hyper and Vit E), with means of 11.11, 48.89 and 44.87%, respectively. It's obvious that the control group frozen by a standard extender with 300 mOsmshowed the lowest total motility values. No significant difference was observed when comparing Hyperosmolar (Hyper) and vitamin E (Vit E) extenders. Concerning the progressive motility (PM), these two treatments showed sensibly the same values (12.82 and 11.11%), values significantly higher than those of the control group (2.85%).

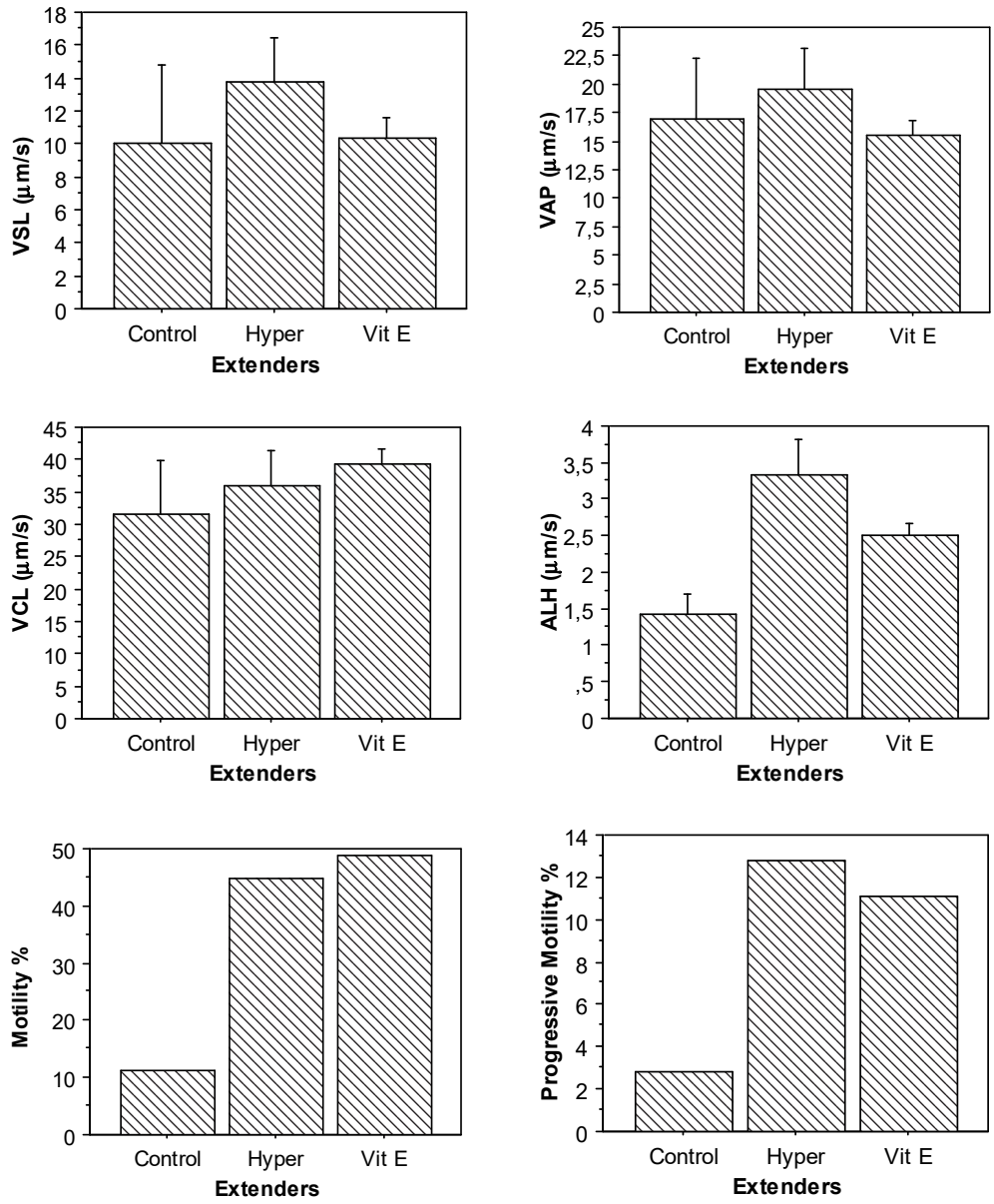

Fig. 1. Percentages (Mean ± S.E.M.) of total motility (TM), progressively motility (PM), curvilinear velocity (VCL), straight linear velocity (VSL), average path velocity (VAP), linearity lateral movement of the head (ALH) after cryopreservation of rooster sperm in 300 mOsm extender (control) hyper-osmolar extender with 450 mOsm (hyper) and extender containing vitamin E (Vit E). Values are presented as Mean ± S.E.M

Kinematic parameters (VCL, VAP, VSL, ALH)

The impact of investigated treatments on VCL, VSL, VAP and ALH are presented in Fig. 1. The lowest values were observed in the control group with 31.58 ± 8.35 µm/s, 10.01 ± 4.78 µm/s, 17.01 ± 5.31 µm/s and 1.43 ± 0.28 µm. Among all treatments, hyperosmolar extender showed the highest values,except for VCL, followed by Vitamin E

extender with 35.93 ± 5.35 μm/s versus 39.18 ± 2.34 μm/s, 13.75 ± 2.69 μm/s versus 10.35 ± 1.19 μm/s, (19.59 ± 3.53 μm/s versus 15.59 ± 1.27 μm/s and (3.33 ± 0.5 versus 2.5 ± 0.16 μm, for VCL, VSL, VAP and ALH, respectively.

The aim of the present experiment was to improve rooster sperm cryopreservation by lowering the amount of intracellular water formation through the use of hyperosmolar extender at 450 mOsm. Two controls were concomitantly used, an isotonic extender with 300 mOsm and an extender containing vitamin E.

Vitamin E is one of the most potent molecules known to inhibit production of ROS and LPO. Vitamin E, a lipophilic molecule, is used both as an antioxidant [27], to combat LPO, and as a stabilizer of plasma membranes [28]. The beneficial effect of vitamin E on sperm was previously observed [29, 30]. Vitamin E (Vit E) increased significantly VCL, VAP, ALH and BCF when compared to the control. This is in accordance with the previous reports where vitamin E was found to increase sperm motility and membrane integrity [31, 32].

The phospholipidic fraction of spermatozoa cell membranes characterized by a high proportion of polyunsaturated fatty acids (PUFA) [33] is essential to membrane fluidity particularly in fusion events including acrosome reaction, sperm egg-interaction and motility [34, 35]. Accordingly, high membrane concentration in PUFA makes sperm cells more susceptible to lipid peroxidation, especially, during the different stages of *in vitro* preservation [36]. In this respect, vitamin E showed in turkey significant positive effect on sperm mobility and viability during storage for 48 h at 5 °C [37] and enhanced fertility outputs [38]. As well, similar positive impacts have been observed in avian diet supplementation with alpha-tocopherol [39]. Similarly, dietary supplementation with other antioxidants, including vitamin E, selenium, vitamin C and enzymatic antioxidant systems improved significantly animal health and sperm quality [40, 41].

In the current experiment hyperosmolar extender showed sensibly the same protective effects as vitamin E treatment. It seems that the rate of water efflux from intracellular compartment was significantly enhanced in these hyperosmolar conditions by increasing permeability to the water in spermatozoa cell membranes. Contrary, in the control group, during cryopreservation the cell may not be able to loseenough intracellular water; the cytoplasm becomes super cooled and can freeze, which usually results in a lethal injury as previously demonstrated [42].

The susceptibility of sperm membrane to cold shock and lipid peroxidation (LPO) depends on cholesterol, a membrane stabilizer at low temperature and PUFA [43]. It is well established that cryopreservation induces cholesterol depletion from the membrane which could exacerbate sperm cold shock damage [44, 45], probably by reducing the amount of intracellular ice formation, such membrane cells alteration could be significantly avoided.

The current results showed that rooster sperm cells could be preserved in the same extent by the use of a potent antioxidant molecule (vitamin E) and by reducing intracellular ice formation. Consequently, these results open interesting alternatives in developing new rooster sperm diluents by a simultaneous use of hyperosmolar solutions at 450 mOsm and antioxidant molecules, mainly vitamin E. However, further research is warranted particularly in terms of fertility outputs after artificial insemination.

CONCLUSIONS

The current results showed that rooster sperm cells could be preserved in the same extent by the use of a potent antioxidant molecule (vitamin E) and by reducing intracellular ice formation. Consequently, these results open interesting alternatives in developing new rooster sperm diluents by a simultaneous use of hyperosmolar solutions at 450 mOsm and antioxidant molecules, mainly vitamin E. However, further research is warranted particularly in terms of fertility outputs after artificial insemination.

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For citation:
Iguer-ouada Mokrane, Norezzine Abdelaziz, Rebouh Nazih Yacer. The interest of hyperosmolar extenders in rooster sperm cryopreservation. *RUDN Journal of Agronomy and Animal Industries*, 2018, 13 (4), 396—404. doi: 10.22363/2312-797X-2018-13-4-396-404.

DOI: 10.22363/2312-797X-2018-13-4-396-404

ГИПЕРПОСМОЛЯРНЫЕ НАПОЛНИТЕЛИ СПЕРМЫ ПЕТУХОВ ПРИ ЕЕ КРИОКОНСЕРВАЦИИ

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Целью настоящей работы было исследование потенциального преимущества гиперосмолярного наполнителя в процессе замораживания—оттаивания спермы петухов. Цель заключалась в том, чтобы свести к минимуму количество внутриклеточной воды и уменьшить влияние внутриклеточного льда в процессе замораживания. В общей сложности у пяти 45-недельных коммерческих бройлеров кросса «Hubbard» были взяты образцы спермы в течение двухнедельного периода. Собранную сперму объединяли и делили на три равные части. Контрольный образец разбавляли трис-наполнителем без дополнительных добавок при 300 mOsm (контроль). Два других образца разбавляли трис-наполнителем при 300 мОм, содержащим витамин Е (Vit E), или с трис-наполнителем без витамина Е, но при осмолярности 450 mOsm (Hyper). После инкубации при 22 °С в течение 15 мин все образцы охлаждали и затем замораживали в жидком азоте. Для исследования влияния на различные параметры моторики был использован анализ спермы с использованием метода (CASA). После оттаивания наполнители с Hyperosmolar (Hyper) и витамином Е показали наилучшие значения с точки зрения сохранения подвижности сперматозоидов. Гиперосмолярный наполнитель (Hyper) показал особенно высокие значения в отношении скорости движения VSL и прогрессирующих подвижных сперматозоидов, известных как показатели качества спермы. Представленные результаты показали, что при использовании наполнителей спермы петухов при осмолярности, равной 450 mOsm, наблюдали существенное улучшение показателей сперматозоидов, которое было даже лучше, чем при использовании витамина Е. Положительное влияние, вероятно, связано с уменьшением внутриклеточного образования льда.

Ключевые слова: птица, сперма, криоконсервация, гиперосмолярный наполнитель, окислительный стресс, холодный поднос, сперматозоиды, внутриклеточная вода