UDC 636.5.084.5.639.09:614.95

PETER F. SURAI, *PhD, DSc, Professor*
Trakia University, Stara Zagora 6000, Bulgaria,
Moscow State Academy of Veterinary Medicine and Biotechnology named after K.I. Skryabin, Moscow, 109472, Russia,
Szent Istvan University, Gödöllo H-2103, Hungary,
Saint-Petersburg State Academy of Veterinary Medicine, St. Petersburg, Russia,
Sumy National agrarian University, Sumy, Ukraine,
Odessa National Academy of Food Technologies, Odessa, Ukraine,
Russian Academy of Sciences, Moscow, Russia

E-mail: psurai@feedfood.co.uk

Vitagenes in poultry production: adaptation to commercially relevant stresses

Abstract. ***Commercial poultry production is associated with various stresses and the vitagene network is responsible for stress adaptation. Indeed, activation of vitagenes via such transcription factors as Nrf2 and HSF leads to an additional synthesis of an array of protective molecules which can deal with increased ROS/RNS production. Therefore, nutritional modulation of vitagenes is considered as a new direction in nutritional research. Therefore, there is an opportunity to activate a range of vitagenes (via Nrf2-related mechanisms: superoxide dismutase, SOD; heme oxygenase-1, HO-1; GSH and thioredoxin, or other mechanisms: Heat shock protein (HSP)/heat shock factor (HSP), sirtuins, etc.) to maximise internal AO protection and maintain redox balance and improve stress resistance. Therefore, the development of vitagene-regulating nutritional supplements is on the agenda of many commercial companies worldwide. Our recent data indicate that vitagene-regulating mixture (PerforMax/Magic Antistress Mix) showed promising results in fighting stresses and found its way into commercial poultry production.***

Key words: *poultry, stress, antioxidants, molecular mechanism, vitagenes*

Introduction

Commercial poultry production is associated with various stresses and molecular mechanisms of stress adaptation is the most important topic of the current poultry research. Indeed, recently a vitagene concept has been developed explaining how stress adaptation proceeds at the molecular level *(Surai and Fisinin, 2016a; 2016b; Surai et al., 2017a; 2019)* and the main aim of the paper is to present an updated view on this concept and its application in poultry production.

1. Vitagene Network

The term "vitagene" was first introduced by Rattan in 1998 to describe various maintenance and repair processes in the cell *(Rattan, 1998)*. Therefore, several important genes coding proteins that regulate the complex network of the so-called longevity assurance processes were suggested to be called "vitagenes". The vitagene concept was first developed in relation to the medical sciences by Calabrese and colleagues *(Calabrese et al., 2014)* and the major pro-survival mechanisms in the body/cells which are under vitagene network control are shown in Figure 1.

As can be seem from Figure 1, the vitagene network operates on four levels: at the molecular level, cellular level, tissue and organ level as well as physiological and redox control level. The vitagene concept was successfully transferred from medical science and applied in poultry and animal production *(Surai, 2018; Surai and Fisinin, 2016a; 2016b; Surai et al., 2017a; Surai et al., 2019)*.

Therefore, the vitagene family includes:

- Heat shock proteins (HSPs): HSP70 and heme oxigesnase-1 (HO-1);
- SOD;
- Thioredoxin system (Trx, Trx peroxidase (peroxiredoxins), Sulfiredoxin and TrxR);
- Glutathione system (GSH, glutathione reductase (GR), glutaredoxin (Grx), GPx); and
- Sirtuins.

Indeed, HSP regulation in avian species has been recently reviewed *(Surai and Kochish, 2017)* and it was concluded that

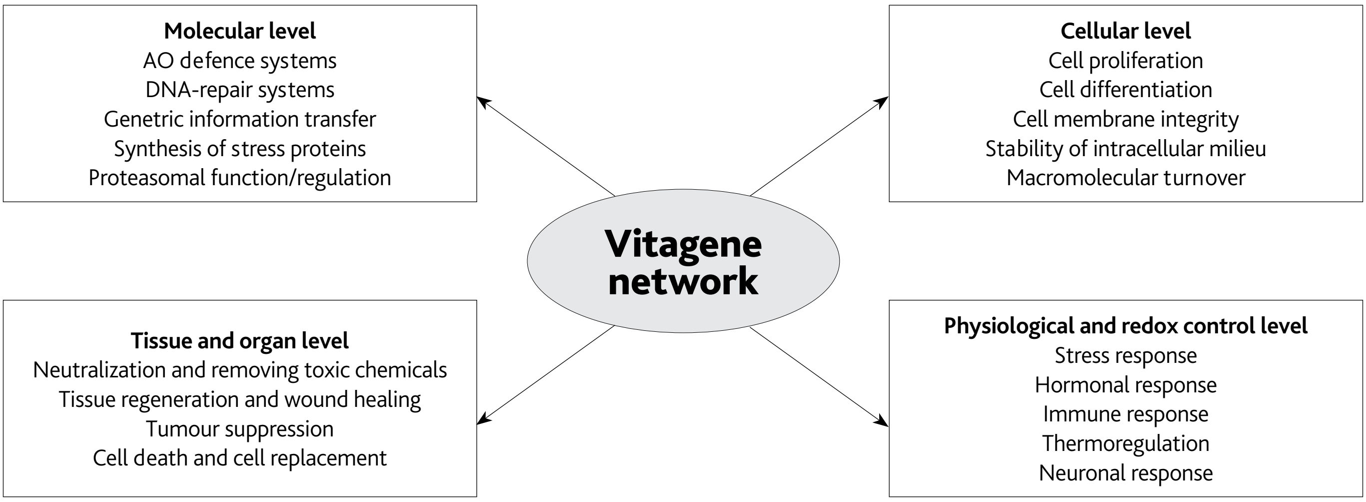

Figure 1. Major components of the vitagene network (adapted from *Surai et al., 2019*).

HSP70 and HO-1 are major players in adaptation to stress. Indeed, additional synthesis of HSP70 in response to various stresses is considered to be an adaptive mechanism to deal with cellular proteostasis. Indeed, most traditional antioxidants (e.g. vitamin E, carotenoids, coenzyme Q, etc.) deal with products of lipid peroxidation while protein structure integrity determines their biological activity. Protein synthesis in stress conditions faces problems of incorrect folding leading to decreased protein functional activities including enzymatic activity, receptor functions and signaling functions. Therefore, HSP70 having chaperone function is of great importance for protein homeostasis in stress conditions. Similarly, adaptive additional synthesis of HO-1 in response to stress also contributes to the adaptation to stress. Indeed, our critical analysis of recent literature shows that HSP70 and HO-1 expression in avian species effectively responds to commercially relevant stressors including heat stress, heavy metal stress, Se deficiency, chicken transportation and increased stocking density *(Surai and Kochish, 2017)*. It is proven that HSP expression can be effectively regulated by nutritional means, including vitamins E, C and D, carnitine, betaine *(Surai et al., 2017a)* and some phytochemicals such as silymarin *(Surai, 2015a)*. Indeed, these vitagenes (HSP70 and HO-1) are important elements responsible for adaptation of poultry to various stresses by maintaining optimal cell proteostasis.

SOD, as important vitagene, is considered to be the main driving force in cell/body adaptation to various commercially relevant stress conditions *(Surai, 2016)*. Since the superoxide radical is the main free radical produced in physiological conditions in the cell *(Surai, 2018)*, SOD is thought to be the key element of the first level of antioxidant defence in the cell. Recently, the protective roles of SOD in avian biology and poultry production have been reviewed *(Surai, 2016)*. It was shown that SOD activity in avian species is tissue-specific and depended on many different factors including genetics, nutrition and various stress-related factors, such as heat, heavy metals, mycotoxins and other toxicants. Indeed, SOD was shown to provide an effective protection against lipid peroxidation in chicken embryonic tissues and in semen *(Surai et al., 2019)*. It should be mentioned that there are complex interactions inside the antioxidant network of the cell/body responsible for to maintenance of homeostasis under stress conditions. In fact, nutritional means of SOD upregulation in poultry production and possible commercial consequences of such upregulation await further investigation. Indeed, in the medical sciences, manipulation of SOD expression and SOD mimics are used as an important tool in disease prevention and treatment.

Thioredoxin and glutathione systems regulated by vitagenes are not well characterised in poultry, but existing evidence clearly shows that these systems are deeply involved in cellular redox balance maintenance and by doing so, they actively regulate poultry adaptation to various stresses *(Surai et al., 2019)*. A thiol redox system consisting of the glutathione system (glutathione/glutathione reductase/glutaredoxin/glutathione peroxidase) and the thioredoxin system (thioredoxin/thioredoxin peroxidase (peroxiredoxins)/sulfiredoxin/thioredoxin reductase are thought to be the major players in redox status regulation. In fact, the thioredoxin system is an important thiol/disulphide redox controller maintaining the redox homeostasis. Recent studies have clearly shown that the thioredoxin system is involved in the redox regulation of the expression of genes regulating various cellular functions, including synthesis of deoxyribonucleotides (DNA synthesis and repair), protein biosynthesis, hormone and cytokine action, apoptosis, etc., being a key element of the anti-stress strategy in the cell/body *(Surai et al., 2019)*. In fact, chicken Trx is shown to be a protein of 105 amino acids with a molecular weight of 11,700. The sequence of the chicken Trx is found to be very similar to the sequences of other thioredoxins. In particular, comparison of the chicken Trx protein sequence with those from bacteria and plants indicated structural features that appear to be essential for activity. At least four different classes of Prx protein have been identified to be evolutionary conserved in chickens. In fact, chicken Prx proteins possess antioxidant activity; however, Prx expression in chickens is not tissue specific, confirming its essentiality as a housekeeping gene in all tissues to protect against oxidative damage (For review see Surai and Fisinin, 2016). Prx1 was shown to be expressed in chicken macrophages, chicken embryonic kidney and chicken jejunum. Furthermore, chicken Prx6 was indicated to be expressed in chick-

en liver and chicken gut *(for review see Surai et al., 2019)*. Acute heat stress was found to upregulate Prx1 and Prx3 in the small yellow follicles of layer-type chickens. Therefore, it seems likely that in poultry Trxs, Prdxs and TrxRs function as signal transduction proteins regulating stress-induced signalling cascades being important antioxidants participating in cellular/organismal adaptation to stress and their upregulation is considered to be an important approach to improve stress resistance of poultry.

It is generally accepted that GSH can be synthesized in poultry from three amino acids (i.e., L-glutamate, L-cysteine and glycine) with glutamate cysteine ligase (GCL) being the rate-limiting enzyme in GSH biogenesis. Therefore, Nrf2-regulated synthesis of GCL is of great importance for the antioxidant defence network efficacy. GSH is exclusively synthesized in cytosol and compartmentalized in different organelles, including nuclei, endoplasmic reticulum (ER) and mitochondria. Interestingly, nuclear GSH is found exclusively in the reduced form and participating in preserving proteins involved in DNA repair and gene transcription. Furthermore, mitochondrial GSH maintains the mitochondrial integrity by controlling mitochondrial ROS generation and apoptotic signalling. Therefore, cellular GSH is a key regulator of different biological processes, including synthesis of DNA and proteins, affecting cell growth and proliferation, apoptosis, immunity, amino acid transport, xenobiotic and endogenous oxidant metabolism/detoxification, redox-sensitive signal transduction, etc. On the one hand, the GSH thiolic group can directly react with and detoxify a range of ROS, including H2O2, superoxide anion, hydroxyl radicals, alkoxyl radicals and hydroperoxides *(for review see Surai et al., 2019)*. There is a range of proteins with GSH-dependent hydroperoxidase activity, including GPx, peroxiredoxins (Prx)-isoforms, some Grx and many GST. Importantly, in stress conditions GSH plays a crucial role as a redox buffer (GSG/GSSG) regulating the redox status of the living cells, responsible for prevention of the loss of protein thiols and providing optimal redox milieu for signalling. Indeed, the ratio of GSH/GSSG reflects redox balance being the main indictor of the cellular redox potential. Under oxidative stress, a decreased redox potential (GSH/GSSG ratio) alters the physiological functions of affected proteins. Molecular mechanisms of GSH-Px action in poultry have been recently reviewed *(Surai et al., 2018b; 2018c)*.

Sirtuins (SIRTs) are a highly conserved family of NAD+-dependent enzymes possessing deacetylases, deacylase, mono-ADP-ribosyltransferase and other activities *(Surai et al., 2019)*. There are seven members of the sirtuin family, SIRT1–SIRT7, which are located in different subcellular compartments and they are ubiquitously distributed from eubacteria to mammals. In particular, SIRTs have been shown to be associated with various cellular and metabolic processes regulating cell plasticity mechanisms of adaptation to various stresses by affecting specific transcription factors. In fact, SIRTs orchestrate cellular stress response and maintain genome integrity and protein stability *(Surai et al., 2019)* and involved in a number of biological processes, including cell growth and differentiations, apoptosis, chromatin condensation, energy transduction and glucose homeostasis, DNA repair and apoptosis, muscle and fat differentiation, neurogenesis, mitochondrial biogenesis, glucose and insulin homeostasis, hormone secretion, cell stress responses, etc. Therefore, sirtuins participate in various stress-related pathways within the complex signalling network responsible for regulation of stress response and restoration of adaptive homeostasis under stress conditions. Recently the expression and regulation of sirtuins in chicken liver have been described *(Ren et al., 2017)*. In particular, chicken SIRTs share the same conserved functional SIR2 domains and they are located in various cellular compartments, including the nucleus (cSIRT3 and cSIRT5), cytoplasm (cSIRT2 and cSIRT4), and in both the cytoplasm and nucleus (cSIRT1, cSIRT6 and cSIRT7). All sirtuins except cSIRT7 possess a deacetylase activity. It seems likely that chicken sirtuins play roles in central intermediary metabolism (cSIRT1, cSIRT2, cSIRT5 and cSIRT6) and in amino acid biosynthesis (cSIRT3). Interestingly, cSIRT4 has been suggested to take part in transcription regulation, with potential regulatory functions. In laying hens, SIRTs were shown to be age-dependently expressed in the heart, liver, pectoralis, kidney, spleen, abdominal fat, duodenum, glandular stomach, pancreas and lungs. An age-related regulation of gene expression (with increasing with sexual maturity) of *cSIRT1*, *cSIRT2*, *cSIRT4*, *cSIRT6* and *cSIRT7* was observed in the chicken liver *(Ren et al., 2017)*. Clearly, sirtuins are expected to be important players in adaptive responses of poultry to stress and this topic awaits further investigation. Research related to assays of SIRT expression in poultry is quite limited, but accumulating evidence indicates that SIRTs are highly conserved among various organisms/ Similar to mammals, stress can induce SIRT expression in birds, e.g., there was an upregulation of SIRT1 in the chicken hypothalamus, liver and muscle in response to 48 h fasting. In contrast, HS was shown to downregulate SIRT1 in the chicken liver, while dietary supplementation of epigallocatechin gallate ameliorated the detrimental effects of HS on SIRT1 expression *(for review see Surai et al., 2019)*.

Accumulating experimental evidence indicates that there is a great opportunity to nutritionally modulate the vitagene network using various nutritional supplements, including phytochemicals *(Surai, 2015a)*, carnitine *(Surai, 2015b; 2015c; 2015d)*, betaine, taurine and vitamins A, E and D *(Surai et al., 2017a)*. In fact, activation of the vitagene network by nutritional means is considered a new fundamental approach for improving animal/poultry resistance to various stresses *(Surai et al., 2017a; 2019)*.

Conclusions

Commercial poultry production has to deal with a range of stresses, from hatching (high temperature and humidity) up to slaughter (catching, transportation and holding). It seems likely that in many cases, it is possible to improve the rearing and welfare conditions of broilers, layers and breeders, but the major limitation is the cost of such improvements. A growing body of evidence indicates that an excess of ROS/RNS production and oxidative stress are major molecular mechanisms of most common commercial stressors in poultry production. During evolution, antioxidant defence systems developed in birds provided them a chance to survive in an oxygenated atmosphere. The antioxidant defence network includes internally synthesised (e.g., antioxidant enzymes, GSH, CoQ) and externally supplied (vitamin E, carotenoids, etc.) antioxidants. It should be taken into account that all antioxidants in the body are working cooperatively together as a team to maintain optimal redox balance in the cell/body, a key element in cell signalling, regulation of the expression of various genes, stress adaptation and homeostasis maintenance in birds. Therefore, cellular ROS concentration is strictly regulated by the antioxidant defence network in conjunction with various transcription factors and vitagenes. For example, activation of such transcription factors as Nrf2 leads to an additional synthesis of an array of protective molecules which can deal with increased ROS/RNS production and oxidative stress. However, when stress is too high to be dealt with by Nrf-2-related mechanisms, other transcription factors including NF-κB become predominant, and inflammation and apoptosis predispose healthy tissues to damage and to the development of various disease states and decreasing the productive and reproductive performances of poultry. Therefore, it is a challenging task for poultry nutritionists is to develop a system of optimal antioxidant supplementation to help growing/productive birds maintain effective antioxidant defences and redox balance in the body. On the one hand, antioxidants, such as vitamin E or minerals, such as Se (a precursor of GPx and other selenoproteins, Mn, Cu and Zn (important parts of SOD), have become a compulsory part of the commercial premixes for poultry and, in most cases, their levels in premixes are sufficient to meet the physiological requirements in these elements independently on their provision with feed ingredients. On the other hand, in commercially relevant stress conditions, there is a need for additional support for the antioxidant system in poultry. The new direction in improving the antioxidant defences of poultry in stress conditions is related to an opportunity to activate a range of vitagenes (via Nrf2-related mechanisms: SOD, HO-1, GSH, Trx; via NF-κB-related mechanisms: SOD, HO-1; or other mechanisms: HSP, sirtuins, etc.). This can maximize an internal AO defences and help maintain optimal redox balance, cell signaling and improves stress adaptation. Therefore, the development of vitagene-regulating nutritional supplements is on the agenda of many commercial companies worldwide. One successful example could be a complex mixture of nutrients called Magic Antistress Mix/PerforMax containing carnitine, betaine, vitamins, minerals, organic acids, etc., which are commercially available for poultry *(Velichko et al., 2013; Shatskikh et al., 2015; Grigorieva et al., 2017)*. Indeed, prevention of the detrimental consequences of stressors and improved performance in broilers *(Velichko et al., 2013; Grigorieva et al., 2017)*, broiler breeders and layers *(Shatskikh et al., 2015)* using vitagene-activation may optimize the antioxidant defence system. Furthermore, the same antistress composition (PerforMax/Feed-Food Magic Antistress Mix) was shown to be effective in pig production *(Surai and Melnichuk, 2012; Gaponov et al., 2012; Surai and Litvinov, 2017; Surai et al., 2017b)*. ■

П.Ф. СУРАЙ, *доктор філософії, доктор біологічних наук*
Тракійскій університет, Стара Загора, Болгарія,
Московська державна академія ветеринарної медицини та біотехнології імені Скрябіна, Москва, Росія,
Університет Святого Іштвана, Годоло, Угорщина,
Санкт-Петербурзька академія ветеринарної медицини, Санкт-Петербург, Росія,
Сумський національний аграрний університет, Суми, Україна,
Одеська національна академія харчових технологій, Одеса, Україна,
Російська академія наук, Москва, Росія
E-mail: psurai@feedfood.co.uk

Вітагени у птахівництві: адаптація до промислових стресів

Анотація. ***Промислове птахівництво пов'язано з різними стресами і вітагени відповідальні за адаптацію до стресів. Загалом, активація вітагенів через такі фактори транскрипції, як Nrf2 і HSF призводить до додаткового синтезу захисних молекул, які захищають від шкідливої дії вільних радикалів. Таким чином,***

активація вітагенів різними нутрієнтами і препаратами розглядається в якості нового напряму в дослідженнях з живлення птиці. При цьому активація вітагенів у стрес-умовах дозволяє підсилити антиоксидантний захист, підтримати редокс-баланс в організмі і підвищити стійкість до стресів. Зокрема, розробка вітаген-регулюючих добавок є надзвичайно важливим напрямом роботи безліч компаній у світі. Наші експериментальні дані свідчать, що вітаген-регулюючий препарат (PerforMax / Меджик Антистрес Мікс) має захисні властивості при різних стресах і вже активно використовується на птахівничих підприємствах.

Ключові слова: птахівництво, стрес, антиоксиданти, молекулярний механізм, вітагени

П.Ф. Сурай

Витагены в птицеводстве: адаптация к промышленным стрессам

Аннотация. *Промышленное птицеводство связано с различными стрессами и витагены ответственны за адаптацию к стрессам. В целом, активация витагенов через такие факторы транскрипции, как Nrf2 и HSF приводит к дополнительному синтезу защитных молекул, которые защищают от повреждающего действия свободных радикалов. Таким образом, активация витагенов различными нутриентами и препаратами рассматривается в качестве нового направления в исследованиях по питанию птицы. При этом активация витагенов в стресс-условиях позволяет усилить антиоксидантную защиту, поддержать редокс-баланс в организме и повысить устойчивость к стрессам. В частности, разработка витаген-регулирующих добавок является важнейшим направлением работы множества компаний в мире. Наши экспериментальные данные свидетельствуют, что витаген-регулирующий препарат (PerforMax/Меджик Антистресс Микс) обладает защитными свойствами при различных стрессах и уже активно используется на птицеводческих предприятиях.*

Ключевые слова: птицеводство, стресс, антиоксиданты, молекулярный механизм, витагены

References

Calabrese, V., Scapagnini, G., Davinelli, S., Koverech, G.,Koverech, A., De Pasquale, C., Salinaro, A.T., Scuto, M., Calabrese, E.J., Genazzani, A.R. (2014). Sex hormonal regulation and hormesis in aging and longevity: Role of vitagenes. J. Cell Commun. Signal. 8. 369–384.

Gaponov, I.V., Fotina, T.I., Surai, P.F. (2012). Physiological and technmological stresses at time of piglet weaning. Protective effect of the antistress preparation. Pig Production of Ukraine. 6. 6-9

Grigorieva, M.A., Velichko, O.A., Shabaldin, S.V., Fisinin, V.I., Surai, P.F. (2017). Vitagene regulation as a new strategy to fight stresses in poultry production. Agric. Biol. (Sel'skokhozyaistvennaya Biologiya) 52. 716–730.

Rattan, S.I. (1998). The nature of gerontogenes and vitagenes. Antiaging e_ects of repeated heat shock on human fibroblasts. Ann. N. Y. Acad. Sci. 854. 54–60.

Ren, J., Xu, N., Ma, Z.; Li, Y., Li, C., Wang, Y.,Tian, Y., Liu, X.; Kang, X. (2017). Characteristics of expression and regulation of sirtuins in chicken (Gallus gallus). Genome. 60. 431–440.

Shatskikh, E., Latipova, E., Fisinin, V., Denev, S., Surai, P. (2015). Molecular mechanisms and new strategies to fight stresses in egg-producing birds. Agric. Sci. Technol. 7. 3–10.

Surai, P.F. (2015a). Silymarin as a Natural Antioxidant: An Overview of the Current Evidence and Perspectives. Antioxidants. 4. 204–247.

Surai, P.F. (2015b). Antioxidant Action of Carnitine: Molecular Mechanisms and Practical Applications. EC Vet. Sci. 2.1. 66–84.

Surai, P.F. (2015c). Carnitine Enigma: From Antioxidant Action to Vitagene Regulation. Part 1. Absorption, Metabolism and Antioxidant Activities. J. Veter. Sci. Med. 3. 14.

Surai, P.F. (2015d). Carnitine Enigma: From Antioxidant Action to Vitagene Regulation Part 2. Transcription Factors and Practical Applications. J. Veter. Sci. Med. 3. 17.

Surai, P.F. (2016). Antioxidant systems in Poultry Biology: Superoxide dismutase. Anim. Nutr. 1. 8.

Surai, P.F. (2018). Selenium in Poultry Nutrition and Health; Wageningen Academic Publishers: Wageningen, The Netherlands. 428.

Surai, P.F., Fisinin, V.I. (2016a). Vitagenes in poultry production. Part 3. Vitagene concept development. Worlds Poult. Sci. J. 72. 793–804.

Surai, P.F., Fisinin, V.I. (2016b). Antioxidant system regulation: From vitamins to vitagenes. In Handbook of Cholesterol; Watson, R.R.; de Meester, F., Eds.; Wageningen Academic Publishers: Wageningen, The Netherlands. 451–481.

Surai, P.F., Fisinin, V.I., Shatskikh, E.V., Latipova, E.N. (2017b). Modern methods of fighting stresses in poultry and pig production: Vitagene concept in action. Sfera. 2. 5. 40–43.

Surai, P.F., Kochish, I.I. (2017). Antioxidant systems and vitagenes in poultry biology: Heat Shock Proteins. In: Heat Shock Proteins in Veterinary; Asea Alexzander, A.A., Punit, K., Eds.; Springer: Basel, Switzerland. 123–177.

Surai, P.F., Kochish, I.I., Fisinin, V.I. (2018b). Glutathione peroxidases in poultry biology: Part 1. Classification and mechanisms of action. Worlds Poult. Sci. J. 73. 185–197.

Surai, P.F., Kochish, I.I., Fisinin, V.I. (2018c). Glutathione peroxidases in poultry biology: Part 2. Modulation of enzymatic activities. Worlds Poult. Sci. J. 73. 239–250.

Surai, P.F., Kochish, I.I., Fisinin, V.I. (2017a). Antioxidant systems in poultry biology: Nutritional modulation of vitagenes. Eur. J. Poult. Sci. 81. 1612–9199.

Surai, P.F., Kochish, I.I., Fisinin, V.I., Kidd, M.K. (2019). Antioxidant Defence Systems and Oxidative Stress in Poultry Biology: An Update. Antioxidants (Basel). 8, 7. pii: E235. doi: 10.3390/antiox8070235.

Surai, P.F., Kochish, I.I., Fisinin, V.I., Grozina, A.A., Shatskikh, E.V. (2018a). Molecular Mechanisms of Gut Health Support in Poultry: Role of Microbiota; Agricultural Technologies: Moscow. Russia.

Surai, P.F., Litvinov A. (2017). From vitamins to vitagenes. Modern method to fight stresses in pig production. Pig Produuction (Russia). 3. 56–58.

Surai, P.F., Melnichuk, S.D. (2012). Mechanisms of protection from stresses in pig production: from vitamins to vitagenes. Pig Production of Ukraine (Kiev), 2.10-15

Velichko, O.A., Shabaldin, S.V., Surai, P.F. (2013). Practical aspects of vitagene concept use in poultry production. Poult. Poult. Prod. (Moscow). 4. 42–45.