INFLUENCE OF HALLOYSITE ADDITIVE IN HENS FEEDING ON BIOLOGICALLY ACTIVE EGG-WHITE COMPONENTS ACTIVITY

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SUMMARY

The use of natural and synthetic aluminosilicates in the animal production has been intensively researched in the recent years. They are characterised by selective absorption and high ion replaceable effect. They could also impact the digestive processes and metabolites binding in which decrease toxic gases emission from the litter without evoking negative changes in physiological parameters.

Research was carried out on lying hens. The aim of the research was to define the influence of halloysite on biologically active eggs' components. Haloysite was given with fodder to animals in 1 and 2%. Research of biological activity of egg-white components was carried out on eggs from each experimental group. The content of lisosyme, cystatine and antitrypsyne activity was marked. Feeding hens by fodder enriched by halloysite has significantly influenced the cystatyne content in egg-white of examined eggs increased also ability to inhibit trypsyne by ovomucoide and ovoinhibitor. Aluminosilicate additive to fodder didn't influence the lysozyme activity, which maintained the same level in all experimental groups.

Keywords: lying hens, aluminosilicates, halloysite, cystatyne, lysozyme, ovomucoide, ovoinhibitor

INTRODUCTION

The use of natural and synthetic aluminosilicates in the animal production has been intensively researched in the recent years. Natural and synthetic sorbents are porous, negatively charged material with sorptive properties depends on the particles' structure and polarisation degree and pore diameters. They have specific catalytic properties. They are also characterised by selective absorption and high ion replaceable effect. Halloysite, montmorillonite, vermiculite, pearlite, bentonite, zeolite, (heulandite and clinoptiolite) and synthetic HSCAS (Hydrated Sodium Calcium Aluminosilicate) are the names that are the most commonly found in the scientific communicates. These substances have sorptive properties towards heavy metals, some gases, mycotoxins and supplement the diet in trace elements. They could also impact the digestive processes and metabolites binding in which decrease toxic gases emission from the litter without evoking negative changes in physiological parameters.

Favourable effects of some aluminosilicates in animals' feeding were proved by many authors. (Kubena et al., 1991; Kyriakis et al., 2002; Papaioannou et al., 2002) In Poland a natural aluminosilicate is halloysite, which has rich deposits not explored before in the vicinity of Legnica. Determining how the addition of halloysite to laying hens' fodder influences health and

consumption safety of eggs will enable applying halloysite as additive to fodder. Hens' eggs are one of the best known products of „designed food" type. Food modifications consisting in putting halloysite (aluminosilicates) additive into fodder may influence the health of hens (Scheideler, 1993) and the components of eggs (Dobrzański et al., 1994). Those substances after isolation from the egg content are often applied as natural food preservatives or they determine durability of products consisting of egg-white.

Egg white contains biologically active components – lysozyme, cystatine, ovomucoid, ovoinhibitor and tripsine (Stevens, 1991**;** Guerin-Dubiard et al., 2006**)**. Lysozyme is an alkaline globular protein, which shows high enzymatic activity. Natural, biological lysozyme functions are aimed at protection of growing, which makes it an antiseptic substance. Moreover, lysozyme has the ability to inactivate viruses through bonding with its DNA, by creating inseparable complexes. As a substance of strong antibacteric and antivirus properties it finds broader and broader application in food industry as biopreservative and in pharmaceutical and cosmetic industry as natural antibiotic (Proctor et Cunningham, 1988). Cystatine shows also antiseptic and antiviral properties (Collins et al., 1998). It's an inhibitor of very strong effect with relation to ficyne and papaine. Furthermore, cystaine has the ability to inhibate cysteine proteinases like catepsine B, H and L (Saxena et Tayyab 1997).

Alterations in the cysteine proteinase inhibitor, cysteine proteinase ratios have been postulated to contribute to the malignant progression of tumours (Calkins et al., 1995). Especially important function of cystatine is connected with intra- and extracellular control of proteine decomposition, that's why it finds great interest in clinic.

The egg-white contains protein called ovomucoid that does not coagulate from solution by heating. There is 11% of ovomucoid in hens' egg-white and 15% in egg-white of ducks and geese. Ovomucoid from eggs of various birds' species can be various ensymes inhibitor, i.e. ovomucoide from hens' egg-white inhibits only trypsyne, but that from ducks or turkeys – trypsyne and chymotrypsyne.

Trypsyne and proteinases of bacterical and mouldical origin are inhibited by compound of protein nature called ovoinhibitor (Broadway, 1997). Ovoinhibitor can also disactivate chymotrypsine but differs from ovomucoide as regards working specificity. Inhibitors mentioned above possess very sophisticated and specific activity. Ovomucoide i ovoinhibitor inhibit the activity of serine proteinases and cystatine inhibites activity of tiolic proteinases. Cystatine i ovoinhibitor inhibit many proteolitic ensymes and ovomucoide only trypsyne (Saxena et Tayyab 1997). All mentioned inhibitors are highly thermostable (Acker et Ternes, 1994). In hens' egg-white their activity is well-known.

MATERIAL AND METHODS

Research was carried out on 60 hens (20 in one group), lasting 8 weeks. The aim of the research was to define the influence of halloysite on biologically active eggs' components. The experiment was conducted in controlled conditions in the animal vivary. Laying hens (ISA SHAVER) were kept in battery system (SPECHT). Provided microclimatic conditions were fitting standard norms established for laying hens. Animals were provided with permanent access to water. The fodder was passed every day at 8 a.m. in the amount of 125g/bird/day. Halloysite used in the experiment was raw (HSD) and activated in the sulphuric acid environment (HAV).

Laying hens were assigned to three groups. Before starting the experiment the animals had been given for 4 moths fodder as follows: control group – full-portion mixture Dolpasz „EXTRA

N-1/0 16%, experimental group I – full-porton mixture Dolpasz „EXTRA N-1/0 16% with 2% HAV addition and experimental group II – full-portion mixture Dolpasz „EXTRA N-1/0 16% with 2% HSD addition.

Research of biological activity of egg-white components was carried out on 30 eggs from each experimental group. The content of lysozyme, cystatine and antitrypsyne activity was marked.

During antitrypsyne activity of egg-white marking (Broadway, 1997) there was applied trypsyne reaction with synthetic substrate BapNA (N-benzoylo-DL arginino p-nitroanilide) as a result of which p-nitroanilin of yellow tint and maximum of absorbance by 412nm is secreted. The inhibitor's ability to inihibit trypsyne was examined by adding appropriate amount of inhibitor to samples, which resulted in drop of absorbance by 412 nm to control examination (without inhibitor).

Cystatine was marked by the test of cystatine to papaine inhibition activity (Siewiński, 1991). Antipapaine test is based on colorimetric marking of amount of freed hydrolyse BANA (chlorowodorek Na-benzoilo-DL-arginylo-B-naftylamidu) products as a result of cysteine proteinase (papaine) activity.

1 unit of inhibitor activity corresponds with 1 unit of papaine enzymatic activity (the amount of enzyme hydrolysing 1,0 mM of substrate per minute in standard conditions (37 degrees Celsius).

Lysozyme was marked with spectrophotometric method. The principle of marking consists in the measurement of dynamism of clouding changes in *Micrococcus lisodeicticus* suspension, which is exposed to incubation with appropriately diluted lysozyme. Measurement is conducted at a stable temperature of 25 degrees Celsius and the wavelength of &=450nm, specifying the fall of absorbance every 60 seconds during 6 minutes.

The results from the research were worked out statistically with the help of Statistica ver.7.0 computer programme. One-way variance analysis was carried out, averages were compared with the help of Duncan test. Differences at the level of $p \leq 0,05$ were considered as statistically significant.

In the study of group results the homogenous results were marked with the same letters, i.e. a, b, c.

RESULTS

Feeding hens by fodder enriched by halloysite has significantly influenced the cystatyne content in egg-white of examined eggs. There was observed twice and threefold increase of cystatine activity (4,62 units control) in the group with 2% HAV addition (12,8 units), and 7,41 units in case of HSD addition (Tab.1). Diverse feeding caused also increased ability to inhibit trypsyne by ovomucoide and ovoinhibitor. Antitrypsyne activity rose in egg-white of hens fed with fodder with additive of HAV up to 14,6 units and HSD up to 16,4 units in relation to control examination and came to 11,1 units per 0,1 mg protein. Aluminosilicate additive to fodder didn't influence the lysozyme activity, which maintained the same level in all experimental groups.

Probable reason of changes could be the fall of fodder contamination by bacteria, fungi and mycotoxines observed in fodder supplemented with halloysite, what has its effect on the health state (Kołacz, 2004).

CONCLUSIONS

1. Feeding laying hens on fodder enriched by halloysite caused twice and threefold twice increase of cystatine activity, which may show its influence on hens' health.
2. Halloysite additive to fodder influenced increasing ability of trypsyne inhibiting by ovomucoid i ovoinhibitor.

Table 1. Biologically active substances in egg-white

Experimental group	Biologically active substances		
	Cystatine [units/3mg of egg-white]	Lisosyme [units/1mg of egg-white]	Trypsyne [units/0,1mg of egg-white]
Control	4,62[a]	9,69[a]	11,1[a]
HAV	12,38[c]	10,01[a]	14,6[b]
HSD	7,41[b]	9,27[a]	16,4[c]

a,b,c – common letter in indexes of two averages indicates lack of statistically significant difference by P=0,05 (n=30)

ACKNOWLEDGEMENTS

The study carried out within the confines of the research grant of KBN No. 2806Z06126

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