Egg white biologically active proteins activity in relation to laying hen's age

E. SWIERCZEWSKA[2], T. SKIBA[1], A. SOKOLOWSKA[3], J. NOWORYTA-GLOWACKA[1], W. KOPEC[1]*, M. KOENIOWSKA[1] and L. BOBAK[1]

[1]Agricultural University of Wrocław, Department of Animal Products Technology, 50375 Wroclaw, Norwida 25 Str, Poland
[2]Warsaw Agricultural University (SGGW), Department of Poultry Breeding, 02786 Warsaw, 8 Ciszewskiego Str, Poland
[3]University of Wroclaw, Institute of Biochemistry and Molecular Biology, 50137 Wroclaw, 2 Tamka Str., Poland
***wkopkop@poczta.onet.pl**

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Abstract

The study was performed in order to determine the activity of biologically active proteins that naturally occur in egg white in relation to the age of the laying hen. For the experiment 240 eggs were collected from 40 hens (20 birds of ISA Brown and 20 birds of Hy-Line). Hens were kept in individual cages and fed with standard fodder. The activity of cystatin, lysozyme, serine proteinases inhibitors (ovomucoid and ovoinhibitor) were measured in egg whites obtained from hens in 35, 40, 45 and 50 weeks of age. The results of the study showed that cystatin had the lowest activity in egg white collected from 35 weeks old hens, whereas eggs collected in 50 weeks of hen's age expressed the highest activity of cystatin. This relation was proved for both bird's crossbreeds. No significant differences in cystatin activity were observed between two experimental lines. Similar to the results obtained for cystatin, the lysozyme activity was the lowest at first time of eggs collection, and then it increased from 244 u/mg protein to 273 u/mg protein for ISA Brown and from 235 u/mg protein to 316 u/mg protein for Hy-Line. Activity of trypsin inhibitors analysed in egg white increased together with the hen's age from 35 to 45 weeks, when the activity of serine proteinases inhibitors reached the maximum point. Activity of mentioned biological components measured in eggs obtained from 50 weeks old hens was significantly lower. A similar trend was estimated for chymotrypsin inhibitor analysed in egg white. Hy-Line birds produced eggs with higher anti-trypsin and anti-chymotrypsin activity than ISA Brown hens. It is concluded that activities of biologically active proteins in egg white increase with increasing age of the hens to 45 weeks in case of trypsin and chymotrypsin inhibitors, and up to 50 weeks in case of cystatin and lysozyme.

Introduction

Lysozyme, conalbumin, ovomucoid, ovoinhibitor and cystatin are biologically active proteins of egg white protecting the embryo during the incubation period. Their activity also prolongs the shelf life of table eggs. Some of the biologically active constituents of egg white, especially lysozyme, have wide novel applications in medicine and/or as a preservative substance in food production. Lysozyme protects the embryo up to the time of producing of its own immunoglobulins and it is also active against viruses and some toxins (Kopec and Trziszka, 1997). Another kind of protection is achieved by cysteine proteinases inhibitors i.e cystatin and by inhibitors of serine proteinases i.e. ovomucoid and ovoinhibitor, which inhibits activity of bacterial enzymes.

Earlier studies showed that activities of biological substances in egg white are affected by conditions and keeping system (intensive, extensive) of laying hens. It was found that the hens managed in an extensive type of housing system laid eggs with a higher activity of lysozyme, cystatin, ovoinhibitor and ovomucoid, as compared to eggs originating from the layers kept in an intensive housing system (Swierczewska *et al*., 2003). Low lysozyme activity in egg whites was observed when the temperature in the premises for the laying hens decreased during winter or increased during summer time beyond the standard range established for the layers (Swierczewska *et al*., 2002).

Aim of the study

In earlier studies an ambiguous relationship between the hen's age and the biological activity of laid eggs was found, so the aim of present studies was to determine the activity of lysozyme, cystatin, ovomucoid and ovoinhibitor in egg whites from hens of different age. The study enables to find the optimal range in laying period for obtaining eggs rich in biological active substances.

Material and methods

The studies were conducted on the 240 eggs derived from 40 laying hens of Isa Brown (20 hens) and Hy-Line (20 hens) cross breeds. Hens were kept in individual cages and fed with a standard feed mixture containing 18% of total protein and 11.5 MJ metabolic energy per kg. Eggs were manually broken and yolk was separated from white, which was the raw material for the study. Eggs were collected at the peak production of laying period i.e. at the hen's age of 35 weeks, 40, 45 and 50 weeks.

Activity of lysozyme was determined by a spectrophotometric (turbidimetric) method at a wavelength of 450 nm. The change in absorbances in an suspension of *Micrococcus lysodeikticus* bacteria, was registered during the 6 minutes reaction of the enzyme with the bacterial cells.

Activity of cystatin was determined as the ability to inhibit enzymatic activity of papain using BANA (N-benzoil-DL arginyl- β-naphtylamide hydrochloride) as a substrate after incubation samples at 37ºC. The reaction was stopped by the addition of DMBA (p-dimethyloaminobenzaldehyde) and the decrease of the absorbance was measured spectrophotometrically at a wavelength of 450 nm.

Anti-trypsin activity of ovomucoid and ovoinhibitor was measured spectrophotometrically at 412 nm after a 10 min incubation of trypsin solution with egg white at 37°C in 0,1 M tris-HCl buffer (pH 8) with the addition of 20 mM $CaCl_2$, using nitroanilide N-benzoil-DL-Arginine (BapNA) as a substrate (Erlanger *et al*., 1961). Anti-chymotrypsin activity of ovoinhibitor was measured spectrophotometrically at 412 nm after 10 min incubation of chymotrypsin solution with egg white at 37°C in 0,1 M tris-HCl buffer (pH 8), using N-Suc-Ala-Ala-Pro-Phe-pNA (SucAAPFpNa) as a substrate. Results obtained in the study were calculated and expressed as units of anti-trypsin activity per 5 mg of egg white protein and anti-chymotrypsin activity per mg of egg white protein. One unit of inhibitory activity was defined as the amount of inhibitor, which reduced the activity of 2 μg of an enzyme to 50% of the original value.

Results and discussion

The effect of the hen's age on the activity of biologically active substances for both of studied breeds was observed (Table 1). At the age of 35 weeks, when the peak of lay was reached, the activity of lysozyme was about 240 u/mg protein and increased to 304 u/mg for ISA Brown and to 284 u/mg for Hy-Line at 40 weeks of age. A significant increase ($P< 0.05$) of the proteins activity was observed at 50 weeks of age and the highest biological activity was determined in whites of eggs laid by Hy-Line hens. More evident influences of the hen's age on the inhibitory activity of egg white proteins towards cysteine proteinases was observed (Table 1). Considerable increase of the activity from about 5 u/mg protein up to over 10 u/mg was noticed between 35 and 50 weeks. At the end of the studied laying period (50 week), the inhibitory activity of cystatin determined for egg whites obtained from eggs laid by ISA Brown hens was significantly higher (12.7 u/mg), than those noticed for egg whites of Hy-Line hens. Collected results showed that during prolongation of the laying period the defence mechanism of the egg had changed. From 40 weeks of age lysozyme activity was high and maintained on the quite stable level, while potency of defence against cysteine proteinases is increased during the laying period up to 50 weeks of age, which closely corresponds to the results reported by Trziszka *et al.,* (2004) in their experiments with Tetra SL breed hens.

Different, than was found for cystatin, relationship between activity of inhibitors of serine proteinases i.e. ovomucoid and ovoinhibitors and hens age was observed. Activity of inhibitors of serine proteinases in egg whites of 2 studied breeds increased between 35 and 45 weeks of age up to almost 1000 u and then dropped to 574 u at 50 weeks for ISA Brown hens. Degree of inhibition of chymotrypsin like proteinases activity by ovoinhibitor was high at the age of hens of 40 to 50 weeks. The highest values of inhibition ability of ovoinhibitor were found especially at the age of 45 weeks. Along prolongation of hens age up to 50 weeks significant decrease in serine proteinase inhibitors activity was noticed. It might be caused by lowered activity of cup cells and tubular glands of oviduct mucosa, which produces some of protein fractions. High anti-trypsin activity was also observed in other studies at the beginning of laying period at the hens age of 25 weeks and decreased at 50

weeks, while activity of ovoinhibitor rose to 50 weeks of age (Swierczewska *et al.,* 2005). Hy-Line hens produced eggs with higher activity of serine proteinases inhibitors than ISA Brown hybrids, but at the peak of activity (45 weeks) the values determined in both kinds of egg whites were similar.

As was mentioned above high activity of the constituents of egg defence system has a great influence on the isolation procedures during production of preparations useful for food and medicinal applications. Even a 2.5 fold increase in activity of cystatin was observed at the end of the laying period as well as high lysozyme activity showed that eggs collected at the age of 50 week is the best raw material to isolate both components. However, this can not be recognised as the optimal for the defence system controlled by serine proteinase inhibitors, because the peak of activity of ovomucoid and ovoinhibitor was observed at the age of 40-45 weeks.

Conclusions

The peak of the serine proteinases inhibition by egg white constituents was observed at the age of laying hens 40-45 weeks and along with the prolongation of the laying period, activities of cystatin and lysozyme in the eggs are increased up to 50 weeks of hens age.

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Table 1 Activity of the biological substances in egg white.

Cross-line Hens age (weeks)	Lysozyme activity [u/mg protein]		Cystatin activity [u/mg protein]		Antytrypsin activity [u/5mg protein]		Antichymotrypsin activity [u/mg protein]	
	ISA Brown	Hy-Line	ISA Brown	Hy-Line	ISA Brown	Hy-Line	ISA Brown	Hy-Line
35	244^{ab}	235^{a}	5.5^{a}	4.4^{a}	730^{b}	809^{bc}	2.4^{a}	2.9^{bc}
40	304^{ef}	284^{def}	7.6^{b}	7.3^{b}	847^{cde}	898^{cd}	3.5^{d}	4.2^{e}
45	253^{abc}	268^{bcd}	7.5^{b}	8.7^{bc}	957^{e}	932^{e}	4.3^{e}	4.4^{e}
50	273^{cde}	316^{f}	12.7^{d}	10.4^{c}	574^{a}	790^{bc}	2.6^{ab}	3.3^{cd}
Analysis of variance	–		–		x		xxx	
cross								
age	xxx		xxx		xxx		xxx	

a,b,c,d,e – the same letter in indices of means shows no significant difference at $P \leq 0.05$
x – variation significant at $P \leq 0.05$
xxx – variation significant at $P \leq 0.001$