STANDARDIZATION OF AN ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) FOR THE DETERMINATION OF AVIDITY OF AVIAN IGY IMMUNOGLOBULIN

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ABSTRACT
Considering the great interest in the use of IgY antibodies in immunotherapy and rapid immunodiagnostics methods, become necessary related studies on physico-chemical and biological properties of this molecule as greed. The techniques for evaluation of greed are based in greater or lesser ease with which the antibodies are decoupled from specific antigenic complex. The determination of avidity is usually based on treatment of antibodies bound to antigen, on a solid support, with a chaotrope that is able to withdraw the lower affinity antibodies. The goal of this work was to standardize an enzyme-linked immunosorbent assay (Elisa) for estimation of IgY antibody avidity produced in laying hens. The determination of avidity is usually based on treatment of antibodies bound to antigen, on a solid support, with a chaotrope that act as destabilising effect of antigen-antibody complex, where the lower antibody affinity if dissociate. The hens were inoculated with four doses of 125 µg and a final dose of poison snake crotalico 50 µg. Eggs after the third and fifth inoculations were collected and separate the egg yolks and stored to -20 40˚c until the moment of use. Various concentrations of Urea and MgCl2 chaotrope agents, with different times of 5 and 30 minutes of incubation. The results obtained with 5 ' hatchery showed that there were significant differences between the samples analysed. While in the presence of MgCl2 significant difference in the greed of samples incubated for 30' ($p<0,05$).
KEYWORDS: egg yolks, ELISA, antibodies, hens

INTRODUCTION
Many studies show that the IgY of birds, presents similar sensitivity and affinity of mammalian IgG. IgY molecules can also be employed as an excellent tool in blood level tests and imunoenzimáticos also reduce interference problems in immunological tests and come as an addition of advantages in imunoenzimáticos tests (Brunda; Sashidhar and Sarin, 2006). Studies of greed have been applied to the diagnosis of diseases caused by protozoa, viruses and bacteria, allowing to differentiate primary infection of chronic infection (Kudo, 2006). The techniques for the assessment of greed are based in greater or lesser ease with which antibodies are specific antigenic complex decoupled and can be accomplished through various methods, such as: Radioimmunoassay, Agglutination, complement fixation test, ELISA, immunofluorescence, immuno-blotting, where denaturing agents added after the formation of Antigen-antibody complex are able to separate low-avidity antibodies (Pajuaba, 2006). His determination is usually based on the processing of the Antigen, antibodies linked to a solid support (plate), with a chaotrope that is able to remove the lower affinity antibodies. After treatment of low avidity antibodies to dissociate from high avidity antibodies as Antigen remain attached to the Antigen (Kudo, 2006; Macre,

2002). Despite the greed test be emerging as a good aid in diagnosis of recent infection, there are methodological problems that imply his best standardization. One such issue is the definition of its varied concentrations and chaotrope, having already been advocated the use of urea, DEA (Diethylamine), and NH4SCN (ammonium Thiocyanate) (KUDO, 2006; MACRE, 2002). Another important factor is whether the method of choice is the dilution of the chaotrope is the serum diluent, or whether the method is the elution (MACRE, 2002), in which the chaotrope is used in washing the plate after incubation, the serum and eluate chosen for this work. Based on reported in the literature for the procedure of determination of tests for greed, and for better understanding of IgY molecule, in front of the small number of works on physical, chemical and biological properties of this molecule, this work aims to standardize a technique imunoenzimática assay (ELISA) for determination of avidity of immunoglobulin of yolk IgY obtained from hens ' eggs.

MATERIALS AND METHODS

Used chicken Gallus Gallus domesticus White Leghorn, lineage with 20 to 70 weeks of life, acquired from farms of egg-producing region of Londrina-PR. animals were kept in the Central Animal House of the Universidade Estadual de Londrina in individual cages, at room temperature, getting clean water and laying ration at will.

5 hens used were chosen randomly. All animals receive 5 doses of imunógeno on 4 points in the pectoral muscle, on days 0, 15, 30, 45 and 90 of the experiment. The first dose applied with full of Freund adjuvant and the remaining doses in incomplete Freund's adjuvant, receiving 4 initial doses of 125 μg of poison crotálico and a final dose of 50 μg.

The eggs were collected and identified as the chicken in 2 days – and – 1 prior to immunization and after seven days of each inoculation, for obtaining of the yolk (IgY). These gems were extracted eggs, separated from clara, and used for the formation of gems pools of hens each group, after first (sample 1), second (sample 2), third (sample 3), fourth (sample 4) and fifth (sample 5) immunizations. The samples were stored in glycerol (vv) – 20° C until the moment of its use in ELISA.

The data obtained were analyzed by ANOVA one way, followed by Tukey test, using the program GraphPad Prism ® 5. The differences were considered significant when P 0.05.

RESULTS AND DISCUSSION

TABLE 1 – Results of Optical Density (OD) in Different Concentrations of $MgCl_2$ and its Incubation Time in Greed of Samples of Antibody from Hens Immunized with Poison Gems Crotálico After the 3rd and 5th Immunization, Respectively.

$MgCl_2$				
	Time			
Concentration (M)	Sample 3		Sample 5	
	5'	30'	5'	30'
PBS (100 %) *	1,651	1,541	1,250	1,265
2,5	0,406	0,251 #	0,345	0,308 #
2	0,574	0,407 #	0,513	0,474 #

1,5	0,761	0,525 #	0,738	0,605 #

The results are expressed as standard deviation average of *P*<0,05%.
Significant difference (#)
PBS total value (100%) of reactivity with no dissociation of antibodies.

Table 2 – Results of Optical Density (OD) in Different Concentrations of Urea and its Incubation Time in Greed of Samples of Antibody from Hens Immunized with Poison Gems Crotálico after the 3rd and 5th Immunization, Respectively.

Urea				
	Tempo			
Concentration (M)	Sample 3		Sample 5	
	5'	30'	5'	30'
PBS (100 %) *	1,651	1,541	1,250	1,265
9	0,540	0,331	0,600	0,439
8	0,627	0,307	0,630	0,420
7	0,983	0,523	0,806	0,621

The results are expressed with standard deviation average *P*<0,05%.
*PBS total value (100%)) of reactivity with no dissociation of antibodies.

Inouye et al., 1984, using 2 M Guanidine hydrochloride for dissociation of low avidity antibodies in immune complex in antibody-Antigen complexes ELISA system, noted a titration curve offset in samples in which the protein denaturant was employed, decreasing the amount of antibody immune complex. The same result could be found in this study, where displacement occurs also in the titration curve, decrease in the values of the O.D, presented in Tables 1 and 2 to the respective agents, urea, caotrópicos MgCl2 and evaluated here.

In relation to the $MgCl_2$, had consistent results to reported by Bollen et al., 1996, where this assessed and compared the avidity of immunoglobulin IgY (chicken) with respect to the avidity of IgG immunoglobulin and mammals (rabbit), using the best performing chaotrope in this work, $MgCl_2$ 2 M, stating that the greed of birds and mammals are similar.

As for urea, is being used more frequently in this type of method, of greed, and widely reported in the literature (Afonso et al., 2004; Kudo, 2006; Macre, 2002; Pajuaba, 2006). However, despite such amplitude in its use, when evaluated in this work for determination of avidity of immunoglobulin IgY, bird, the urea is not reached expected effect cited by most authors consulted, using Urea as a chaotrope in imunoenzimáticas for determination of IgG reactions of mammal being not mentioned its use for immunoglobulin IgY.

This way, after you get the result that $MgCl_2$ was more effective than urea for the proposed Protocol, assessed the avidity of IgY found in the blood of the yolk IgY. Unlike that reported by Woolley and London, 1995, in this work, the IgY obtained from blood and showed no yolk has a similar greed, where the blood was a hoarder IgY material in relation to the IgY found in the yolk.

CONCLUSION

None of the various concentrations used and incubation times influenced so that the results achieved with Urea were significant.

On the other hand, showed significant results in any MgCl2 concentrations tested in this work, and it was possible to observe just when the same was incubated for 30 minutes, showing that the incubation time significantly interferes with the result.

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