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G BIZANOV

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IgY extraction and purification from chicken egg yolk

Bižanov G.

Department of Biomodels, State Research Institute Center for Innovative Medicine, 08409 Vilnius, Lithuania

ABSTRACT. The interest for immunoglobulin Y (IgY) isolation arises from the possible applications of these immunoglobulins in diagnostics and therapeutics. Powered whole eggs or yolks have been used in veterinary medicine as an inexpensive immunoglobulin Y source for the treatment of enteric diseases. Incorporating feed grade egg yolk antibodies into animal diets has been examined extensively in an attempt to limit pathogenic diarrhea causing by *Escherichia coli* in swine, and limit *Salmonella* establishment in calves and mice, as well as *Campylobacter*, *Clostridium*, and *Salmonella* in poultry. Thus, therapeutic administration of the IgY might reduce the clinical use of antibiotics, and minimize the risk of developing bacterial antibiotic resistance. Methods of immunoglobulins isolation and purification from hen yolk are reviewed. For a large-scale production, one of the problems is separating the water-soluble protein fraction from the lipids and other hydrophobic substances. Precipitation or aggregation of lipid occurs under various conditions: (1) by use of water dilution under acidic conditions; (2) by means of acids; (3) combination of solvents (chloroform, acetone) which selectively solubilize the lipids; (4) using of 3.5% polyethylene glycol and (5) by applying natural gums (polyanionic polysaccharides). Further purification of IgY after crude extraction can be achieved by selective precipitation. IgY precipitation can be carried out by salt precipitation using saturated solutions of sodium or ammonium sulphate, which dehydrate proteins. Furthermore, selective precipitation is achieved by using 8.8% sodium chloride or at 12% of polyethylene glycol. The methods comparison on the basis of IgY yield, showed that the mean IgY yield obtained by water dilution method or precipitation with salts, or by using polyethylene glycol was 5.6 mg/ml of egg yolk, 6.3 mg/ml of egg yolk and 8.7 mg/ml of egg yolk correspondingly and did not significantly differ between them. Water dilution method seemed to offer the best IgY recoveries. Moreover, this method in combination with chromatography and filtration can be applied easily in an industrial environment. Filtration technology offers the best opportunities for industrial applications while precipitation with polyethylene glycol or salts provides a cheap and easy methodology for laboratory use. In the production of safer foods, an important strategy is to exploit natural antimicrobial agents as alternatives to conventional synthetic chemical preservatives. In this regard, the IgY is of much interest for its potential application in fortified foods, such as administration for prevention of enteric diseases. The use of IgY is cost-effective.

Keywords: Extraction and purification, immunoglobulin, chicken egg yolk

Corresponding Author:

G. Bižanov

Department of Biomodels

State Research Institute Center for Innovative Medicine

Address: 29, Moletų plento LT- 08409 Vilnius, Lithuania

Telephone: 3705 246 92 43, Fax: 3705 246 92 10

E-mail: gazim.bizanov@imcentras.lt

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INTRODUCTION

During the last decade, antibodies (Abs) have increasingly been used in veterinary medicine as well as in the new context of the so-called functional foods.

The advantage of obtaining Abs *via* laying hens instead of mammals has improved animal welfare. This is in complete accordance with the principle of the 3 R's – reduction, refinement and replacement (Russel and Burch, 1959).

Powered whole eggs or yolks have been used in veterinary medicine as an inexpensive immunoglobulin Y (IgY) source for the treatment of enteric diseases. Incorporating feed grade egg yolk Abs into animal diets has been examined extensively in an attempt to limit pathogenic diarrhea caused by *Escherichia coli* in swine, and limit *Salmonella* establishment in calves and mice, as well as *Campylobacter*, *Clostridium*, and *Salmonella* in poultry (Al-Adwani et al., 2013).

Furthermore, it was shown that anti-bovine rotavirus (BRV) IgY containing yolk provided up to 80% protection in neonatal calves and therefore may be a promising strategy to prevent BRV-related mortality (Vega et al., 2011).

Thus, therapeutic administration of the IgY might reduce the clinical use of antibiotics, and minimize the risk of developing bacterial antibiotic resistance.

The production of safer foods, is now an important strategy. It is necessary to exploit natural antimicrobial agents as alternatives to conventional synthetic chemical preservatives. In this regard, IgY is of much interest for its potential application in fortified foods, such as the IgY administration for prevention of enteric diseases. Moreover, the use of IgY is cost-effective as IgY cost less than \$10/gram (Dubie et al., 2015).

The extraction and purification of specific antibodies from chicken from egg yolk are increasingly attracting the interest of the scientific community as demonstrated by the significant growth of interest regarding IgY in literature. However, purity and yield vary greatly from method to method thus making the optimization for each experiment a necessity (Carlander, 2002).

Therefore, in the present paper various IgY extractions from egg yolk and purification methods were described.

COMPOSITION OF THE EGG YOLK

In general, egg yolk contains approximately 50% water. The dry weight of egg yolk is made up of 2/3 of lipids, and 1/3 of proteins (Shenstone, 1969). In fact, egg yolk proteins are distributed in two particular parts: the granules and the plasma in which the former are suspended. Granule proteins are composed of α - and β -lipovitellins (70%), phosvitine (16%) and low-density lipoproteins (12%) (Burley and Cook, 1961). Plasma proteins consist of α -, β - and γ -livetins and low-density proteins (McCully et al., 1962; Williams, 1962; Kovacs-Nolan et al., 2005). The α - and β -livetins were identified as chicken serum albumins and α_2 -glycoprotein, respectively, (Hatta et al., 1980), whereas IgY is the predominant fraction of γ -livetins (Kovacs-Nolan et al., 2005). In particular, all livetins are water-soluble and they correspond to serum proteins (Williams, 1962).

For a large-scale production, one of the problems is separating the water-soluble proteins from the lipids and other hydrophobic substances. Therefore, several methods are used for purifying IgY and are based on the strategy of separation of proteins (livetins) from lipoproteins (lipovitellins) and the rest of the yolk lipids using various chemical substances.

Practical applications of hen egg antibody may require removal of lipid, leaving the antibody in the water soluble protein fraction (WSPF). The main components of WSPF are IgY, α - and β -livetins, low density lipoproteins, and albumin (Kim and Nakai, 1998; De Meulenaer et al., 2002).

METHODS FOR IgY EXTRACTION

Water dilution method

Precipitation or aggregation of lipid from WSPF by different methods are reviewed.

This method is based on the use of water in order for the proteins to be transferred from the emulsion (condition: water in lipid) (Kates, 1972). The use of water dilution method for the extraction of IgY was

Table 1. The yield of IgY after application of the water dilution method

IgY mg/ml of egg yolk	References
5.4	Akita and Nakai, 1992
1.2	Ko and Ahn, 2007
12.6-25.0	Kitaguchi et al., 2008
5.1	Araújo et al., 2010
4.5	Mahdavi et al., 2010
13.9	Sunwoo et al., 2010
54.3	Liou et al., 2011
23.1	Kassim et al., 2012
3.3	Nafea, 2012

introduced by Jensenius et al., 1981. In this study, a property of yolk lipids was used for aggregation under low ionic strength and neutral pH conditions while also it was found that the immunoglobulin was isolated together with the lipid at acidic pH values. In another study, a ten-fold water dilution of the yolk was used in order to obtain the IgY soluble fraction (Jensenius and Koch, 1993).

The adjustment of pH to 5.0 combined with ten-fold yolk dilution was found to be crucial for the removal lipids or lipoproteins from the WSPF. In particular, 94.5% of IgY was recovered after WSPF pH adjustment to 5.1, while lower pH values of diluted egg yolk solution resulted in a clear WSPF (Akita and Nakai, 1992). Similar recovery was obtained earlier (Kwan et al., 1991).

Chang et al., (2000) and Ko and Ahn (2007) reported that lipid content at pH 6 was 1.0%, whereas that adjusted to pH 5.0 was 0.08%. In study conducted later, the lipid content at pH 5.0 was 1.75% (Nafea, 2012).

Results of the IgY extraction by water dilution method are listed in Table 1. As seen from Table 1, the yield varied from 1.2 to 25.0 mg/ml of egg yolk.

Thus, water dilution method seems to offer the best IgY recoveries. Moreover this method can be applied easily in an industrial environment.

Extraction of IgY with different chemical substances.

In studies the chemical substances were used to induce removal of the lipoproteins from the egg yolk.

In particular, phosphotungstic acid (Vieira et al., 1986), trichloroacetic acid (Asemota et al., 2013) successfully applied for elimination of lipids. For example, 3.46 mg/ml egg yolk was purified by using of the caprylic acid alone (Araújo et al., 2010).

Bade and Stegemann (1984) used cooled isopropanol to precipitate the water soluble proteins and further removed lipids by consecutive isopropanol and acetone extractions. In other studies, propan-2-ol, acetone and chloroform were used in order to eliminate the lipids (Bizhanov and Vyshniauskis, 2000; Deignan et al., 2000; Bizhanov et al., 2004; Al-Edany, 2011). As a result, the yield varied from 7.11 to 12.9 mg of IgY/ml of egg yolk.

In another study, Polson et al., (1980a) were the first

Table 2. The yield of IgY after single or double precipitation by ammonium sulfate

IgY mg/ml of egg yolk	Ammonium sulfate, %		References
3.8	34.0	34.0	Bižanov and Jonauskienė, 2003
13.0	50.0	-	Ruan et al., 2005
4.7-9.2	55.0	31.7	Dai et al., 2012
4.0	19.0	-	Júnior et al., 2012
0.84	24.0	15.2	Parma et al., 2012
9.2	50.0	30.0	Xu et al., 2013

which introduced the use of 3.5% polyethylene glycol (PEG) for extraction of IgY from egg yolk. Moreover, method was improved by increasing the dilution by buffer from a 2:1 to 4:1 ratio (Polson et al., 1985).

In a study conducted by Hatta et al., (1990) was systematically compared the use of twelve natural gums. Xanthan and λ -carragenan seemed to be able to remove most of the lipids. As a result, the yield of IgY was about 6.8 mg/g egg yolk.

These various IgY crude extraction methods give different results considering recovery and purity of levels obtained. The selectivity of precipitation directly depends on the type of precipitating agent applied. So far, for the immunoglobulin extraction the organic solvents (for routine and fast analysis) were applied. However, several precipitation steps using organic solvents there are necessary in order to obtain a purified protein.

Polysaccharides, as safe and nontoxic substance, can be applied for isolation of the IgY in biomedical field.

Purification of IgY

Further purification of IgY after crude extraction can be achieved by selective precipitation.

Salt precipitation is a traditional technique in pro-

tein purification. For IgY, mainly, ammonium and sodium sulfate have been applied. The results in Table 2 show the yield of IgY after precipitation with ammonium sulfate at various concentrations. As seen from Table 2 the yield is considerably different.

In other works, double precipitation of IgY by using dextran and sodium sulfate in combination with calcium chloride was applied (Jensenius et al., 1981; Akita and Nakai, 1993). In particular, the yield varied from 7.5 to 15.0 mg of IgY/ml of egg yolk.

Because of its bio-compatibility, sodium chloride was used for the precipitation instead of the other precipitants described in the literature. Hernández-Campos et al., 2010 found that sodium chloride at 0.88% and 8.7% decreased the selectivity and purity factor. In another study, it was observed that the optimal IgY precipitation occurred at NaCl concentration between 0.3%-9.36% (Ko and Ahn, 2007; Hodek et al., 2013). As a result, the yield varied from 2.7 to 8.9 mg of IgY/ml of egg yolk.

The precipitation with sodium and ammonium sulfate, sodium chloride as well as polyethylene glycol offers a cheap and easy methodology and can be used in laboratory practice. Furthermore, the purity of IgY, obtained after precipitation with sodium sulfate was 94% (Akita and Nakai, 1993).

Table 3. The yield of IgY after precipitation by polyethylene glycol

IgY mg/ml of egg yolk	References
4.4	Bizhanov and Vyshniauskis, 2000
4.4	Bizhanov et al., 2004
9.7	Shahbazi et al., 2009
4.6	Al-Edany, 2011
4.8	Marcet et al., 2011
5.43	Sudjarwo et al., 2012
8.6	Wen et al., 2012
2.87	Sampaio et al., 2014

The most successful precipitation method with respect to IgY recovery and purity consists of adding PEG at a 12% level as a the method of Polson et al., 1985. For the most part, the PEG may be removed by precipitation of the IgY either with 40% precooled ethanol at -20°C or by 2 M ammonium sulfate (Polson et al., 1980b).

The results of the IgY yield after application the PEG precipitation are summarized in Table 3. As seen from the data, the yield peaked at 9.7 mg/ml of egg yolk. As follows from data represented in three tables, the mean yield of IgY obtained by three methods did not significantly differ between them.

The precipitation with polyethylene glycol or salts offers a cheap and easy methodology for the laboratory use.

Various chromatographic techniques can be used to further purify the IgY. These techniques include the following methods, such as anion exchange chromatography (Bade and Stegemann, 1984), affinity chromatography (Ntakirutimana et al., 1992), thio-philic chromatography (Hansen et al., 1998), or ion exchange chromatography (Ko and Ahn, 2007). As a

result, the yield of IgY was not more than 1 mg/ml of egg yolk (Hassl and Aspöck, 1988; McCannel and Nakai, 1990; Verdoliva et al., 2000). Furthermore, it was reported that purity of IgY obtained by cation exchange chromatography was not high and contained too many contaminating proteins.

Thus, column chromatography is an expensive and impractical for large-scale production of antibodies (Ko and Ahn, 2007).

Filtration methods by using traditional funnel filtration or otherwise column- and ultra- filtration offer another possibility for IgY purification. These methods for separation are based on using different membranes (Kim and Nakai, 1996; 1998; Hernández-Campos et al., 2010) and columns (Kim and Nakai, 1996). However, by using synthetic membranes it is necessary considered that only under certain conditions (pH and ionic strength solution) can be obtained good results of purification of IgY.

It was noted that ultrafiltration seems more suitable method for the large industrial applications because of lower operation costs and direct scale up. (Akita and Nakai, 1992). These methods also allowed

the isolation of immunologically active IgY which can be used for further downstream immunotechnological processes.

CONCLUSION

IgY extraction methods require an initial delipidation step prior to extraction of insoluble lipids and lipoproteins.

The water dilution method was found to be most efficient for the extraction of IgY from the WSPF. This technique is a simple and rapid and also it is available for large scale production of IgY production. Moreover this method can be applied easily in an industrial environment.

Various IgY crude extraction methods give different results considering recovery and purity of levels obtained. The selectivity of precipitation directly depends on type of precipitating agent applied.

Further purification of IgY after crude extraction can be achieved by selective precipitation.

The precipitation with sodium or ammonium sulfate, sodium chloride as well as polyethylene glycol offer a cheap and easy methodology and can be used in laboratory practice.

Column chromatography and ultrafiltration are expensive and impractical for large-scale production of antibodies.

The knowledge of this method can be used for the development and refinement of a new methodology approach. The extraction of IgY from aqueous solution can be achieved using several methods, resulting in variations in the yield of the extract. However, each method has a specific purpose and can be improved.

The choice of a suitable method depends on the yield and purity desired, final application of the IgY as well as material cost, technology, labor skills and scale of extraction. ■

REFERENCES

- Akita EM, Nakai S (1992) Immunoglobulins from egg yolks: isolation and purification. *J Food Sci* 57:629-634.
- Akita EM, Nakai S (1993) Comparison of four purification methods for the production of immunoglobulins from eggs laid by hens immunized with enterotoxigenic *E. coli* strain. *J Immunol Methods* 160:207-214.
- Al-Adwani SR, Crespo R, Shah DH (2013). Production and evaluation of chicken egg-yolk-derived antibodies against *Campylobacter jejuni* colonization-associated proteins. *Foodborne Pathog Dis* 10: 624-631.
- Al-Edany AA (2011) Effect of immunoglobulin y purified from immunized hen eggs on the growth of *Staphylococcus aureus*. *Bas J Vet Res* 10:85-96.
- Araújo AS, Lobato ZIP, Chávez-Olórtegui C, Velarde DT (2010) Brazilian IgY-Bothrops antivenom: Studies on the development of a process in chicken egg yolk. *Toxicon* 55: 739–744.
- Asemota H, Curtello S, Vaillant AAJ, Mohammed W, Vuma S, Chalapathi Rao AV, Kurhade A, Kissoon S, Smikle M, Wisdom B, Kurhade G (2013) Purification of Avian IgY with trichloroacetic acid (TCA). *J Chromatogr Separ Technique* 4:205. doi:10.4172/2157-7064.1000205.
- Bade H, Stegemann H (1984) Rapid method of extraction of antibodies from hen egg yolk. *J Immunol Methods* 72: 421-426.
- Bizhanov G, Vyshniauskis G (2000) A comparison of three methods for extracting IgY from the egg yolk of hens immunized with Sendai virus. *Vet Res Commun* 24: 103-113.
- Bižanov G, Jonauskienė I (2003) Production and purification of IgY from egg yolk after immunization of hens with pig IgG. *B Vet I Pulawy* 47:403-410.
- Bizhanov G, Jonauskienė I, Hau J (2004) A novel method, based on lithium sulfate precipitation for purification of chicken egg yolk immunoglobulin Y, applied to immunospecific antibodies against Sendai virus. *Scand J Lab Anim Sci* 31:121-130.
- Burley RW, Cook WH (1961) Isolation and composition of avian egg yolk granules and their constituent α and β -lipovitellins. *Can J Biochem Physiol* 39:1295-1307.
- Carlander D (2002) Avian IgY antibody: in vitro and in vivo. PhD Thesis. University of Uppsala, Uppsala, Sweden.
- Chang HM, Lu TC, Chen CC, Tu YY, Hwang JY (2000) Isolation of immunoglobulin from egg yolk by anionic polysaccharides. *J Agr Food Chem* 48:995-999.
- Dai YC, Wang YY, Zhang XF, Tan M, Xia M, Wu XB, Jiang X, Nie J (2012) Evaluation of anti-norovirus IgY from egg yolk of chickens immunized with norovirus P particles. *J Virol Methods* 186:126-131.
- Deignan T, Kelly J, Alwan A, O'Farrelly C (2000) Comparative Analysis of Methods of Purification of Egg Yolk Immunoglobulin. *Food Agr Immunol* 12:77-85.
- De Meulenaer B, Baert K, Lanckriet H, Hoer VV, Huyghebaert A (2002) Development of an enzyme-linked immunosorbent assay for bisphenol A using chicken immunoglobulins. *J Agr Food Chem* 50:5273-5282.
- Dubie T, Sisay T, Zeru F, Gebru M-u, Muktar Y (2015) The potential application of avian egg antibodies with emphasis on immunotherapeutic and immunodiagnostic purpose. *J Vet Med Anim Health* 7: 145-158.
- Hansen P, Scoble JA, Hanson B, Hoogeraad NJ (1998) Isolation and purification of immunoglobulins from chicken eggs using thiophilic chromatography. *J Immunol Methods* 215:1-7.
- Hassl A, Aspöck H (1988) Purification of egg yolk immunoglobulins. A

- two-step procedure using hydrophobic interaction chromatography and gel filtration. *J Immunol Methods* 110:225-228.
- Hatta H, Kim M, Yamamoto T (1990) A novel isolation method for hen egg yolk antibody, 'IgY'. *Agr Biol Chem* 54:2531-2535.
- Hernández-Campos FJ, Brito-De la Fuente E, Torrestiana-Sánchez B (2010) Purification of egg yolk Immunoglobulin (IgY) by ultrafiltration: effect of pH, ionic strength, and membrane properties. *J Agr Food Chem* 58:187-193.
- Hodek P, Trefil P, Simunek J, Hudecek J, Stiborova M (2013) Optimized protocol of chicken antibody (IgY) purification providing electrophoretically homogenous preparations. *Int J Electrochem Sci* 8:113-124.
- Jensenius JC, Andersen I, Hau J, Crone M, Koch C (1981) Eggs: conveniently packaged antibodies. Methods for purification of yolk IgG. *J Immunol Methods* 46:63-68.
- Jensenius JC, Koch C (1993) On the purification of IgG from egg yolk. *J Immunol Methods* 164:141-142.
- Júnior ÁF, Santiago FM, Silva MV, Ferreira FB, Júnior AGM, Mota CM, Faria MS, Siva Filho HH, Silva DAO, Cunha-Júnior JP, Mineo JR, Mineo TWP (2012) Production, characterization and applications for *Toxoplasma gondii*-specific polyclonal chicken egg yolk immunoglobulins. *PLoS One* 7:1-10.
- Kassim N, Mtenga AB, Shim WB, Chung DH (2012) The in vitro and in vivo efficacy of hen IgY against *Vibrio parahaemolyticus* and *Vibrio vulnificus*. *J Microbiol Biotechnol* 22:1423-1431.
- Kates M (1972) Techniques of lipidology. Isolation, analysis and identification of lipids. North-Holland Publishing, Amsterdam.
- Kim H, Nakai S (1996) Immunoglobulin separation from egg yolk: a serial filtration system. *J Food Sci* 61:510-512.
- Kim H, Nakai S (1998) Simple separation of immunoglobulin from egg yolk by ultrafiltration. *J Food Sci* 63:485-490.
- Kitaguchi K, Minoura M, Noritake M, Mizutani M, Kinoshita K, Horio F, Murai A (2008) Determination of immunoglobulin Y concentration in yolk extract prepared by water dilution method: comparisons among three strains of chickens. *J Poult Sci* 45:82-87.
- Ko KY, Ahn DU (2007) Preparation of immunoglobulin Y from egg yolk using ammonium sulfate precipitation and ion exchange chromatography. *Poultry Sci* 86:400-407.
- Kovacs-Nolan J, Phillips M, Mine Y (2005) Advances in the value of eggs and egg components for human health. *J Agr Food Chem* 53:8421-8431.
- Kwan L, Li-Chan N, Helbig N, Nakai S (1991) Fractionation of water soluble and insoluble components from yolk with minimum use of organic solvents. *J Food Sci* 56:1537-1541.
- Liou JF, Shiau JW, Tai C, Chen LR (2011) Production of egg yolk immunoglobulin against *Escherichia coli* from white Leghorn and Lohmann chickens. *J Anim Vet Adv* 10:2349-2356.
- Mahdavi AH, Rahmani HR, Nili N, Samie AH, Soleimani-Zad S (2010) Egg yolk antibody (IgY) powder against *Escherichia coli* O78:K80. *J Anim Vet Advan* 9:366-373.
- Marcet I, Laca A, Paredes B, Díaz M (2011) IgY isolation from a watery by-product obtained from an egg yolk fractionation process. *Food Bioprod Process* 89:87-91.
- McCannel AA, Nakai S (1990) Separation of yolk immunoglobulins into subpopulations using DEAE-ion exchange chromatography. *Can Inst Food Sci Technol J* 23:42-46.
- McCully KA, Mok CC, Common RH (1962) Paper electrophoretic characterization of proteins and lipoproteins of hen's egg yolk. *Can J Biochem Physiol* 40:937-952.
- Nafea NM (2012) Isolation, purification and conjugation of polyclonal antibodies type Y from immunized hens against Hepatitis B surface antigen. MS Thesis. University of Baghdad, Baghdad, Iraq.
- Ntakirutimana V, Demedts P, Van Sande M, Scharpé S (1992) A simple and economical strategy for downstream processing of specific antibodies to human transferrin from egg yolk. *J Immunol Methods* 153:133-140.
- Parma YR, Chacana PA, Lucchesi PM, Roge A, Granobles Velandia CV, Krüger A, Parma AE, Fernández-Miyakawa ME (2012) Detection of Shiga toxin-producing *Escherichia coli* by sandwich enzyme linked immunosorbent assay using chicken egg yolk IgY antibodies. *Front Cell Infect Microbiol* 2:1-8.
- Polson A, Von Wechmar MB, Van Regenmortel MVH (1980a) Isolation of viral IgY antibodies from yolks of immunized hens. *Immunol Commun* 9:475-493.
- Polson A, Von Wechmar MB, Fazakerley G (1980b) Antibodies to proteins from yolk immunized hens. *Immunol Commun* 9:495-514.
- Polson A, Coetzer T, Kruger J, Von Maltzahn E, Van der Merwe KJ (1985) Improvements in the isolation of IgY from the yolks of eggs laid by immunized hens. *Immunol Invest* 14:323-327.
- Ruan GP, Ma L, He XW, Meng MJ, Zhu Y, Zhou MQ, Hu ZM, Wang XN (2005) Efficient production, purification, and application of egg yolk antibodies against human HLA-A*0201 heavy chain and light chain (β 2m). *Protein Expres Purif* 44:45-51.
- Russel WMS, Burch RL (1959) The principles of humane experimental technique. Methuen, London.
- Sampaio LC, Baldissera MD, Grando TH, Gressler LT, Capeleto DdeM, de Sa MF, de Jesus FP, dos Santos AG Jr, Anciuti AN, Colonetti K, Stainki DR, Monteiro SG. (2014). Production, purification and therapeutic potential of egg yolk antibodies for treating *Trypanosoma evansi* infection. *Vet Parasitol* 204:96-103.
- Shahbazi P, Shayan P, Ebrahimzadeh E, Rahbari S (2009) Specific egg yolk antibody against recombinant *Cryptosporidium parvum* P23 protein. *Iran J Parasitol* 4:15-24.
- Shenstone FS (1968) The gross composition, chemistry and physico-chemical basis of organization of the yolk and white. In: (ed.: Carter TC) *Egg quality a study of hen's egg*, Oliver and Boyd, Edinburgh, pp.26-58.
- Sudjarwo SA, Sudjarwo KE, Koerniasari (2012) Purification and characterization protein of anti-dengue specific immunoglobulin Y for diagnostic kit of dengue. *J Appl Pharm Sci* 2:7-12.
- Sunwoo HH, Lee EN, Gujral N, Suresh MR (2010) Growth inhibition of *Escherichia coli* 987P by neutralizing IgY antibodies. *Open Immunol J* 3:1-8.
- Verdolina A, Basile G, Fassina G (2000) Affinity purification of immunoglobulins from chicken egg yolk using a new synthetic ligand. *J Chrom B* 749:233-242.
- Vieira JG, Oliveira MA, Maciel RM, Mesquita CH, Russo EM (1986) Development of an radioimmunoassay for the synthetic amino terminal (1-34) fragment of human parathyroid hormone using egg yolk-obtained antibodies. *J Immunoassay* 7:57-72.
- Vega C, Bok M, Chacana P, Saif L, Fernandez F, Parreño V (2011) Egg yolk IgY: protection against rotavirus induced diarrhea and modulatory effect on the systemic and mucosal antibody responses in newborn calves. *Vet Immunol Immunopathol* 142: 156-169.
- Wen J, Zhao S, He D, Yang Y, Li Y, Zhu S (2012) Preparation and characterization of egg yolk immunoglobulin Y specific to influenza B virus. *Antiviral Res* 93:154-159.
- Williams J (1962) Serum proteins and the livetins in hen's-egg yolk. *Biochem J* 83:346-355.
- Xu JJ, Ren CZ, Wang SS, Liu DD, Cao LQ, Tao JP (2013) Protection efficacy of multivalent egg yolk immunoglobulin against *Eimeria tenella* infection in chickens. *Iran J Parasitol* 8:449-458.