HENS' ANTIBODIES PRODUCTION STIMULATION BY ADMINISTRING *ESCHERICHIA COLI* F5 K99 FIMBRIAL ANTIGENS

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Summary

The studies evaluated the interference of *Calendula officinalis* extracts with antibacterial antibodies production and IgY transovarian transfer in extensive raised hens.We obtained fimbrial *E.coli* antigens and we used four hens groups raised in extensive system divided as following: witness group inoculated with salina, the second group was inoculated with antigen, the third group with antigen and alcohol and the fourth group with antigen and alcoholic extract of *Calendula officinalis.* All the groups were inoculated three times in the days 0, 21st and 45th of the experiment. IgY sinthesis dinamics in serum and yolk was followed by microaglutination reactions. Formoled *E. coli* B41 F5K99 strain proved to have good antigenic properties inducing Ig sinthesis in inoculated hens organisms with an ascendent courve after the first inoculation to all the groups inoculated, with the higher value after 45 days from the first inoculation. An active Ig transfer from serum to yolk was registred starting with 5 days after the second inoculation. High yolk aglutinants antibodies (IgY) levels were detected (over 1/11658) starting with 21 days – the moment of the second inoculation. Hidro-alcoholic *Calendula officinalis* extract may costitue a hen's colibacilar fimbrial adjuvant alternative, and yolk rich in specific Ig may be an alternative in treating and preventing bacterial digestive diseases.

Key words: Adjuvant, *Calendula officinalis, Escherichia coli* F5 K99, fimbrial antigens

The investigations evaluated the interferance between *Calendula officinalis* extracts with antibacterial antibodies production and IgY transovarian transfer in hens raised in extensive system. Antibodies production means complex mecanisms of cell cooperation and sequentional modifyings of the humoral factors, the researches followed the dynamic evaluation of the immune cells function involved in detecting and processing the antigen but also of the serum and vitelus effector levels. Thus we delimited the levels and stages where the vegetal principies are acting. The experiment was conducted in order to estimate the environmental factors effect, inoculation stress, the solvent used to prepare the extract and the immune reactivity reaction t the extract.

Considering these aspects, the paper followed:

- Hyper immunization fimbrial antigen obtaining;

- IgY sinthesis dynamic;
- Yolk IgY high titres obtaining after hyper immunization with *E.coli* F_5 K_{99} fimbrial antigens;

This protocole is to be used in treating colibacilosis in different birds and mammel species using yolk or the egg in total.

Material and methods

The experiment was realized on 16 hens of Transylvanian crossbreed with ages between 8 and 18 months raised in extensive system.

The birds were randomized divided in 4 groups as following:

- group 1 –negativ witness, inoculated with salina
- group 2 –pozitiv witness, inoculated with antigen
- group 3 -. Inoculated with antigen and alcohol
- group 4 – inoculated with antigen and alcoholic extract of *Calendula officinalis*

The hens were mantained during the experiment in extensive raising systam.

The antigen was represented by a *E. coli* Copenhaga strain suspension with the antigenis formula $B_{41=}$0101.K-:H- F41. The germs were cultivated on Minca medium at 37°C for 24 hours.

The *Calendula officinalis* extract was prepeared from plants collected from the N-W part of Romania. The impure extract was filtred and kept at 4^0C.

The experimental protocole used different treatment on groups at 21 days and and blood samples at 0, 21 and respectively 45 days, while the eggs were collected every 5 days for two months.

A bacterial suspension with 2 milion germs/ml was prepeared for the inoculation.

For the 3 antigen inoculation, we also used equal volums of salina, alcohol and vegetal alcoholic extract of *Calendula officinalis*, and the administration path was similar to the antigen one.

From the samples collected we performed:

- Total serum and vitelin Ig determination using turbidimetric test with zinc sulphate
- IgY extraction by serial dilutions in distilled water
- Slow serum agglutination reaction.

Results and discussions

Results obtained for total serum Ig determination

At the begining of the experiment maximum average value was calculated for the hens inoculated with antigen and extract (5.99±-0.27 [$\log_2$]/mg/dl), and the minimum for inoculation with bacterian suspension (4.74±0.76[$\log_2$]/mg/dl)

Table 1.

Serum Ig medium values ([log_2]/mg/dl)

Collection	Experimental group			
	Group 1	Group 2	Group 3	Group 4
I (day 0)	5.23±1.44	4.74±0.76	5.64±1.93	5.99±0.27
II (day 21)	5.11±1.38	5.28±1.58	5.43±1.22	6.54±0.67
III (day 45)	6.57±0.63	6.53±0.27	6.65±1.12	6.78±0.33

For the second collection we detected a increase of the serum Ig concentrations at the bacterial suspension groups. Ig concentartion values at the antigen stimulated hens together with phito pharmaceutical product administration reaches the maximum value per collection (6.54±0.67 [log_2]/mg/dl). After the re-contact with the bacterian antigen, the Ig serum concentrations are presentins variations for all the groups. Medium maximum value is revealed also for the fourth group (6.78±0.33 [log_2]/mg/dl), while the minimum for the bacterial inoculated group is very close to the witness group. We detected that at the end of the experiment, the interval with medium values for the four groups is restrained, with omogen values.

Firstly the alcohol inhibits the anticorpogenesis. The serum concentrations are inferior to those recorded at the begining of the experiment after the antigenic stimulation. After the bacterian suspension reinoculation, the solvent determines a spectacular growth of the imunoglobuline serum level, the values registered being similar to those calculated for the group treated with extract and maximum within the tests. It's effect is unsteady.

The vegetal extract compensates the solvent activity and proves during the tests a stimulative effect upon the imunoglobuline synthesis. The results are sustained by the dates of some studies that characterize the carotenoid activity, the major compound in *Compositae species,* over the imunoglobuline synthesis (Jynouchi et al., 1994).

The results obtained at the vitelline imunoglobuline dosing

At the birds inoculated with the bacterian suspension the minimum value per experiment is regestered in day 10 namely 3.32 [log_2]/mg/dl. consecutive to the first antigenic stimulation the vitellin of IgY grows (5.32[log_2]/mg/dl), being maximum for this group during the test.

Table 2.

Vitellin Ig values ([log_2]/mg/dl)

Collection day	Experimental groups			
	Group 1	Group 2	Group 3	Group 4
0	5.80	5.28	5.55	4.16
10	4.39	3.32	5.28	4.52
15	4.95	5.32	4.70	4.32
21	5.35	4.80	5.80	4.70
30	4.90	4.52	5.28	4.80
35	5.32	4.70	5.32	4.39
45	5.55	4.95	5.24	4.39
50	4.90	4.75	5.45	4.58
60	5.39	4.39	5.16	4.08

The initial imunoglobuline concentration in group 4 represents the smallest calculated value for the 4 experimental groups from the begining of the experiment (4.16[log_2]/mg/dl), but this value is not minimum per group, towards the end of the testing being registered an iminoglobuline level of 4.08 [log_2]/mg/dl. Both after the first antigenic stimulation and after the booster we observed a growth of the vitelline concentration of imunoglobuline. The initial Ig concentration at the fourth group is the lowest value for the four experimental groups from the begining of the experiment (4.16[log_2]/mg/dl). Also, this value is not minimum for the group, at the end of the tests a value of 4.08 [log_2]/mg/dl Ig level being registred. Both after the first antigenic stimulation and after the booster, a increase of the vitellin concentration is observed, especially after the booster (4.52 şi respectively 4.70[log_2]/mg/dl).

Analysing the IgY values for the four groups, we observed that at the beginning of the experiment they never had a uniform repartition. Right after the bacterian suspension inoculation, the Ig ovular concentration is increasing at the fourth group and decreasing at the groups inoculated with antigen but also treated different. After the booster a increase of the ovular level for the third and fourth group is detected, registring maximum values per experiment in these groups (5.80 şi respectively 4.70 [log_2]/mg/dl).

The statistic calculation established significant statistic differences between the witness group and ant the group inoculated with antigen ($p< 0.05$). The differences between the first and the fourth group are distinctly significant, while for the third and the fourth groups are very significant ($p<0.001$).

The obtained data, corelated with the time necessary to the IgY ovular transfer are indicating a fast stimulating effect induced by the *C. officinalis* alcoholic extract to the Ig mobilization at the folicular level (Erhard, 1997).

IgY ovarian folicul transfer is realized by a active Fc receptors and hinge region mediate process, more specific the molecular structure of the second and the third fragments of the IgY heavy chains (Morris et al., 2002).

The results obtained at the determination of the serum and vitellus Ig concentration using slow agglutination reaction

For the witness group the serum anti-phimbrial antibodies titres medium maximum values were registred at the third inoculation (3.25±1.7). This titre is increasing constantly during the experiment (Tabel 3). At the third group inoculated with antigen a constant increase of the titre from the initial value of 1.5±0.57, to 2±1.5 at the second collection and at the end to a maximum of 2.6±0.5. The agoup stimulated with antigen is presenting the same medium values of the anti phimbrial antibodies titres with the withness group.

Table 3.

Anti phimbrial serum antibodies titres values

Collection	Experimental group			
	Group 1	Group 2	Group 3	Group 4
I	2.25±0.5	1.5±0.57	1.5±0.57	2.25±0.5
II	2.75±1.7	2±1.15	2	3.75±0.5
III	3.25±1.7	2.67±0.57	2.67±0.57	3.5±0.57

T he group treated with antigen and alcoholic extract of *Calendula officinalis,* the medium maximum value was observed during the primary immune response (3.75±0.5). The booster leeds to a slight decrease of the anti phimbrial antibodies serum titres. The treatment with vegetal extract is corelated with maximum values of the medium after the first contact with the bacterian suspension, but also after the booster (3.75±0.5 şi respectiv 3.5±0.57).

At the beginning of the experiment the hens presented similar values of vitellus anti phimbrial antibodies titres (Table 4). The witness group is not presenting important differences, the two months while the eggs were collected we detected discreet increases of the titre after the booster. For the second group we observed a decrease of the titres after the first stimulation. After the booster the titres are increasing but with no relevant differences respect to the first group.

Table 4

Vitellus anti phimbrial antibodies titres values

Day	Group 1	Group 2	Group 3	Group 4
0	2	2	3	3
10	1	2	3	3
15	1	1	2	1
21	1	1	2	4
30	1	3	2	3
35	1	2	3	2
45	2	1	2	4
50	2	3	3	4
60	1	2	3	4

For the groups inoculated with alcohol and extract we observed similar variations to the antigen witness. The only differences are the higher values for the second and the third groups respect to negativ and respectively positive are statistically secured at $p<0.01$ and respectively $p<0.01$.

There is continous interest in finding adjuvants who's aplication to induce higher antibodies titres and that the inoculation is not followed by local and general signs. The recent studies are describing the use of plants extracts in order to increase the immune capacity of vaccinal antigens both in mammels (Hu et al., 2003, Rivera et al., 2003) and birds (Hoshi et al., 1999, Qureshi et al., 1996). The vegetal adjuvants characterized nowadays are presenting high bio-disponibility, with better results respect to the classic ones (oily adjuvants).

Conclusions

After administring *E.coli* F5 K99 antigens in order to stimulate IgY sinthesis at hens raised in extensive sysyem, we conclude:

- *E. coli* B41 F5K99 formoled strain proved to have good antigenic properties inducing Ig sinthesis in the organism of the inoculated hens.
- Aglutinant antibodies titre in the vaccinated hens serum registred a ascendent increase for all the groups, the higher value being registred after 45 days from the first inoculation.
- Yolk aglutinants antibodies titre registred values over 1/11658 starting with 21 days (booster day). Hidro alcoholic extract of *Calendula officinalis* may be used as adjuvant for the hens' phimbrial colibacilar vaccines.

- High yolk aglutinin levels registred proved to be a favourable point in using IgY in preventing and treating various infectious contagious diseases with digestive localizations.

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