

# EFFECT OF FERMENTED WHEAT GERM EXTRACT (FWGE) ON SHEDDING *SALMONELLA* *INFANTIS* AND IMMUNREACTIONS OF BROILERS

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## SUMMARY

170 broilers have been infected with *Salmonella infantis* and vaccinated against IBD, (infectious bursal disease) IB, (infectious bronchitis) ND. (Newcastle disease) Feed of 85 broilers has been completed with FWGE (fermented wheat germ extract) Shedding-characteristics of *Salmonella infantis* and immune reactions after vaccinations were investigated with both broiler groups. Intensity and dynamics of shedding of bacteria by cloacal swabs proved to be equal with both groups and stopped between 18-19<sup>th</sup> day after infection, but cecal content remained positive even on 31-33 days after infection.

FWGE increased the IBD VN GMT (virusneutralization geometric mean titers) 7 days after vaccination by 26%, but 21 days after immunization there was no difference in the titres. IB ELISA MMT (mathematical mean titers) 28 and 42 days after vaccination have been increased with the FWGE treated group by 820 and 180% compared to the control. ND HI GMT (haemagglutination inhibition geometric mean titers) proved to be 100% higher with the FWGE treated group on the 42<sup>nd</sup> day of rearing.

## INTRODUCTION

EU has banned AGP-s (antibiotic growth promoters) in 2006 and researches has been carried out to find alternatives to control and prevent colonization of pathogen bacteria e.g. *Salmonellas* in the intestines modulating gut microbiota [Ribeiro et al, 2007]. Broilers produced in EU, must be free of *Salmonellas* since 2003. *Salmonella infantis* infection of broiler flocks and contamination of chicken meat came to the front and became almost exclusive.

Beneficial effect of FWGE has been verified in Hungarian poultry production among others with the production parameters, immune reactions, mycoplasmosis and coccidiosis of broilers [Kósa et al, 2003, Stipkovits et al, 2004] production level and vaccination reactions with parent flocks of table egg layers [Kósa, E., Bajcsy, E., 2008] and histo-morphology of intestinal mucosa with broilers [Ózsvári et al, 2010].

## MATERIAL AND METHODS

In two fully separated and climatized compartments each of them 14 m<sup>2</sup>, 85 (group No I, experimental group) 85 (Group No II, control group) Ross 308 day old broilers originated from *Salmonella* free parent stock have been settled. Before settling floor, walls and all equipment including feeders, drinkers, door, etc. of the premises have been thoroughly cleaned first mechanically and by high pressure water secondly. After drying up of the premises all of the surfaces and equipment have been disinfected by 200 ml/m<sup>2</sup> of 1% Virocid solution with high pressure spray. After complete drying up floor of the premises has been bedded by 10 cm thickness with heat treated, chopped straw closed into plastic sacks at the producer.

been used as lighting program with 20 lux. Both of the experimental and control flocks have been fed by a commercial broiler starter feed till the 14<sup>th</sup> day and grower feed till the end of rearing to the 42<sup>nd</sup> day, ad libitum. Starter feed of the experimental group have been completed by 3 g/kg, grower feed by 2 g/kg of FWGE. Feed of the control group did not contain FWGE.

Each broilers of experimental and control groups has been orally infected by culture of live *Salmonella infantis* containing 10 000 000 bacteria/individual dose on the 4<sup>th</sup> day of rearing.

Each compartment has been equipped by 3 small drinkers till the end of 2<sup>nd</sup> week and 2 bell drinkers till the end of rearing. In the first second weeks broilers were fed from the chicken boxes placed on the litter, and after 2 of 1,5 m long trough feeders have been placed in the compartments. Microclimate of the premises has been fitted by the usual broiler technology and controlled automatically. 1 hour light and 23 hours dark period have

Both groups has been vaccinated by coarse spray against ND and IB by Nobilis Vaccine Ma5+Clone 30 on the 1<sup>st</sup> day, against IBD by drinking with Nobilis Gumboro D78 vaccine on the 21<sup>st</sup> day and against ND by coarse spray with Nobilis vaccine Clone 30 on the 28<sup>th</sup> day.

*Environmental samples* were taken by agar sausage method for *Salmonella* investigation before settling of birds from the surfaces of compartments, and from the covering papers of chicken boxes by the regulation of

home veterinary authority 180/2009.(XII.29.) FVM, based on Commission regulation (EC) No.1003/2005.

*Blood samples* (10/groups) were taken by puncturing from cubital vein for determining of humoral antibody level of ND and IB on the 14<sup>th</sup> day, and determining of ND, IB, IBD humoral antibody level on the 28<sup>th</sup> day, finally ND, IB and IBD humoral antibody level on the 42<sup>nd</sup> day. Antibodies of ND have been tested by HI, IB by ELISA, IBD by VN.

*Cloaca tampon samples* (10/groups) were taken on the 5, 6, 7, 9, 10, 15, 17, 22, 23, 29<sup>th</sup> day of rearing for Salmonella cultivation by enrichment method.

*Cecal samples* were taken from 5 sacrificed birds/ group on the 35<sup>th</sup> and 37<sup>th</sup> day of rearing. Cecum has been

removed after double ligation of the organ at the ileum avoiding contamination of its content.

*Drinking water samples*, 1 l were taken in both compartments for Salmonellas before settling of flocks from the water supplying tap by the hygienic rule of sampling.

*Feed samples*, 1 kg were taken for presence of Salmonellas from the starter feed on the 1<sup>st</sup> day of rearing and from the grower feed 16<sup>th</sup> and 30<sup>th</sup> day of the rearing with the experimental and control flock.

*Mortality* has been noticed and the cause of it has been diagnosed.

## RESULTS

### Environmental samples

a) 32 agar sausage and 11 drag swab samples taken from the surfaces of the two compartments proved to be negative for Salmonellas as well as the 500 g of bedding material (chopped straw).

b) 5x5 g of covering papers spoiled by droppings (meconium) proved also to be negative for Salmonellas in the bacteriological investigation.

### Serological investigations

a) *IBD VN geometrical mean titres* are shown in the Table 1.

Table 1:

Age at the blood sampling/day	FWGE+	FWGE-
28	317	251
42	141	158

b) *IB ELISA mathematical mean titres* are shown in the Table 2.

Table 2:

Age at the blood sampling/day	FWGE+	FWGE-
14	32	470
28	559	68
42	493	176

c) *ND HI geometrical mean titres* are shown in the Table 3.

Table 3:

Age at the blood sampling/day	FWGE+	% negative	FWGE-	% negative
14	12		18	
28	8		8	
42	549	0	274	0

### Bacteriological investigation

a) *Cloaca-tampon samples*. Percentage of positive samples is shown in the Table 4.

Table 4.

	Days of sampling										
	5	6	7	9	10	15	16	17	22	23	29
FWGE+	90	10	70	10	90	50	40	50	30	0	0
FWGE-	50	50	90	10	90	30	70	60	0	0	0

b) *Cecal samples*

The cecal samples taken on the 35<sup>th</sup> and 37<sup>th</sup> day proved to be positive for *Salmonella infantis* with both flocks.

c) *Drinking water* was *Salmonella* negative on the day of settling of the birds as well as the *feed samples* taken with both flocks on the 1<sup>st</sup>, 16<sup>th</sup> and 30<sup>th</sup> day of rearing.

### Mortality

In the group fed by *FWGE* supplementation died 5 birds (5.9 %) because of omphalitis, 1, mechanical suffocation 1, polyserositis 1, pneumonia 2 birds. On the group fed

without *FWGE* supplementation died 4 birds (4,7%), because of omphalitis 1, pericarditis 1, polyserositis 1, and pneumonia 1 broiler.

### DISCUSSIONS

*Prebiotics* are non-digestible carbohydrates containing feed ingredient a substrate for multiplication of useful bacteria in microbiota, prevent or at least suppress colonization of pathogen microflora including *Salmonellas* [Patterson, J.A., Burkholder, K.M., 2003] and influence the immune status of farm animals beneficially [Soltan, M.A., 2009].

The colonization of *Salmonellas* has been decreased with poultry fed by a diet completed with *prebiotics* by decreasing harmful effects of stress factors and decrease shedding of pathogens [Line, J.E., Bailey, J. S., Cox, N.A., 1997, Patterson, J.A., Burkholder, K.M., 2003]. In our investigation could not be demonstrated any difference neither in intensity nor dynamics in shedding *Salmonella infantis* between the *FWGE+* and *FWGE-* groups but from the 23<sup>rd</sup> day both flocks proved to be negative by the cloaca tampon test (Table 4) though the cecal content remained positive with both flocks even on the 35<sup>th</sup> and 37<sup>th</sup> day of rearing.

As far as the immune responses are concerned it has been stated, that inclusion of MOS (mannan oligosaccharide) in broiler diets increased the immune response to

vaccination against IBDV and NDV. It has also been determined that PKC (palm kernel cake) containing NSP (non starch polysaccharid) added to the broiler feed increased HI antibody level to ND vaccine and relative weight of immune organs [Soltan, M.A., 2009] *FWGE* increased humoral antibody levels after ND, IBD, EDS and AE immunisation with egg layer parents [Kósa, E., Bajcsy, E., 2008]. *FWGE* influenced IBD VN antibody level of broilers, because on the 28<sup>th</sup> day, 7 days after vaccination it proved to be 26% higher compared to the control., but 21 days after vaccination this difference could not be shown out. (Table 1)

Beneficial effect of *FWGE* on the IB ELISA titres can be seen in the Table 2, which proves that the titres on the 28<sup>th</sup> day (28 days after vaccination) 820% and on the 42<sup>nd</sup> day (42 days after vaccination) 180% higher antibody titres. (Table 2)

As far as the ND HI titres are concerned as result of two vaccinations (1<sup>st</sup> day, 28<sup>th</sup> day) titres of blood samples taken on the 42<sup>nd</sup> day proved to be 100% higher with the *FWGE +* (Table 3) group compared to the controls and all samples proved to be positive.

### CONCLUSIONS

Bacteriological investigation of cloaca swabs proves, that *FWGE* in the feed did not influence neither of dynamics nor intensity shedding of *Salmonella infantis*, but it can be stated, that while the cloaca-swabs became negative on the 23<sup>rd</sup> day of rearing (19 days after infection) cecal content proved to be positive even on the 37<sup>th</sup> day, so the flock remained salmonella positive in spite of negative tampon-results.

Humoral immune response after vaccination has been positively influenced by *FWGE* in case of IBD vaccination till the 28<sup>th</sup>, in case of IB and ND till the 42<sup>nd</sup> day of rearing (slaughtering time). On the basis of the results of our study the conclusion may be drawn that *FWGE* has no positive effect in prevention of *Salmonella infantis* infection, but increases the immune responses after vaccination in broilers remarkably.

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