Testing the Efficacy of Fermented Wheat Germ Extract Against *Mycoplasma gallisepticum* Infection of Chickens

L. Stipkovits,*[,1] K. Lapis,† M. Hidvégi,‡ E. Kósa,§ R. Glávits,‖ and Á. Resetár#

*Veterinary Medical Research Institute of the Hungarian Academy of Sciences, Budapest, Hungary; †First Institute of Pathology and Experimental Cancer Research, Semmelweis Medical University, Budapest, Hungary; ‡Department of Biochemistry and Food Technology, Technical University of Budapest and Biromedicina Company, Budapest, Hungary; §Szent István University, Faculty of Veterinary Science, Budapest, Hungary; and ‖Central Veterinary Institute, Budapest, Hungary; and #Biromedicina Company, Budapest, Hungary

ABSTRACT The effect of fermented wheat germ extract (FWGE, Immunovet-HBM) was studied in chickens challenged with *Mycoplasma gallisepticum*. Ninety *M. gallisepticum*- and *M. synoviae*-free 3-wk-old chickens were exposed to aerosol infection of *M. gallisepticum*. One group (30 birds) was treated with FWGE, a second group with tiamulin, and a third group was untreated. The fourth group was exposed to PBS aerosol as a negative control. On d 9, all chickens were slaughtered and examined for the presence of gross and histological lesions, the presence of the challenge strain in the organs and specific antibodies in the serum. Body weight gains and feed conversion rates were recorded. In the groups treated with FWGE and with tiamulin, the chickens remained clinically healthy: their BW gains were 441.7 g and 446.8 g, respectively. Feed conversion ratios were 1.72 and 1.71 for FWGE- and tiamulin-treated birds, respectively. Control birds had BW gain of 480.8 g, and feed conversion ratio of 1.78. The numbers of birds with gross lesions (15 and 11, respectively) and lesion scores (25 and 25, respectively) of the FWGE- and tiamulin-treated groups were significantly lower than in the infected untreated group (25 birds, lesion score of 190). No mycoplasma was reisolated from brain, liver, spleen, heart, or kidneys of the FWGE-treated birds, and the number of mycoplasma isolations from the respiratory tract samples was less frequent (10) than from the infected untreated group (64). In addition, 35 samples from other internal organs were also positive. Twenty percent of the birds treated with FWGE showed serological response with a 5.0% reaction score, whereas in the infected untreated group, 83.3% of birds were reactors, with a 62.5% reaction score.

(*Key words*: broiler, fermented wheat germ extract treatment, *Mycoplasma gallisepticum* infection)

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INTRODUCTION

The chronic respiratory disease of chickens and turkeys associated with *Mycoplasma gallisepticum* (*M. gallisepticum*) infection causes significant economic losses to the poultry industry throughout the world (Mohamed et al., 1987; Kleven, 1990; Kleven et al., 1991; Jordan, 1996). This includes increases in mortality and feed conversion, decreases in growth, high carcass condemnation, decreases in egg production and hatchability. *Mycoplasma gallisepticum*, through the inhibition of immune functions, predisposes birds to other infections such as *Escherichia coli*, and *Haemophilus paragallinarum* (Stipkovits, 1968; Jordan, 1996), leading to further economic losses. To prevent these losses, the application of various antibiotics has been proposed (Hannan et al., 1989; Jordan et al., 1989). However, in recent years, the European Union has been making efforts to reduce the use of antibiotics, especially those that are used in human medicine. In view of this, an alternative solution would be to use immunomodulators. In this study, we examine the effect of an immunomodulator, fermented wheat germ extract (FWGE), for the treatment of *M. gallisepticum* infection of chickens. This immunomodulator has proved to be effective in human therapeutic applications (Jakab et al., 2000).

Fermented wheat germ extract is a standardized extract of fermented by *Saccharomyces cerevisiae*, optimized to a yield of 0.4 mg/g 2,6-dimethoxi-p-benzoquinone. When the extract was tested on sublethally irradiated or cyclophosphamide-treated mice, it significantly increased the number of reticulocytes and thrombocytes in treated animals. In another experiment, FWGE inhibited tumor pro-

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[1]To whom correspondence should be addressed: stipkovits@linux.vmri.hu.

Abbreviation key: FWGE = fermented wheat germ extract; RPA = rapid plate agglutination.

gression and metastasis in the in vivo models 3 LL-HH, B16, and HCR-25 (Hidvégi et al., 1999b). Pretreatment of mice with FWGE increased the blastic transformation of peripheral T lymphocytes induced by Concanavalin A. When used in the treatment of thymectomized mice (C57 BI10), FWGE significantly decreased the graft survival time, showing an immunostimulatory effect (Hidvégi et al., 1999a). Treatment with FWGE resulted in a significant decrease in the number of metastases in various tumor models, including Lewis lung carcinoma (3 LL-HH), HCR25 human colon carcinoma, and B16 melanoma. It synergistically enhanced the metastasis inhibiting effect of antineoplastic agents in C38 mouse colon carcinoma and B16 mouse melanoma models (Hidvégi et al., 1999b). Co-administration of vitamin C with FWGE gave the strongest metastasis inhibitory effect in most of the tumor models (Hidvégi et al., 1998). Administration of FWGE proved to be effective as supportive therapy to surgery plus chemotherapy for a colorectal cancer patient (Jakab et al., 2000). Furthermore, it did not show any toxic effects (Hidvégi et al., 1999b). Considering these results, we believe that the effect of FWGE on chickens experimentally infected with *M. gallisepticum* should be investigated.

MATERIALS AND METHODS

Experimental Birds

Arbor Acres chicks, free of *M. gallisepticum* (n = 140), were transported to our facility upon hatching and were reared in isolated conditions. The parent flock of chickens had been tested throughout the rearing and laying period by regular serological tests including rapid plate agglutination (RPA) with stained Nobilis *M. gallisepticum* and Nobilis *M. synoviae* antigens, and blocking ELISA (MyGa and MySy tests[2]) (Czifra et al., 1993, Németh et al., 1993) for antibodies against *M. gallisepticum* and *M. synoviae* infections, with negative results. The negative status of experimental chickens was tested serologically by ELISA with sera collected from 20-d-old slaughtered chicks to exclude the presence of maternal antibodies. No maternal antibodies could be detected. No mycoplasma was detected in the nasal cavities, trachea, lungs, or air sacs of these chicks on medium B (Ernø and Stipkovits, 1973). The chicks were fed a standard diet containing diclazuril as an anticoccidial.

Challenge

At the age of 3 wk, the chickens were marked individually, weighed, and distributed into 4 groups of 30 chickens each. The groups were organized so that the average BW of the groups did not differ by Student's *t*-test. Chickens were placed into a 200-L volume box. The groups were treated as follows. Group 1 was not infected. Ten milliliters of sterile medium was sprayed into the box; the birds were exposed for 20 min, and then transferred to a separate room. These chickens were considered the negative (control) group. Group 2 was infected with an aerosol of *M. gallisepticum* strain 1226, cloned 3 times and filtered through a 450-nm Millipore filter. The strain was lyophilized and stored at –20°C. Before challenge, the strain was propagated in medium B to a concentration of 9.5×10^8 cfu/mL. Chickens were placed in the same box as Group 1. Ten milliliters of *M. gallisepticum* broth culture was sprayed into the box; after exposure for 20 min, the birds were transferred to a separate room. This group received no treatment. Group 3 was challenged in the same way as group 2 and placed in another separate room. The chickens were treated with FWGE[3] via the feed, at a concentration of 3.0 g/kg of feed for 9 d. Based on preliminary studies, the optimal level of FWGE was in the range of 0.3 to 3.0 g/kg of feed. Group 4 was infected as group 2, and was transferred into a separate room and treated with 80% tiamulin[4] at a concentration of 200 mg of active substance (tiamulin hydrogen fumarate) per kg of feed for 9 d.

To avoid cross-contamination, different persons attended each of the groups. Feeding and management of all groups were the same. On d 9 of the experiment, chickens were slaughtered and examined for gross and histopathological lesions as well as for the presence of *M. gallisepticum* antibodies and mycoplasmas in the respiratory tract and inner organs.

Testing the Efficacy of Treatment

The chickens were examined daily for the development of clinical signs. Body weights of individual chickens were obtained before challenge and on d 9. Average BW of the groups were compared by Student's *t*-test. In each group, feed consumption was recorded and divided by the weight gain of the group. All chickens were slaughtered on d 9 after infection. They were examined for the presence of pathological lesions induced by *M. gallisepticum*. The pathological lesions of left and right air sacs and peritoneum were scored from 0 to 4 as follows: 0 = no lesions; 1 = cloudy air sac wall; 2 = small quantity of serous exudate in air sac or peritoneum; 3 = serous-fibrinous exudate, thickened air sac wall; and 4 = large quantity of fibrinous mass in air sacs and peritoneum. The numbers of birds with lesions and the sum of lesion scores in the groups were compared statistically by χ^2-test. Lungs, spleen, and bursa of Fabricius were examined histologically. Tissue slides were stained with hematoxylin and eosin. Lungs were examined for the presence of foci of interstitial pneumonia, lymphohistiocytic bronchitis, catarrhal pneumonia, or pleurisy. The severity of lesions was scored as follows: 0 = no lesions; 1 = slight; 2 = medium; and 3 = severe lesions. The spleen and the bursa of Fabricius were also examined for the presence of follicles rich in lymphocytes, and for the severity of lymphocyte depletion.

[2]MyGa and MySa tests, Diagnosticum Co. Ltd., Budapest, Hungary.
[3]Biromedicin Co. Ltd., Budapest, Hungary.
[4]Tiamulin, Biochemie GmbH, Kundl, Austria.

TABLE 1. Initial BW, BW gains, and feed conversion ratios of chickens challenged with *Mycoplasma gallisepticum* and treated with fermented wheat germ extract (FWGE) or tiamulin

Group/treatment	Initial BW (g)	Average BW gain (g)	Feed conversion ratio
1. Noninfected/untreated	380.8 ± 56.6	480.8 ± 85.1[a***]	1.78
2. Infected/untreated	369.6 ± 36.6	391.7 ± 51.6[b,c***]	2.23
3. Infected/FWGE	362.0 ± 36.6	441.7 ± 87.7	1.72
4. Infected/tiamulin	377.3 ± 34.8	446.8 ± 63.5	1.71

[a]Difference between noninfected and infected untreated groups.

[b,c]Difference between infected untreated group and groups infected and treated with FWGE or tiamulin, respectively.

***Difference by Student *t*-test at level of $P < 0.001$.

Test for Mycoplasma Infection

Swab samples from the organs placed on solid medium B were used to reisolate *M. gallisepticum* from the trachea, air sacs, lung, brain, liver, spleen, kidney, and heart of the chickens. Isolates were identified by epifluorescence examination (Bradbury, 1998) of colonies using conjugated anti-*M. gallisepticum* hyperimmune serum and species-specific PCR (Kempf et al., 1993). The proportion of reisolation in groups was compared by χ^2-test. Sera of chickens collected at slaughter were examined for the presence of specific antibodies against *M. gallisepticum* by the RPA test. The intensity of reactions was scored from 0 to 4. The number of reactors and the sum of scores of RPA in the groups were compared statistically.

RESULTS AND DISCUSSION

Clinical Performance

The noninfected control chickens remained healthy, whereas chickens infected and untreated began to show clinical signs (sneezing, rales, coughing, lacrimation) on d 6 of the experiment. On d 9, most of the chickens were sitting and breathing through their open mouths. On both d 7 and 9 one bird died in this group. No clinical signs were seen in the groups treated with FWGE or tiamulin.

Before the experiment, average BW of the groups did not differ from each other. During the experiment, lower ($P \leq 0.001$) BW gains were recorded in the infected untreated group compared with the control noninfected group and the group infected and treated with tiamulin or FWGE (Table 1). Average BW gains of the latter 3 groups did not differ from each other. The feed conversion ratio increased by 0.45 to 0.52 in the infected untreated group compared with the noninfected control and treated groups (Table 1).

Pathological Examinations

All of the noninfected control birds were free from any pathological lesions. In the infected untreated birds, air sacculitis and peritonitis of varying severities were observed. In contrast, the numbers of birds with lesions were lower ($P \leq 0.001$) in the FWGE- and tiamulin-treated groups (15 and 11, respectively) than in the infected untreated group (25). No differences were observed between the FWGE- and tiamulin-treated groups in the number of birds with lesions; lesion scores were also similar. Lesion scores were higher in the infected untreated group (190) compared with the treated groups (25 and 25) (Table 2). Lymphohistiocytic bronchitis or foci of interstitial pneumonia in the lungs of noninfected chickens were found very rarely (in 6 and 2 cases, respectively). Catarrhal pneumonia and pleurisy were not detected. The incidence of lymphohistiocytic bronchitis, interstitial pneumonia, and catarrhal pneumonia in the infected untreated group was high (43, 33, and 12, respectively), whereas incidences in the FWGE- and tiamulin-treated groups was lower ($P \leq 0.001$) (12, 14, and 0, respectively, in group 3 and 17, 8, and 0, respectively, in group 4), and were not different from the noninfected control group. There was no difference between the treated groups.

Lymphocyte depletion in the bursa of Fabricius and spleen occurred very rarely in the noninfected group, and in the groups infected and treated with FWGE or tiamulin. In all birds, follicles rich in lymphocytes were observed. Because of the infection, the number of follicles decreased ($P \leq 0.001$) in the infected untreated group.

Mycoplasma Infection

No mycoplasmas were reisolated from birds of the noninfected group. In contrast, mycoplasmas were isolated from most of the birds of the infected untreated groups, mainly from the trachea, lungs, and air sacs. Mycoplasmas were found relatively often (35 cases) even in other inner organs, such as the liver, spleen, heart, and kidneys. The mycoplasma reisolation rates from respiratory tract were lower ($P \leq 0.001$) in the FWGE- and the tiamulin-treated groups (10 and 3, respectively) than in the infected untreated group. The tiamulin-treated group had fewer mycoplasma reisolates than the FWGE-treated group ($P \leq 0.05$). Reisolation from other inner organs was not successful in these groups.

Birds in the noninfected group remained serologically negative. In the infected untreated group, 25 birds became seropositive with a reaction score of 75 (Table 3). In the treated groups, lower ($P \leq 0.001$) numbers of birds (6 and 8) showed serological reaction with scores of 6 and 11

TABLE 2. Air sacculitis, peritonitis, and histological lesions in lung, spleen, and bursa of Fabricius and their scores in chickens challenged with *Mycoplasma gallisepticum* and treated with fermented wheat germ extract (FWGE) or tiamulin

Lesions	Groups/treatment			
	Noninfected/ untreated	Infected/ untreated	Infected/ FWGE	Infected/ tiamulin
Air sacculitis and peritonitis (no. of birds)	0^{abc***}	25^{de***}	15	11
Scores of gross lesions	0^{abc***}	190^{de***}	25	25
Histology of lung				
Lymphohistiocytic bronchitis	6^{a***}	43^{de***}	12	17
Foci of interstitial pneumonia	$2^{a***b**}$	33^{de***}	14	8
Catarrhal bronchopneumonia	0^{a***}	12^{de***}	0	0
Pleurisy	0	14	0	0
Histology of spleen				
Follicles rich in lymphocytes	25^{a***}	7^{de***}	25	25
Lymphocyte depletion	0^{a***}	25^{de***}	2	5
Histology of bursa Fabricii				
Follicles rich in lymphocytes	25^{a***}	6^{de***}	25	25
Lymphocyte depletion	0^{a***}	26^{de***}	2	5

[a–c]Difference between noninfected group and infected untreated group or groups infected and treated with FWGE and tiamulin, respectively.

[d,e]Difference between infected untreated group and groups infected and treated with FWGE and tiamulin, respectively.

,*Difference by χ^2-test at levels of $P < 0.01$ and $P < 0.001$, respectively.

for groups 3 and 4, respectively. There was no difference between the treated groups.

In the experiment presented in this paper, chickens exposed for 20 min to 9.5×10^8 cfu/L of *M. gallisepticum* became infected and showed clinical signs of respiratory disease, which was of fatal outcome in some birds. In the field, birds are often exposed to such conditions for several weeks. In the study birds following infection, gross and histopathological lesions were found that were characteristic of *M. gallisepticum* infection. The birds became serologically positive. These data confirmed the observations of other authors (Jordan et al., 1989, Kleven et al., 1991; Jordan, 1996).

It is well known that tiamulin is one of most effective antimycoplasma drugs (Jordan et al., 1989). After treatment with tiamulin, infected birds remained clinically healthy: gross and histopathological lesions developed in fewer birds, and lesions were much less severe than those in the infected untreated group. The mycoplasma reisolation rate was much lower and mycoplasmas could be reisolated from the trachea only, whereas in the infected untreated group, mycoplasma was isolated from the trachea, lungs, air sacs, liver, spleen, kidney, heart, and brains. Serological responses were observed in a few birds only in the tiamulin- and FWGE-treated groups.

Based on the experimental data obtained, FWGE, like tiamulin, was able to protect birds from *M. gallisepticum* infection. Infected birds treated with FWGE did not develop clinical signs, and no mortality was recorded. In the slaughtered birds, pathological lesions were mild and rarely seen. According to the results of histological examination, catarrhal pneumonia and pleurisy did not occur; lymphohistiocytic bronchitis and foci of interstitial pneumonia were recorded rarely and in a mild form. The rate

TABLE 3. Reisolation of *Mycoplasma gallisepticu*m from the inner organs, and results of the slide agglutination test of sera from chickens challenged with *Mycoplasma gallisepticum* and treated with fermented wheat germ extract (FWGE) or tiamulin

Reisolation	Group/treatment			
	Noninfected/ untreated	Infected/ untreated	Infected/ FWGE	Infected/ tiamulin
Total reisolation from respiratory tract	0^{abc***}	64^{de***}	10^{f*}	3
Total reisolation from other organs[1]	0^{abc***}	35^{de***}	0	0
No. of serologically reacting birds	0^{abc***}	25^{de***}	6	8
Scores of RPA[2]	0^{abc***}	75^{de***}	6	11

[a–c]Difference between noninfected group and infected untreated group or groups infected and then treated with FWGE or tiamulin, respectively.

[d,e]Difference between infected non-treated group and groups infected and treated with FWGE or tiamulin, respectively.

[f]Difference between groups infected and treated with FWGE and infected and treated with tiamulin.

[1]Brain, liver, spleen, kidneys, and heart.

[2]RPA = rapid plate agglutination.

*, **, ***Difference by χ^2-test at levels of $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

of mycoplasma reisolation and detection of serological response were very low. These parameters were similar to the tiamulin-treated group.

In conclusion, these findings have demonstrated the positive effects of the immunomodulator, FWGE. The effects are probably due to activation of macrophages, the induction of transcription of cytokine genes, the release of inflammatory cytokines, the increase of blastic transformation of lymphocytes (Hidvégi et al., 1998, 1999a,b), and the reduction of MHC class I expression, thus exposing mycoplasmas to natural killer cell activity, which has been demonstrated in various tumor experiments.

These findings are of considerable importance as they show that immunomodulators such as FWGE are effective against *M. gallisepticum* infection, similar to the well-known antimycoplasma drug, tiamulin. *Mycoplasma gallisepticum* infection is widespread throughout the world, and FWGE could be used to reduce the economic losses caused by mycoplasma infection. This is especially relevant as the agriculture industry seeks to reduce its use of use of antibiotics.

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