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EFFECT OF Cd AND LACTOBACILLUS LEVELS ON IRON CONCENTRATION IN DIFFERENT ORGANS AND MEAT OF BROILER CHICKEN

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ABSTRACT

The aim of this study was to determine the effect of levels of cadmium and the lactobacillus complex on iron concentration in liver, kidney, meat (leg, breast) of broiler chicken. Two hundred seven day old broiler chicks were evenly distributed into twenty groups. It was found that the increasing of cadmium concentration in diets lead to decreasing of iron concentration in meat (leg, breast). Significant differences were found between the control group and the groups 20, 30, 40 ppm Cd, P≤ 0.05 for leg meat. Significant differences were also found between the control group and the groups 30, 40 ppm Cd, P≤ 0.05 for breast meat. While increasing of cadmium concentration in diets lead to increasing of iron concentration in kidney and liver, this increasing was significant when comparing groups 20, 30, 40 ppm Cd with the control group P≤ 0.05. The study showed that the lactobacillus complex lead to decreasing of negative effects of cadmium on the iron distribution in the studied organs and tissues, especially for groups which have at least 20 ppm cadmium in their diets.

Keywords: *broiler chicken , cadmium,* Lactobacillus, *liver, kidney, leg, breast, iron.*

INTRODUCTION

Elements have two functions in animal bodies: the first is structural function and the second is organizational. Elements can be divided into major elements as: Ca, P, Mg, K, Na, etc. and trace elements as: Fe, Cu, Zn, I, Co, etc., in addition to non-essential elements as Pb, Hg, As, Si and Cd [1]. Cadmium is a heavy metal unnecessary for the body and the work of its organs, and it is highly toxic for a wide range of microbes, plants, animals and humans, because it causes damages to liver, kidney, lungs, heart and the nervous system [2, 3]. It was reported that the maximum tolerable dietary Cd level for domestic animals was 0.5 ppm, dietary concentrations of 1 ppm result in undesirable effect, while 5 ppm cause adverse health effects [4]. Cadmium is of grave cancered [5]. The main risk posed by cadmium as an environmental pollutant is its easy access to the food chain [6]. It may cause disorders of the metabolism, and traces of elements in the body lead to accumulation of zinc, with increase in the liver, and decrease in the hair, when cadmium levels increase in the diets of many animals [7]. Cadmium accumulation had effects on tissue structures, it caused decreasing of Fe and Hb (hemoglobin) levels in the tissues [8]. Cadmium causes many damages of the digestive apparatus, especially small intestines that are expressed as red spots [9, 10]. Glutathione was used for binding heavy metals (Cd, Pb, Ni) [11] and Aflatoxins B1 [12, 13]. Probiotics lead to improving of meat quality, animals immunity, reduction of toxic effects on bird growth [14, 15], also the health benefit of lactic acid bacteria have been attributed to their production of EPS (exoplolysaccharides) which has antiulcer, immunomodulating, or cholesterol-lowering activity [16,17]. There is significant evidence that probiotics, such as specific types of lactobacillus bacteria, and bifidobacteria can lower the three major risk factors for coronary heart disease and stroke: excessive cholesterol, high blood pressure, and high triglyceride levels. Lactobacillus bacteria lowered total blood cholesterol by 22 %, and triglycerides - by 33 % [18]. Besides the specificity of the bacterial strain, the bacterial concentration influences the AFB1(Aflatoxins B1) removal. Different minimum concentrations had been reported such as 5.10^9 CFU/ml of either L.acidophilus or B.longum to remove only 13 % of the AFB1 within one hour [19], or 2.10^9 CFU/ml of lactobacilli and propionibacterium to remove 50 % of free AFB1, but higher binding occurred at 10^{10} CFU/ml [20]. When the bacteria are subjected to various chemical and physical treatments, their ability to remove AFB1 can be increased significantly. Autoclaved cells of L.casei remove significantly more AFB1 from phosphate buffered saline (PBS), compared to viable bacteria [21]. Heat treatment (boiling for 1 hour), and acid treatment also significantly enhanced AFB1 binding by GG and LC-705 [20]. Peltonen and colleagues compared the binding ability of various strains of viable and heat-treated Bifidobacteria, and found that the viable bacteria bound 4-56 %, while heat-treated bacteria bound 12-82 % of the AFB1[22]. In contrast, Lankaputra and Shah found that viable bacteria bound more dietary mutagens (including AFB1) than heat-treated bacteria [23]. As heat-treated bacteria are often more efficient to remove AFB1 than viable cells, metabolic degradation cannot be the mechanism responsible for AFB1 removal. It seems more likely that the toxin is bound to the bacterial surface. The aflatoxin-bacteria complex was therefore studied to test the stability of the interaction, and toxin release was observed after washing the AFB 1-bacteria complex with water [24,25]. This finding led to the conclusion that the bacteria only reversibly bound AFB 1 However, one study reported that bound AFB1 was only extractable from heat-killed bacteria, but not from viable ones [23].

The aim of the present study was to investigate the effects of levels from cadmium on iron concentration in liver, kidney, leg meat, and breast meat of broiler chicken.

EXPERIMENTAL

Material and Methods

Two hundred unisex chickens (Ross 308), have been randomly allotted to 4 parts as follows:

First part: Four groups (each group containing 10 birds), cadmium was added to their diets with levels 10, 20, 30 and 40 ppm as cadmium chloride, successively.

Second part: Three groups (each group containing 10 birds), lactobacillus complex was added to their diets with levels 5, 7 and 9 kg/ton of diet, successively.

Third part: Twelve groups (each group containing 10 birds), lactobacillus complex was added to their diets with levels 5, 7 and 9 kg/ton of diet and cadmium as cadmium chloride with levels 10, 20, 30 and 40 ppm.

Fourth part: control group (contains 10 birds), birds of the control group were fed diets without addition of cadmium or lactobacillus complex.

Before the experiment 10 samples of diets were analyzed for cadmium concentration by atomic absorption spectrometry, the average of cadmium concentration in these diets was $1.0^9 \pm 0.42$.

The Lactobacillus complex contained L.b.acidophillus and L.b.burglaricous at about $8\ .10^8$ CFU/g.

The experiment birds were fed these diets from 7 days age until 42 days.

The protein in the diets was about 22 % and energy was 3200 Kcal.

At the end of the experiment, samples were taken from organs and tissues, iron was determined by the humid method, described in WHO, 1992.

Statistical evaluation

The results obtained were evaluated by ANOVA as a completely randomized design. Statements of statistical significance were based on P?0.05.

RESULTS AND DISCUSSION

The mean results with standard error for iron concentration in liver, kidney, leg meat, and breast meat are presented in the following tables. Table 1 refers to the effect of cadmium on iron concentration in organs and tissues of broiler chicken. From Table 1 follows that the increase of cadmium concentration in diets lead to a decrease of the iron concentration in leg meat and

Table 1. Iron concentration (ppm) in organs and tissues of birds that took only different levels (10 , 20 , 30 , 40 ppm) of cadmium by diets .

	Control	Cadmium concentration in diets, ppm			
		10	20	30	40
Leg meat	±14.012 2.279 a	±13.032 1.941 a b	±11.946 2.242 b c	±11.25 2.066 b c	±10.452 2.119 c
Breast meat	±13.516 1.980 a	±12.624 2.382 a b	±11.698 2.377 a b c	±10.893 2.348 b c	±10.017 1.916 c
Liver	±59.877 5.366 d	±63.08 5.431 c d	±65.944 4.802 b c	±69.154 4.681 a b	±72.879 4.834 a
Kidney	±54.96 5.645 d	±58.699 4.159 c d	±62.483 3.964 b c	±65.616 4.432 a b	±68.78 4.268 a

a,b: Mean results within a row with no common superscript differ significantly.

breast meat, and an increase in liver and kidney, confirming the findings of [8, 26].

Significant differences of iron concentration in leg meat were found between the control group and each groups with 20, 30, 40 ppm Cd. Also significant differences were observed between group 10 ppm Cd and group 40 ppm Cd at $P \leq 0.05$.

For breast meat significant differences of iron concentration were found between the control group and each group with 30 and 40 ppm Cd; also between group 10 ppm Cd and group 40 ppm Cd at $P \leq 0.05$.

For liver and kidney, significant differences of iron concentration were found between the control group and each group with 20, 30 and 40 ppm Cd, also between group 10 ppm Cd and groups 30 and 40 ppm Cd, and between group 20 ppm Cd and group 40 ppm Cd at $P \leq 0.05$.

It is difficult to explain the mechanism of cadmium effects on metabolism, and accumulation of metals in tissues and organs. But many studies suggest that cadmium maybe causes decreased absorption of metals in the small intestine by its competition with these metals for their carriers. For example, cadmium

Table 2. Iron concentration (ppm) in organs and tissues of birds that took different levels (5, 7, 9 kg) of lactobacillus complex/ton fodder by its diets.

	Control	Lactobacillus complex concentration in diets, kg/ton of diet		
		5	7	9
Leg meat	±14.012 2.279 a	±14.47 1.944 a	±14.873 2.295 a	±14.631 2.018 a
Breast meat	±13.516 1.980 a	±14.17 2.316 a	±14.457 2.110 a	±14.164 1.762 a
Liver	±59.877 5.366 a	±60.483 5.114 a	±61.252 5.320 a	±61.176 4.108 a
Kidney	±54.96 5.645 a	±54.679 4.669 a	±54.951 4.419 a	±55.145 4.591 a

a,b: Mean results within a row with no common superscript differ significantly.

Table 3. Iron concentration (ppm) in some organs and tissues of birds that took different levels (5, 7, 9) kg of lactobacillus complex/ton fodder with 10 ppm Cd in diets.

	Control	10 ppm Cd at diet	Lactobacillus complex with kg / ton cadmium at diets with ppm+		
			5 complex 10 Cd +	7 complex 10 Cd +	9 complex 10 Cd +
Leg meat	±14.012 2.279 a	13.032± 1.941 a	±13.247 1.972 a	±13.584 2.070 a	±13.751 2.077 a
Breast meat	±13.516 1.980 a	12.624± 2.382 a	±12.731 2.733 a	±13.246 2.224 a	±13.329 2.274 a
Liver	±59.877 5.366 a	63.08± 5.431 a	±62.811 4.569 a	±61.683 4.628 a	61.227± 5.065 a
Kidney	±54.96 5.645 a	58.699± 4.159 a	±58.824 4.261 a	±57.46 4.290 a	56.938± 4.369 a

a,b: Means within a row with no common superscript differ significantly.

Table 4. Iron concentration (ppm) in some organs and tissues at birds that took different levels (5, 7, 9) kg of lactobacillus complex/ton fodder with 20 ppm Cd in its diets.

	Control	20 ppm Cd at diet	Lactobacillus complex with kg / ton + cadmium at diets with ppm		
			5 complex +Cd 20	7 complex +Cd 20	9 complex +Cd 20
Leg meat	±14.012 2.279 a	±11.946 2.242 b	±11.726 1.880 b	±12.452 2.004 a b	±12.677 2.375 a b
Breast meat	±13.516 1.980 a	±11.698 2.377 a	±11.549 1.939 a	±12.108 2.297 a	±12.295 2.558 a
liver	±59.877 5.366 b	±65.944 4.802 a	±65.694 5.255 a	±64.914 5.086 a	±64.326 5.220 a b
Kidney	±54.96 5.645 b	±62.483 3.964 a	±62.148 5.353 a	±61.630 4.918 a	±60.912 4.455 a

a,b: Means within a row with no common superscript differ significantly.

competes with Fe, Mn, and Cu for FPN1 (Ferroprotin1), and DMT1 (Divalent metal transporter1) [27]. These carriers are proteins with sites for metals [28]. Also many researchers found that cadmium causes damages of the small intestine as red spots, may be this has a role in reduction of metals absorption [9,10]. Table 2 refers to effects of the lactobacillus complex on iron concentration in some organs and tissues of broiler chicken.

Table 2 shows that the lactobacillus complex lead to increasing of iron concentration in liver, leg meat, breast meat compared to the control, but no significant differences were observed between the studied groups at $P \leq 0.05$. A slight improvement of iron concentration in these organs and tissues, was noted with the increase of lactobacillus complex in diets. The ability of the lactobacillus for keeping bird intestines from

Table 5. Iron concentration (ppm) in some organs and tissues of birds that took different levels (5, 7, 9) kg of lactobacillus complex/ton fodder with 30 ppm Cd in diets.

	Control	30 ppm Cd at diet	Lactobacillus complex with kg / ton + cadmium at diets with ppm		
			5 complex +Cd 30	7 complex +Cd 30	9 complex +Cd 30
Leg meat	±14.012 2.279 a	±11.25 2.066 b	±10.845 2.184 b	±11.032 2.457 b	±11.598 2.374 b
Breast meat	±13.516 1.980 a	±10.893 2.348 b	±10.582 2.093 b	±11.104 2.196 b	±11.35 2.174 b
liver	±59.877 5.366 b	±69.154 4.681 a	±68.896 4.584 a	±67.924 4.157 a	±67.542 3.829 a
Kidney	±54.96 5.645 b	±65.616 4.432 a	±64.952 3.586 a	±65.168 3.735 a	±64.469 4.576 a

a,b: Means within a row with no common superscript differ significantly.

some damages, in addition to its contribution to improvement of digestion by enzymes, and biological materials, maybe is the reason for improvement of the iron concentration in these organs and tissues. This is supported by the findings of [29], that lactobacillus improve digestibility of protein and some metals as Ca, P, Fe, Mg and Zn, and serves as substrates in the intermediary metabolism [30]. The following Tables (3, 4, 5, 6) refer to effects of the lactobacillus complex on iron concentration in some organs and tissues of broiler chicken, fed with diets polluted with different levels of cadmium.

Table 3 does not show significant differences for iron concentration with addition of the lactobacillus complex to diets with 10 ppm Cd at $P \leq 0.05$. Table 4 displays significant differences for iron concentration in leg meat between the control group and groups 20 Cd, 20 Cd+5 complex, 20 Cd+7 complex, 20 Cd+9 complex. No significant differences were found for breast meat.

For iron concentration in liver significant differences were found between control group and groups 20Cd, 20Cd+5 complex, 20Cd+7 complex, and between the control group and groups 20Cd, 20Cd+5 complex, 20Cd+7 complex, 20Cd+9 complex in kidney at $P \leq 0.05$.

Table 5 shows significant differences for iron concentration in liver, kidney, leg meat, and breast meat between the control group and each of the groups 30Cd, 30Cd+5 complex, 30Cd+7 complex, 30Cd+9 complex, while no significant differences are observed for liver, kidney, leg meat, and breast meat, when comparing groups 30Cd, 30Cd+5 complex, 30Cd+7 complex, 30Cd+9 complex between each other at P?0.05.

In Table 6 significant differences are found for iron concentration in liver, kidney, leg meat, and breast meat between the control group and groups 40Cd, 40Cd+5 complex, 40Cd+7 complex, 40Cd+9 complex, while there are no significant differences for liver, kidney, leg meat, and breast meat when comparing groups 40Cd, 40Cd+5 complex, 40Cd+7 complex, 40Cd+9 complex between each other at P?0.05.

From Tables 3-6 for the addition of lactobacillus complex to diets, which contain different levels of cadmium it follows that the complex decreases negative effects of cadmium on iron concentration, especially for diets which contain the cadmium about 20 ppm and less, while no visible effect was found on iron concentration by the lactobacillus complex when cadmium concentration was about 30 ppm and more.

May be this can be explained by the ability of lactobacillus to bind toxins, as shown by [24,25,31], or by lactobacillus secretions such as biological complexes, which have a main role for improvement of

Table 6. Iron concentration (ppm) in some organs and tissues of birds that took different levels (5, 7, 9) kg of lactobacillus complex/ton fodder with 40 ppm Cd in diets.

	Control	40 ppm Cd in diet	Lactobacillus complex with kg / ton +cadmium in diets, ppm		
			5 complex +Cd 40	7 complex +Cd 40	9 complex +Cd 40
Leg meat	±14.012 2.279 a	±10.452 2.119 b	±10.234 1.831 b	±10.565 2.112 b	±10.845 2.087 b
Breast meat	±13.516 1.980 a	±10.017 1.916 b	±10.148 1.721 b	±10.612 1.538 b	±10.529 1.824 b
Liver	±59.877 5.366 b	±72.879 4.834 a	±72.182 5.592 a	±71.701 5.433 a	±72.36 4.988 a
Kidney	±54.96 5.645 b	±68.78 4.268 a	±67.754 4.164 a	±68.09 4.093 a	±68.354 3.960 a

a,b: Means within a row with no common superscript differ significantly.

growth, keep animal health and increase immunity [32-34].

Increase of the lactobacillus activity was found for decreasing negative effects of cadmium with increase of lactobacillus concentration at diets, and decrease of cadmium concentration were observed [35,20,36]. Their studies showed that the concentration of lactobacillus in due conditions have a main role for the lactobacillus activity for toxins binding.

CONCLUSIONS

The study showed that the lactobacillus complex lead to decreasing of the negative effects of cadmium on iron distribution in the studied organs and tissues, especially for groups which have Cadmium less than 20 ppm in their diets. We advise to add of lactobacillus complex to poultry diets for keeping of the birds healthy and increasing production.

REFERENCES

1. K.M. Nicola, Animal Nutrition, Faculty of Agriculture, Albaath University, 2000, 22–27.
2. M. Anke, E. Losch, J. Hubschmann, K. Kramer, Die nickelbelastungen der Nδhrungskette Von pflanze , tier und Mensch in Deutschland, 2 .Auswirkung der nickelbelastungenbei der Fauna. Mengen und Spurenelemente, 13. Arbeistagung, Jena, 1993, 382–399; Wiss . publ . Karl–Mrx – Unvi., Leipzig .
3. E. Hurna, S . Hurna, Cytotoxicity and genotoxicity of Cd investigated on a rat Livery epithelial cell line and hamster lung fibroblast, Vet. Med. Czech., **43**, 1998, 365-371 .
4. L.R. McDowell, Minerals in animals and Human nutrition, Academic Press, New York, 1992, 359 – 361.
5. I.M. McKenna, T. Gordon, L.C. Chen, M.R. Anever, M.I. Woolkes, Toxical Appl. Pharmacol., **153**, 1998, 169–172.
6. M. Ondrašovič, O. Ondrašovičová, M. Vargová, A. Kočišová, Environmental problems in veterinary practice, Edition of the University of Veterinary medicine, Košice, Data Help, Slovak Republic, 1997, p. 142. (in Slovak).
7. M. Anke, A. Hennig, H.J. Schneider, V. Gargen, W.H. Schlegal, Bkh Trace element metabolism in animals, Ed. C.F Mills E. and S. Livingston, Edinburgh and London, 1970.
8. K. Swiergosz, Cadmium accumulation and effects in growing pheasants phasianus Colchicus (L). Avian Research Inst. Izantnagar, India, J. Poultry, **52**, 8,

2000, 23–26.

9. A. EL-Sebai, M. Szilagyi, I. Szalay, M. Sankari, I. Pais, Physiological and Biochemical Parameters in Chicken Exposed to Cadmium, Defzite und Überschüsse an Mengen – und Spurenelementen in der Ernährung, 14 Arbeistagung 1994, Jena, Germany, 223-258 .
10. E. Battikh, Studding of broiler chicken diets pollution by cadmium and lead, and its effects on some biological parameters, and snowball effect in some parts of carcass, Ms. Thesis, Agriculture College, Aleppo University, 2005, 120-140.
11. A. Pompella, A. Visvikis, A. Paolicchi, V. De Tata, A.F. Casini, The changing faces of glutathione, a cellular protagonist, Biochem. Pharmacol., 66, 2003, 1499–1503.
12. D.L. Eaton, E.P. Gallagher, Mechanisms of aflatoxin carcinogenesis, Ann. Rev. Pharmacol. Toxicol., 1994, 34, 135-172.
13. T.E. Massey, R.K. Stewart, J.M. Daniels, L. Liu, Biochemical and Molecular Aspects of Mammalian Susceptibility to Aflatoxin B1 Carcinogenicity, Proc. Society Experiment Biol. Medicine, **208**, 1995, 213-227.
14. N.B. Inciong, Test of EM Toxicity to Chicken, In Proceedings of the Third Conference on Effective microorganisms (EM), held at Kyusei Nature farming Centre, Saraburi, Thailand, 16-19 November, 1994, International Nature Farming Research Centre, Atami, Japan, 8.
15. National Research Council, Nutrient Requirements of Poultry, 9th Revised Edition. National Academy of Science Press, Washington, 1994, p. 156.
16. P. Ruas Madiedo, J. Hugenholtz, P. Zoon, An overview of the functionality of exopolysaccharides produced by lactic acid bacteria, Int. J. Dairy, **12**, 2002, 163-71.
17. A.D. Welman, I.S. Maddox, Exopolysaccharides from lactic acid bacteria: perspectives and challenges, Trends Biotechnol., **21**, 2003, 269-274.
18. M. Taranto, Effect of Lactobacillus reuteri on the prevention of hypercholesterolemia in mice, Journal of Dairy Science, **83**, 1999, 401-403.
19. F. Bolognani, C.J. Rumney, R. RowlandI, Influence of carcinogen binding by lactic acid-producing bacteria on tissue distribution and in vivo mutagenicity of dietary carcinogens, Food Chem. Toxicol., 35, 1997, 535-545.
20. H. El-Nezami, P. Kankaanpää, S. Salminen, J. Ahokas, Ability of dairy strains of lactic acid bacteria to bind a common food carcinogen, aflatoxin B1. Food Chem. Toxicol., **36**, 1998, 321- 326.
21. N. Thyagaraja, A. Hosono, Binding properties of lactic acid bacteria from 'Idly' towards food-borne mutagens, Food Chem. Toxicol., **32**, 1994,805-809.
22. K. Peltonen, H. El Nezami, C. Haskard, J. Ahokas, S. Salminen, Aflatoxin B1 binding by dairy strains of lactic acid bacteria and bifidobacteria, J. Dairy Sci., **84,** 2001, 2152-2156.
23. W.E. Lankaputhra, N.P. Shah, Antimutagenic properties of probiotic bacteria and of organic acids, Mutat. Res., **397,** 1998, 169-182.
24. J.T. Oatley, M.D. Rarick, G.E. Ji, J.E. Linz, Binding of aflatoxin B1 to bifidobacteria in vitro, J. Food Prot., **63,** 2000, 1133-1136.
25. Y.K. Lee, H. El-Nezami, C.A. Haskard, S. Gratz, K.Y. Puong, S. Salminen, H. Mykkanen, Kinetics of adsorption and desorption of aflatoxin B1 by viable and nonviable bacteria, J. Food Prot., **66,** 2003, 426-430.
26. M.R. Fox, Cadmium metabolism –A review of aspects pertient to evaluating dietary cadmium intake by man, In A.S. Prasad, Ed. Trace elements and human health and disease, v. 2, 1976, p. 401, Academic press, New York.
27. O. Helena, O. Agneta, L. Thomas, S. Staffan, T. Jonas, Increased cadmium absorption in iron supplemented suckling piglest-swedisk, University of Agriculture Sciences, Uppsala, Sweden, 2007.
28. WHO, (World Health Organization), Cadmium (EnvIronmental Health Criteria 134), Geneva, 1992.
29. M.P. Mokoena, P.K. Chelule, N. Gqaleni, The toxicity and decreased concentration of aflatoxin B in natural lactic acid fermented maize meal, J. Appl. Microbiol., **100,** 2006, 773-777.
30. M. Kirchgessner, M.X. Roth, Ergotrope Efekte durch organische Suaren in der Ferkelaufzucht und Schweinemast, Ubersichten zur Tierernahrung, **16,** 1988, 93-108.
31. H. El-Nezami, H. Mykkänen, P. Kankaanpää, S. Salminen, J. Ahokas, Ability of Lactobacillus and Propionibacterium strains to remove aflatoxin B1, from the chicken duodenum, J. Food Prot., **63,** 2000, 549-552.

32. M. Vanbelle, E. Teller, M. Focant, Probiotics in Animal Nutrition: A Review. Archives of Animal Nutrition., **40,** 1990, 543-567.
33. K. Grunewald, Serum cholesterol level in rats fed skim milk fermented by Lactobacillus acidophilus, Journal of Food Science, **47,**1982, 2078-2079.
34. D. Knorr, Technology aspects related to microorganisms in functional foods, Trends Food Sci., **9,**1998, 295-306.
35. I.M. Bovee-Oudenhoven, M.L. Wissink, J.T. Wouters, R. Van der Meer, Dietary calcium phosphate stimulates intestinal lactobacilli and decreases the severity of a salmonella infection in rats, J. Nutr., **129,** 1999, 607–612.
36. G.W. Tannock, Probiotics - time for a dose of realism, Curr. Iss. Intest. Microbiol., **4,** 2003, 33-42.