Keep bacteria under control: Dietary modulation of gut microflora in farm animals by use of hen egg yolk antibodies

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INTRODUCTION

The gut microflora constitutes a dynamic and ecologically diverse environment. Normal microbial populations present in the gut provide an efficient barrier to invading microorganisms. Protection mechanisms involve the competition for nutrients and adhesion sites, the production of antimicrobial compounds, the lowering of intestinal pH through production of short-chain fatty acids and the stimulation of the immune system [1]. An imbalance in this bacterial consortium can favor the colonization by undesirable agents [2]. Therefore, the control of the bacterial balance in the gut of farm animals is interesting in order to limit the risk of infection and maximize performance.

The easiest way to modulate bacterial populations is the use of antibiotics. However, on January 2006, European Union banned the feeding of all sub-therapeutic antibiotics to livestock for growth promotion purpose and there is a trend towards elimination or reduced use of these compounds in other countries. The animal feed industry must cope with this withdrawal and try to develop alternatives to antibiotics to modulate microflora (for a review, see [3]).

For this purpose, the use of hen egg yolk antibodies (called IgY) has recently gained interest. Indeed, while the well-known prebiotics can stimulate bacteria of interest in the intestine, antibodies will instead limit the growth of undesirable targeted bacteria. Major source of human foodborne infections such as salmonellosis or campylobacteriosis, the hen could thus become an ally to fight these pathogens in the future, thanks to its ability to produce massive amounts of antibodies specific of targeted bacteria.

This review article focuses on the use of IgY in therapy or prophylaxis of diseases in veterinary medicine as well as in the context of functional foods to modulate microflora in animal. Other aspects such as molecular properties of IgY or production procedures are reviewed in detail in Schade *et al*. [4].

IMMUNOGLOBULIN Y (IgY)

The hen transmits protective antibodies to its offspring via the egg, as observed in mammals via colostrum ingestion and/or placental transfer. Three classes of immunoglobulins are transferred in this way. IgA and IgM are present in the egg white at very low concentrations while large amounts of IgY (8-25 mg/ml) are present in the egg yolk [5]. These antibodies are specific of microbes the hen encountered during her life and are thus characteristic of the microbial environment of the henhouse. The natural transfer of antibody from hen to chick can be exploited to produce IgY specific of a given pathogen. Indeed, the concentration of IgY in the egg is proportional to that in maternal serum. Therefore, by immunizing laying hens with a specific target antigen, we can direct their immune system and lead to the export of high levels of specific IgY in their eggs [4]. Feeding these specific antibodies to other animals (chickens but also other species) can be seen as en extension of the passive maternal protection [6]. Furthermore, it can be speculated that these orally distributed IgY could allow a microbial control in the gut by binding, immobilising and inhibiting the growth of selected bacteria.

PROMISING RESULTS OF IgY IN ANIMAL DIETS

IgY are used in various research fields, including immunoassays, immunohistochemistry, diagnostics etc. [7]. Specific antibodies are also considered as a promising tool to modulate microflora.

Treatment of intestinal infections

Passive immunization is the most reported application of IgY in animal feed. It consists in a transfer from an individual to another of active humoral immunity through preformed antigen-specific antibodies. The antibodies can be transmitted intravenously or orally. This last option is however widely preferred for the antibody treatment of infections localized in the digestive tract of animals.

The mechanism of action of these antibodies at the intestinal level is not yet fully understood. Several *in vitro* studies showed that specific IgY have a growth inhibitory effect on targeted bacteria [8, 9]. For example, we demonstrated that specific IgY reduce the growth of *Salmonella* in a liquid medium. In the same study, anti-*Salmonella* IgY prevented the attachment of the bacterium at the intestinal Caco-2 cells [9]. These results indicate that IgY are able not only to alter the development of bacteria, but also to thwart their attachment to the intestinal epithelium.

This antibacterial potential has been largely studied in veterinary research with the aim to prevent or treat gastrointestinal infections caused by enteric pathogens in a wide variety of animal species: chicken [10, 11, 12], calf [13, 14], duck [15], pig [16, 17, 18], sheep [19], rabbit [20] and even fishes [21, 22]. Although very promising effects have been obtained against various enteric pathogens, results reported are not always consistent. In a study conducted in our laboratory, specific IgY failed to reduce the cecal colonization by *Salmonella* Enteritidis and *Salmonella* Typhimurium in co-challenged broilers [11]. In this study, hyperimmune eggs were obtained from laying hens immunized using *Salmonella* Enteritidis and *Salmonella* Typhimurium outer membrane proteins. This led to high levels of IgY directed against the two serovars in egg yolks [23]. Powders were obtained from these hyperimmune eggs by freeze-drying the whole yolk. *Salmonella*-free one-day-old chicks received supplemented feed (5 % egg yolk powder) at five levels of concentration in specific IgY-enriched yolk powder (0 – 5%). Three days following the initiation of feed treatments, birds were co-challenged with 1×10^6 cfu of *Salmonella* Enteritidis and *Salmonella* Typhimurium. Positive (challenged and untreated) and negative (unchallenged) control groups were considered. At 4 days post-infection, there was a trend towards a nearly significant difference ($P < 0.1$) between treatment groups concerning the *Salmonella* spp. cecal colonization. This trend was associated with a linear effect ($P < 0.05$) of specific IgY concentration. Nevertheless, this effect was not significant thereafter. In the same trial, we also observed an improvement in growth performance for the groups receiving the yolk enriched diets when compared with the untreated control group ($P < 0.05$). However, the challenged treated birds never reached the performance levels of unchallenged birds ($P <$

0.05) and the growth performance enhancement was not associated with a specific IgY concentration effect.

If IgY are able to remove some targeted bacteria in the gut, it could be expected that other groups or species could then develop because of substrate and adhesion sites availability. Therefore, it could be interesting to associate probiotics with an IgY treatment in order to colonize the empty space. Such combination products have been investigated with success in the recent years. Tellez *et al*. [24] reduced the colonization by *Salmonella* Enteritidis in broilers using a combination of *Lactobacillus acidophilus* and *Streptococcus faecium* (Avian Pac Plus, Loveland Industries Inc., Greeley, CO), and IgY directed against *Salmonella* Enteritidis, *Salmonella* Typhimurium, and *Salmonella* Heidelberg. Similarly, IgY orally distributed worked synergistically with probiotics (*Lactobacillus acidophilus*, *Lactobacillus bulgaricum*, *Lactobacillus reuteri*, and *Bifidobacterium bifidum*) to prevent *Salmonella* Enteritidis infection in challenged ducklings [15]. The synergistic effect is not always reported. The use of IgY combined with a probiotic mixture of *Lactobacillus acidophilus*, *Lactobacillus casei*, *Enterococcus durans*, and *Bifidobacterium thermophilus* (Primalac, Star Labs/Forage Research, Clarcksdate, MO) in broilers led to results similar to those obtained with the antibodies alone [10].

The antimicrobial and growth promoter properties of hyperimmune egg yolk have been largely described, but the potential efficacy of nonimmune egg yolk has also been reported [11, 25]. It could be, at least in part, related to the presence of nonspecific IgY that are able to improve the intestinal immune system of the animal in a nonspecific way. Moreover, the egg yolk is rich in potentially beneficial components, both for the immune system of the animal (mainly immunoglobulins, but also low-density lipoproteins or sialyloligosaccharides) and for its growth (e.g. omega-3 fatty acids or phospholipids) [26].

Modulation of the normal gut microflora

Beyond the control of pathogens, antibodies could also be envisaged to modulate the normal gut microflora. This has been recently studied for example in ruminants, to decrease the risk of ruminal acidosis or to reduce methanogenesis in the rumen.

Ruminal acidosis is a nutritional disorder commonly occuring in cattle fed with high-grain diets containing large amounts of rapidly fermentable carbohydrates. The resulting modification of the rumen microflora causes an accumulation of volatile fatty acids and lactate and a risk of acidosis. The consequences are an alteration in rumen fermentation patterns and in health of the animal [27]. Given the close link between the microbial balance in the rumen and the risk of acidosis, scientists developed means to manipulate this complex ecosystem. For example, an oral administration of lactate-utilizing bacteria (*Megasphaera elsdenii*) as a probiotic was successful in decreasing lactate concentration and increasing ruminal pH in the rumen of beef cattle rapidly changed from a forage-based to a grain-based diet [28]. On the opposite, vaccination against bacteria involved in the production of lactate (*Streptococcus bovis*) maintained higher pH and decreased lactate concentration in rumen [29]. IgY antibodies have also been investigated to control the ruminal ecosystem. Passive immunization through oral administration of IgY specific of *Streptococcus bovis* (lactate producer) or *Fusobacterium necrophorum* (involved in the development of liver abscesses in acidotic animals) reduced rumen concentrations of target bacteria in steers fed high-grain diets [30, 31]. The doses of antibody preparations used were as low as 2.5 mL per daily meal and were thus very low compared with the total volume of rumen. It was however sufficient to increase significantly the ruminal pH level with benefits on ruminal patterns. Indeed, both proteolytic and cellulolytic activities are pH-dependent so that fiber and protein digestibility might be improved by maintaining the rumen pH above 6.0 [32]. Moreover, such IgY treatment also exhibited a positive effect ($P < 0.05$) on feedlot performances [31, 33].

The use of passive immunization through IgY has also been proposed to control other ruminal bacteria such as methanogens. Over the last decades, researchers have explored a variety of approaches to decrease methane production in ruminants due to its contribution to the greenhouse effect and its cost as dietary energy to the ruminant [34]. Treatments using egg yolk antibodies have shown promising results in this area. Indeed, IgY specific of methanogenic bacteria (*Methanobrevibacter smithii*, *Methanobrevibacter ruminantium* and *Methanosphaera stadtmanae*) decreased CH_4 production *in vitro* ($P < 0.05$) but this effect was only transient and by 24 h, CH_4 levels in all treatments were similar ($P > 0.05$) to the untreated control [35]. However, these results suggest that IgY represents a promising tool of controlling the production of methane by cattle.

CURRENT CHALLENGES AND FUTURE PROSPECTS

There already exists some commercial products using the concept of passive immunization through IgY in the marketplace (e. g. Protimax®, Trouw Nutrition, Highland, IL ; Globigen®, EW Nutrition GmbH, Visbek, Germany) and others are currently in development in link with patented applications. Indeed, the use of egg proteins in animal feed is authorized by the current european legislation [36] and such egg-derived products are classified as GRAS (generally recognized as safe). Nevertheless, even if not prohibited, the use of egg proteins in animal feed, especially in the case of poultry feed, could still be rejected by consumers as are animal flour in cattle feed since the mad cow crisis. Other aspects could explain why a wide distribution of hyperimmune egg-derived products to commercial farm animals remains a difficult goal to achieve.

As discussed above, the results reported for these passive immunization strategies are not always consistent. This can be attributed to the variability in protocols investigating these effects, including both *in vitro* and *in vivo* methods, and studying the effects of IgY on a variety of bacterial agents, in various animal species.

Farmers might be interested to control multiple gut microbes. Among them, there are infectious agents but also bacteria composing the normal gut microflora that we could want to regulate. Nevertheless, most studies have been made against a certain bacterium in order to control its development and colonization. The feed industry would benefit more of antibodies preparations targeting a variety of bacteria. Enlarging the activity spectrum of products would promote their commercial application [37]. For example, we have been able to produce IgY simultaneously directed against several *Salmonella* serovars in the same egg yolk [23] with a proven efficacy in *in vitro* models [9]. In some cases (e. g. with *Salmonella*), it is possible to take advantage of the cross-reactivity especially when immunizing the hens with isolated proteins shared by several serovars [23]. But in most of the cases, the specificity of developed IgY to a particular epitope will induce the need to produce IgY on a case-by-case basis with obvious cost consequences [6].

The form in which the antibodies are delivered to animals is also an area that requires further development. Indeed, administer antibodies in a stomach-resistant formula is important in order to prevent inactivation by the acidic pH of the stomach or by proteases before the intestine. Studies conducted with broilers [38, 39, 40] have shown that IgY activity remained detectable at the end of the gut. Moreover, the proteo-lipidic matrix of the egg yolk is effective in protecting the antibodies from the digestive process both *in vitro* [39, 41] and *in vivo* [39, 40]. Nevertheless, even though IgY were protected in the egg yolk proteo-lipidic matrix, this protection seems to be insufficient with regard to the decrease of activity [40]. The same problems of gastric degradation have been reported in pigs [42]. It is somewhat different when looking for an activity in the rumen of polygastric species.

Microencapsulation may be an effective method for protecting IgY from gastrointestinal inactivation [42, 43, 44, 45]. The proposed encapsulation procedures often involve pH-sensible matrix. The release of antibodies to the place where their action is desired in the digestive tract of the animal will be a crucial point for the effectiveness of these protecting products. This is particularly true with regard to poultry, where the role of the crop, in which the pH may be close to that of the intestine, cannot be neglected.

In conclusion, IgY may be a useful and attractive alternative to antibiotics in order to modulate microbial populations in the gut of farm animals. Results are already promising including beneficial impacts on animal performances and health and possible actions where this tool could be used is still vast. This technology, even if needing some further research particularly on the protection-release aspect, is thus likely to be of great value to the animal feed industry in the coming years.

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REFERENCES

1. Bourlioux, P., B. Koletzko, F. Guarner and V. Braesco. 2003. Am. J. Clin. Nutr. 78:675-683.
2. Marcq, C., E. Cox, I. M. Szalo, A. Théwis, and Y. Beckers. In Press. Poult. Sci. doi:10.3382/ps.2010-01017.
3. Vandeplas, S., R. Dubois Dauphin, Y. Beckers, P. Thonart, and A. Théwis. 2010. J. Food Prot. 73:774-785.
4. Schade, R., E. Gutierrez Calzado, R. Sarmiento, P. A. Chacana, J. Porankiewicz-Asplund, and H. R. Terzolo. 2005. ATLA Altern. Lab. Anim. 33:129-154.
5. Rose, M. E., E. Orlans, and N. Buttress. 1974. Eur. J. Immunol. 4:521-523.
6. Berghman, L. R., D. Abi-Ghanem, S. D. Waghela, and S. C. Ricke. 2005. Poult. Sci. 84:660-666.
7. Narat, M. 2003. Food Technol. Biotechnol. 41:259-267.
8. Sunwoo, H. H., E. N. Lee, K. Menninen, M. R. Suresh, and J. S. Sim. 2002. J. Food Sci. 67:1486-1494
9. Chalghoumi, R., A. Théwis, Y. Beckers, C. Marcq, D. Portetelle, and Y.-J. Schneider. 2009. Foodborne Pathog. Dis. 6:593-604.
10. Rahimi, S., Z. R. Shiraz, T. Z. Salehi, M. A. K. Torshizi, and J. L. Grimes. 2007. Int. J. Poult. Sci. 6:230-235.
11. Chalghoumi, R., C. Marcq, A. Théwis, D. Portetelle, and Y. Beckers. 2009. Poult. Sci. 88:2081-2092.
12. Mahdavi, A. H., H. R. Rahmani, N. Nili, A. H. Samie, S. Soleimanian-Zad, and R. Jahanian. 2010. Poult. Sci. 89:484-494.
13. Ikemori, Y., M. Kuroki, R. C. Peralta, H. Yokoyama, and Y. Kodama. 1992. Am. J. Vet. Res. 53:2005-2008.
14. Yokoyama, H., R. C. Peralta, K. Umeda, T. Hashi, F. C. Icatlo, M. Kuroki, Y. Ikemori, and Y. Kodama. 1998. Am. J. Vet. Res. 59:416-420.
15. Fulton, R. M., B. N. Nersessian, and W. M. Reed. 2002. Poult. Sci. 81:34-40.
16. Imberechts, H., P. Deprez, E. Van Driessche, and P. Pohl. 1997. Vet. Microbiol. 54:329-341.
17. Marquardt, R. R., L. Z. Jin, J.-W. Kim, L. Fang, A. A. Frohlich, S. K. Baidoo. 1999. FEMS Immunol. Med. Microbiol. 23:283-288.
18. Letellier, A., S. Messier, L. Lessard, and S. Quessy. 2000. Can. J. Vet. Res. 64:27-31.
19. Cook, S. R., J. S. Bach, S. M. L. Stevenson, R. DeVinney, A. A. Frohlich, L. Fang, and T. A. McAllister. 2005. Can. J. Anim. Sci. 85:291-299.

20. O’Farelly, C., D. Branton, and C. A. Wanke. 1992. Infect. Immun. 60:2593-2597.
21. Lee, S. B., Y. Mine, and R. M. W. Stevenson. 2000. J. Agric. Food Chem. 48:110-115.
22. Arasteh, N., A.-H. Aminirissehei, A. N. Yousif, L. J. Albright, and T. D. Durancea. 2004. Aquaculture. 231:23-36.
23. Chalghoumi, R., A. Théwis, D. Portetelle, and Y. Beckers. 2008. Poult. Sci. 87:32:40.
24. Tellez, G., V. M. Petrone, M. Escorcia, T. Y. Morishita, C. W. Cobb, and L. Villaseñor. 2001. J. Food Prot. 64:287-291.
25. Kassaify, Z. G., and Y. Mine. 2004. Poult. Sci. 83:1497-1506.
26. Mine, Y., and J. Kovacs-Nolan. 2004. J. Poult. Sci. 41:1-29.
27. Krause, K. M., and G. R. Oetzel. 2006. Anim. Feed Sci. Technol. 126:215-236.
28. Klieve, A. V., D. Hennessy, D. Ouwerkerk, R. J. Forster, R. I. Mackie, and G. T. Attwood. 2003. J. Appl. Microbiol. 95:621-630.
29. Gill, H. S., Q. Shu, and R. A. Leng. 2000. Vaccine. 18:2541-2548.
30. DiLorenzo, N., F. Diez-Gonzalez, and A. DiCostanzo. 2006. J. Anim. Sci. 84:2178-2185.
31. DiLorenzo, N., C. R. Dahlen, F. Diez-Gonzalez, G. C. Lamb, J. E. Larson, and A. DiCostanzo. 2008. J. Anim. Sci. 86:3023-3032.
32. Kovacik, A. M., S. C. Loerch, and B. A. Dehority. 1986. J. Anim. Sci. 62:226-234.
33. Dahlen, C. R., L. J. Smith, N. DiLorenzo, and A. DiCostanzo. 2003. Minnesota Cattle Feeder Report. B-495:53-58.
34. Johnson, K. A., and D. E. Johnson. 1995. J. Anim. Sci. 73:2483-2492.
35. Cook, S. R., P. K. Maiti, A. V. Chaves, C. Benchaar, K. A. Beauchemin, and T. A. McAllister. 2008. Aust. J. Exp. Agr. 48:260–264.
36. Regulation (CE) no. 1192/2005. Official Journal of the European Union. L205:0003-0011.
37. Sugita-Konishi, Y., K. Shibata, S. S. Yun, Y. Hara-Kudo, K. Yamagushi, and S. Kumagai. 1996. Biosci. Biotechnol. Biochem. 60:886-888.
38. Wilkie, D. C., A. G. Van Kessel, T. J. Dumonceaux, and M. D. Drew. 2006. Prev. Vet. Med. 74:279-292.
39. Marcq, C., V. Graide, Y. Beckers, D. Portetelle, and A. Théwis. 2008. Proceedings of the Thirteenth Conference on Food Microbiology. 11-12 September 2008. University of Ghent. Belgium. pp. 106-107.
40. Marcq, C., D. Portetelle, A. Théwis, R. Chalghoumi, and Y. Beckers. 2009. 17th European Symposium on Poultry Nutrition. 23-27 August 2009. Edinburgh, Scotland. p. 181.
41. Jaradat, Z. W., and R. R. Marquardt. 2000. Food Agric. Immunol. 12:263-272.
42. Kovacs-Nolan, J., and Y. Mine. 2005. J. Immunol. Meth. 296:199-209.
43. Chang, H.-M., Y.-C. Lee, C. C. Chen, and Y.-Y. Tu. 2001. J. Food. Sci. 67:15-20.
44. Cho, Y.-H., J.-J. Lee, I.-B. Park, C.-S. Huh, Y.-J. Baek, and J. Park. 2005. J. Food Sci. 70:E148-E151.
45. Li, X.-Y., L.-J. Jin, J.-E. Uzonna, S.-Y. Li, J.-J. Liu, H.-Q. Li, Y.-N. Lu, Y.-H. Zhen, and Y.-P. Xu. 2009. Vet. Immunol. Immunopathol. 129:132-136.