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Dietary *Chlorella* supplementation effect on immune responses and growth performances of broiler chickens exposed to post hatch holding time

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Abstract

The study was carried out to investigate the effect of dietary *Chlorella* sp. supplementation on immune response and growth performance of broiler chickens exposed to post hatch holding time. Allotted in 36 pens, a total of 180 newly hatched chicks were assigned in a 3 × 2 factorial design, with dietary *Chlorella* administration (0, 5 and 10 g kg^{-1}) and feeding time post hatch (hour 0 and 48) as the factors. The *Chlorella* supplemented diets were provided to chicks either immediately (*early*) or after 48 hours (*late*) post hatch until day 35.

Irrespective of the post hatch feeding times, *Chlorella* supplementation increased (P< 0.05) total IgA concentration in the intestinal mucosa of broilers. Birds withheld from feed for 48 hours after hatching had lower (P< 0.05) final body weight (BW) than those of fed immediately.*Chlorella* supplementation had no influence on the final BW, but abdominal fat content of broilers was lowered (P< 0.05) by *Chlorella*, regardless of the different feeding times post hatch. In conclusion, post hatch holding time resulted in lower final BW of broilers. Although feeding 1% *Chlorella* could not alleviate the retarded growth rate in feed withheld-birds, the treatment may be useful to improve the immune response and to decrease the abdominal fat content of broilers.

Keywords: *abdominal fat, prebiotics, probiotics, stress*

Introduction

The benefits of early post hatch feeding on final body weight (BW) and immunological performance of broilers have been reported (Juul-Madsen et al 2004). Under practical conditions, the newly hatched chicks are however often withheld from feed (*ca.* 48 hours) due to e.g., transportation from hatchery to commercial farms and time interval in the hatching window. Such condition may be unfavorable to the growth, viability and immune performance of broilers (Cherian 2015). The use of feed additives in broiler diets for instance mannanoligosaccharides (MOS), acidifiers (Ao et al 2012), organic acid blends (Cengiz et al 2012), probiotics (Daşkıran et al 2012) and prebiotics (Koksal et al 2013) has been expected to alleviate the adverse effects of delayed feeding after hatching. However, dietary supplementation with such additives have not yet ameliorated the negative effects of post hatch delayed feeding in birds (Ao et al 2012; Cengiz et al 2012; Daşkıran et al 2012; Koksal

et al 2013).

Marine microalgae have long been recognized for their valuable health effects for both human and animals. Among the marine algae, *Chlorella* has gained much attention in animal and veterinary use (Kang et al 2013). This microalga is known to have high contents of n-3 long-chain polyunsaturated fatty acids (LC-PUFAs) (Xue et al 2012), fiber and protein peptides (Kang et al 2013), which may act as health-promoting substances for birds (An et al 2008). In addition, *Chlorella* contains natural growth factors (Liang et al 2004) and high quality of protein (Kang et al 2013), which are essential for promoting the growth rate of broiler chickens. Taking these beneficial properties of *Chlorella* into consideration, supplementation of broiler diets with this microalga seemed to be a desirable tool for ameliorating the depressed immune response and growth performance of broilers withheld from feed after hatching. The aim of the present study was to investigate the effect of dietary *Chlorella* supplementation on immune response and growth performance of broiler chickens exposed to post hatch holding time.

Materials and Methods

A total of 180 1-day-old chicks with uniform BW (46.76 ± 0.81g) were obtained from commercial hatchery at 0 to 2 hours post hatch and transported within ~15 minutes to the experimental unit. The birds were allotted to 36 wire floors pens with 2 male and 3 female chicks in each. The experiment was conducted according to 3 × 2 factorial design, with 3 levels of *Chlorella* included in the diets and 2 feeding time post hatch. Each treatment was replicated 6 times with 5 chicks per replicate. The levels of *Chlorella* in the diets were 0, 5 and 10 g kg^{-1} feed (Table 1).Water was offered throughout the experiment and the diets (supplemented with *Chlorella*) were provided either immediately (*early*) or after 48 hours (*late*) of hatching until day 35. The dried *Chlorella* sp. meal (crude protein 1.70% and total fat 15.04%, on a dry matter basis) used in this study was obtained from Brackishwater Aquaculture Development Centre Situbondo, East Java, Indonesia. The experiment complied with the guideline of the Faculty of Animal and Agricultural Sciences, Diponegoro University with respect to experimentation and care of animals under study.

Table 1. Composition of experimental diets

Items	*Chlorella* levels		
	0 g kg^{-1}	**5 g kg^{-1}**	**10 g kg^{-1}**
Ingredients (g kg^{-1})			
Maize	562	562	562
Soy bean meal	245	245	245
Meat bone meal	60.0	60.0	60.0
Corn gluten meal	30.0	30.0	30.0
Palm kernel meal	15.0	15.0	15.0
Distiller dried grain soluble	9.00	9.00	9.00
Others[1]	78.7	78.7	78.7
Dried *Chlorella* meal	0.00	5.00	10.0
Analyzed composition[2]			
Metabolisable energy (MJ 100 kg^{-1})	1,243	1,189	1,214
Crude protein (%)	20.8	20.0	20.8
Total fat (%)	5.92	6.19	7.46

[1] *Amino acids, vitamins and mineral-mix*

[2] *Feed analyses were performed by Eurofins Steins Laboratorium A/S, Odense, Denmark*

At hours 0 and 48 (before feeding) and day 35, all chicks were weighed individually. Feed intakes were recorded weekly for each pen. The birds were vaccinated with the inactivated Newcastle Diseases (ND) vaccine and live vaccine against ND, infectious bursal diseases

(IBD) vaccine and live vaccine against ND at days 6, 13 and 17, respectively. At days 13, 20 and 24, birds from each replicate were blood sampled (from wing vein). The same birds were slaughtered at day 35. Abdominal fat, heart, liver and small intestine were obtained immediately after slaughter. Intestinal mucosa and bile were then collected. Immunoglobulin (Ig) concentrations were measured with commercial kits (chicken Ig ELISA Quantification Kit, Bethyl Laboratories, Montgomery, TX, USA) according to the manufacturer's protocols. At day 34, blood was collected from the wing vein of male broiler from each replicate. The serum total cholesterol was determined based on an enzymatic method using Auto analyzer-Advia 1650 (Bayer Diagnostics, Leverkusen, Germany).

The data were analyzed based on General Linear Models Procedure in SAS (SAS Inst. Inc., Cary, NC, USA). Effect of different levels of *Chlorella* in the diet, time of feeding post hatch and their interaction were analyzed. Since no interaction (P> 0.05) between the treatments was observed for all measured parameters in the present study, the results are presented as least square means and standard error for the treatment effects.

Results

Irrespective of the post hatch feeding times, dietary *Chlorella* supplementation increased (P< 0.05) total IgA concentration in the intestinal mucosa of broilers (Table 2). However, the treatments did not affect (P> 0.05) the serum concentrations of IgG and IgM and IgA in bile. Birds withheld from feed for 48 hours after hatching showed lower (P< 0.05) final BW than those offered feed immediately (Table 3).

Table 2. Concentrations of Igs in the serum, intestinal mucosa and bile[1]

Items	*Chlorella* levels			SE	P-value
	0 g kg^{-1}	5 g kg^{-1}	10 g kg^{-1}		
Total serum IgG (mg mL^{-1})					
Day 13	0.45	0.40	0.43	0.08	0.68
Day 20	1.85	1.48	1.72	0.39	0.46
Day 24	3.30	2.62	2.65	0.45	0.10
Total serum IgM (mg mL^{-1})					
Day 13	0.11	0.08	0.10	0.03	0.33
Day 20	0.12	0.12	0.17	0.05	0.14
Day 24	0.17	0.17	0.19	0.04	0.75
Mucosal IgA (mg g^{-1})	16.7^{a}	20.7^{ab}	37.6^{b}	9.32	0.04
Biliary IgA (mg mL^{-1})	4.10	4.12	6.39	1.73	0.29

[1] Data are presented as the effect of Chlorella levels since there was no effect (P> 0.05) of holding time post hatch on Igs concentrations.
[a,b] Values with different letters within the same row were significantly different
SE= standard error

Table 3. Performances of broilers[1]

Items	Feeding time post hatch		SE	P-value
	Early	*Late*		
Final BW (g)	2,039^{a}	1,928^{b}	55.2	<0.01
Accumulative feed intake (g)	2,939	2,774	287	0.13
FCR	1.44	1.45	0.84	0.95

[1] Data are presented as the effect of feeding time post hatch since there was no effect (P> 0.05) of dietary Chlorella supplementation on the performances of broilers.
[a,b] Values with different letters within the same row were significantly different
SE= standard error

Chlorella supplementation could not ameliorate (P> 0.05) the depressed final BW in broiler

exposed to post hatch holding time. Abdominal fat content of broilers was lowered (P< 0.05) by *Chlorella*, regardless of the different feeding times post hatch (Table 4). However, feeding *Chlorella* did not affect (P> 0.05) the total concentration of cholesterol in serum and the relative weight of liver and heart. Post hatch feeding times did not influence the organ weights or serum cholesterol.

Table 4. Percentage of abdominal fat and serum total cholesterol level of broilers[1]

Items	*Chlorella* levels			SE	P-value
	0 g kg^{-1}	5 g kg^{-1}	10 g kg^{-1}		
Abdominal fat (%)	1.83^{a}	1.52^{ab}	1.50^{b}	0.19	0.03
Cholesterol level (mmol L^{-1})	3.39	3.49	3.34	0.38	0.80
Liver (%)	2.26	2.35	2.18	0.15	0.31
Heart (%)	0.53	0.52	0.52	0.02	0.74

[1] Data are presented as the effect of Chlorella levels since there was no effect (P> 0.05) of holding time post hatch on the above parameters.

[a,b] Values with different letters within the same row were significantly different

SE= standard error

Discussion

Post hatch holding time has been reported to impair the immunological performance of chicks (Cherian 2015). However, results in the present study showed that post hatch holding did not affect the concentrations of Igs in the serum, intestinal mucosa and bile of broilers. Indeed, similar findings were reported by Juul-Madsen et al (2004) and Engberg et al (2013) who did not observe any effect of *early* or *late* feeding post hatch on the serum and plasma IgA and IgM concentrations as well as specific IgG response to a vaccination against IBD virus. According to Bar-Shira et al (2005), the activity of gut-associated lymphoid tissue (GALT) in broilers which is retarded due to post hatch holding has recovered after 2 weeks of age. Taking this into view, since Igs concentrations were determined at 2 weeks of age and later, the differences of these immune parameters between the two feeding time groups could not be monitored in this present study and others (Juul-Madsen et al 2004; Engberg et al 2013). Irrespective of the different time of *Chlorella* provision post hatch, feeding *Chlorella* resulted in higher concentration of total IgA in the intestinal mucosa of broiler in the present work. This finding was concomitant with that of reported by Kang et al (2013), in which *Chlorella* administration significantly increased the plasma IgG, IgA and IgM concentrations in broilers. The mechanism by which *Chlorella* enhanced IgA level in the intestinal mucosa of broilers is still unclear. However, An et al (2008) revealed that *Chlorella* may increase the production of interferon (IFN)-γ and interleukin (IL)-2 that in turn increase the production of IgA. Concomitant with the latter inference, Kang et al (2013) assumed that fiber and protein peptides contained in *Chlorella* may stimulate Igs-producing B cells in the GALT resulting in elevated production of IgA in the intestinal mucosa. Moreover, the LC-PUFAs contained in *Chlorella* seemed also to promote the Igs production in broilers through modulation of the cytokines expression (Kang et al 2013).

Regardless of the *Chlorella* supplementation, birds withheld from feed had lower final BW than those of fed immediately post hatch. Similar result was reported by other authors (Juul-Madsen et al 2004; Engberg et al 2013). Feeding *Chlorella* was actually expected to alleviate the retarded growth rate in feed withheld-birds, given that *Chlorella* was able to improve BW gain of broiler in the study of Kang et al (2013). However, *Chlorella* administration failed to do so in the present study. It has been known that dietary fatty acid composition may affect nutrient partitioning in broiler chickens, in which feeding n-3 LC-PUFAs resulted in a leaner bird (Newman et al 2002). The same effect was observed for dietary fiber (Mohiti-Asli et al 2012). Owing to the fact that *Chlorella* contains high amount of n-3

LC-PUFA (Xue et al 2012) and fiber (Kang et al 2013), the potential of *Chlorella* to partition of energy into lean rather than into fat tissue perhaps therefore attenuated the potential of *Chlorella* in promoting the growth of broiler *per se*, and may partly explain that dietary *Chlorella* supplementation resulted in lower abdominal fat content of broilers in the present study.

In conclusion, post hatch holding time impaired final BW of broilers. Although feeding 1% *Chlorella* could not alleviate the retarded growth rate in feed withheld-birds, the supplementation may be useful to improve the immune functions and to decrease the abdominal fat of broilers.

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