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3 **Dietary supplementation with modified arabinoxylan rice bran (MGN-3) modulates**  
4 **inflammatory responses in broiler chickens**

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6 Appropriate Scientific Section: feed and Nutrition

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15 **Running Head: Immunomodulation by arabioxyylan rice bran**

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24

25        **ABSTRACT**

26        The objective of this study was to investigate the effect of dietary supplementation with modified  
27        arabinoxylan rice bran (MGN-3) on the immune system and inflammatory response in broiler  
28        chickens. The levels of cluster of differentiation 3 (CD3), interleukin (IL)-2 and interferon (IFN)- $\gamma$   
29        mRNA in the spleen of chickens increased with the supplementation of MGN-3 at 100 ppm in diet,  
30        while those expression levels in the foregut did not change. Mitogen-induced proliferation of  
31        splenic mononuclear cells (MNC) and blood MNC phagocytosis in chickens fed  
32        MGN-3-supplemented diets were significantly greater than in chickens fed a basal diet (control).  
33        These results provide the first evidence that the use of dietary MGN-3 supplementation induces the  
34        T-cell immune system in chickens. Two hours after *Escherichia coli* (*E. coli*) lipopolysaccharide  
35        (LPS)-induced immune stimulation, the levels of mRNA encoding pro-inflammatory cytokines,  
36        such as IL-2, IFN- $\gamma$  and tumor necrosis factor-like ligand 1A (TL1A), in the spleen of chickens fed  
37        a MGN-3-supplemented diet were significantly lower than those in chickens exposed to other  
38        treatments. The levels of toll-like receptor -4 and -7 mRNAs in the foregut of chickens fed MGN-3  
39        supplemented diets were lower than those in control chickens at 2h after injection of LPS. The  
40        plasma ceruloplasmin concentration in chickens fed a MGN-3-supplemented diet was significantly  
41        lower than in controls at 24h after injection of LPS. These results show that MGN-3 might be useful  
42        as an immunomodulator to stimulate T-cells in growing broiler chickens, thereby protecting  
43        chickens from disease, particularly colibacillosis, without reducing growth performance.

44        (*Key words: broiler chickens, immunomodulation, inflammatory response, modified arabinoxylan,*  
45        *rice bran*)

46

47 **INTRODUCTION**

48 The meat production in broiler industry is to decrease or stop the use of antibiotics which are  
49 used to prevent disease and thereby promote growth in poultry (Ferket 2004). An alternative way to  
50 avoid the use of antibiotics is the control of the immune system that enhances humoral immunity  
51 and minimizes immunological stress in chickens (Klasing 1998). We have previously showed that  
52 dietary supplementation with nutrients enhance immunological function in chickens (Takahashi *et*  
53 *al.*, 2008; Sato *et al.*, 2009). These results suggest that immunomodulators could protect chickens  
54 from disease without decreasing growth performance by enhancing the immune system and could  
55 be used as a substitute for antibiotics. As a result, it is important to identify new supplements which  
56 act as immunomodulators in chickens for efficient meat production without antibiotics.

57 Modified arabinoxylan rice bran (MGN-3), which is a denatured hemicellulose obtained by  
58 reacting rice bran hemicellulose with multiple carbohydrate hydrolyzing enzymes from the Shiitake  
59 mushrooms, consists a xylose in its main chain and an arabinose polymer in its side chain  
60 (Ghoneum, 1998). It has been reported that MGN-3 increases natural killer (NK), T, and B cell  
61 functions both *in vitro* and *in vivo* in mammalian species (Ghoneum and Gollapudi, 2003; Badr  
62 El-din *et al.*, 2008). In addition, supplementation of MGN-3 in the diet improves the antioxygenic  
63 potential and protects against oxidative stress in mice (Noaman *et al.*, 2008). Thus, MGN-3 has a  
64 potential for immunomodulator in mammals. It is suggested that MGN-3 is not digested but is  
65 partially absorbed directly into the blood through the intestinal wall to interact with NK cells and  
66 macrophages (Badr El-din *et al.*, 2008), and then it may modulate the immune responses in not only  
67 gut-associated lymphoid tissue but also spleen. Moreover, arabinoxylan from wheat bran inhibits  
68 Salmonella colonization in broiler chickens (Eeckhaut *et al.*, 2008), while the different structure  
69 was reported among arabinoxylan from wheat bran and rice bran (Rose *et al.*, 2010). Hence, it is  
70 possible that dietary arabinoxylan rice bran may affect inflammatory responses in chickens.

71 In the present study, we have investigated whether MGN-3 enhances the expression of

72 T-cell-related mRNAs (including cluster of differentiation 3 (CD3), interleukin (IL)-2, interferon  
73 (IFN)- $\gamma$ ) and toll-like receptors (TLRs) in the foregut and spleen, as well as phagocytes of blood  
74 mononuclear cells (MNC), mitogen (concanavalin A (Con A) -induced proliferation of splenic  
75 MNC of growing broiler chickens. In addition, the present study also examined the inflammatory  
76 response resulting from *E. coli* LPS-induced immune stimulation in chickens fed  
77 5-ALA-supplemented diets. As inflammatory response parameters, plasma ceruloplasmin (Cer) and  
78 the mRNA expression levels of IL-1 $\beta$ , IL-6, CD3, IL-2, IFN- $\gamma$ , TLRs, and tumor necrosis factor  
79 -like ligand 1A (TL1A) in the spleen were examined.

80

## 81 **MATERIALS AND METHODS**

### 82 ***Animals, diet, blood sampling and lipopolysaccharide treatment***

83 Unvaccinated 1-d-old male broiler chicks (Ross 308 strain) obtained from a local hatchery  
84 (Matsumoto hatchability, Ibaraki, Japan) were used in all experiments. For experiments 1, 2 and 3,  
85 birds were housed in electrically-heated battery brooders and fed on a corn-soybean meal-based diet  
86 (basal diet; 230g crude protein/kg and 3,100 kcal metabolisable energy/kg; Murakami *et al.*, 1995)  
87 *ad libitum* for 14d. 14-d-old chicks were selected that were as close in body weight as possible to  
88 ensure body-weight uniformity and individually reared in stainless-steel wire cages (one bird in one  
89 chase) in a temperature (25°C) and light (23h/d)-controlled room. **MGN-3 was provided from**  
90 **Chiryoku Co.**, and the main chemical structure of MGN-3 is arabinoxylan with a xylose in its main  
91 chain and an arabinose polymer in its side chain (Ghoneum, 1998). All experimental diets were  
92 formulated to contain essential nutrients that met or exceeded recommended levels (Japanese  
93 Feeding Standards for Poultry, 2004).

94 In experiment 1, the effect of graded MGN-3 supplementation of the basal diet with on the  
95 expression of T-cell related mRNAs in the spleen was determined. Eighteen chicks (14d of age) in  
96 individual cages were divided into three groups of six chicks and each group was provided with one

97 of three experimental diets for 14d *ad libitum*. The experimental diets were prepared by simply  
98 supplementation of basal diet with MGN-3 at 0 (Control), 100 or 1,000 ppm. Diet and water were  
99 freely provided. At age 28d, the chickens were then sacrificed by cervical dislocation, and the  
100 foregut (about 1g, from the end of the duodenum to the middle section of the jejunum) spleen and  
101 bursa fabricius samples were collected. Tissue samples were frozen in liquid N<sub>2</sub> and were stored at  
102 –80°C until analysis.

103 In experiment 2, to determine the levels of phagocytosis of blood MNC and of mitogen (Con  
104 A)-induced proliferation of splenic MNC in chickens fed MGN-3-supplemented diets, 12 chicks  
105 (14d of age) in individual cages were divided into three groups of four chicks and provided with  
106 basal diets supplemented with MGN-3 at 0 (Control), 100 or 1,000 ppm. The feeding schedule and  
107 conditions were the same as for experiment 1. Following sample collection, MNC were prepared as  
108 described below.

109 The aim of experiment 3 was to determine the gene expression profiles and plasma Cer  
110 concentrations after LPS-induced immune stimulation in chickens fed MGN-3-supplemented diets.  
111 Thirty-six chicks (14d of age) in individual cages were divided into three groups of 12 chicks and  
112 provided with basal diet supplemented with 0 (control), 10 or 100 ppm MGN-3. At age 28d, the  
113 chickens were intraperitoneally injected with *E. coli* LPS (serotype 0127:B8) at 1.5 mg/kg body  
114 weight, dissolved in sterile saline at a concentration of 500 µg/ml. Twenty-four hours after injection  
115 of LPS, body weight gain, feed intake and rectal temperature were measured for six chickens in  
116 each dietary group. A blood sample was taken from the wing vein 24h after LPS injection from six  
117 chickens in each dietary group, and plasma was stored at –80°C until further analysis. The other six  
118 chickens in each dietary group were killed by decapitation at 2h after LPS injection, and spleen  
119 samples were collected as in experiment 1. The sampling times were according to our previous  
120 study (Takahashi *et al.*, 2008).

121 All of the procedures were approved by the Animal Care and Use Committee of the Tokyo  
122 University of Agriculture and Technology.

123

#### 124 ***Quantitation of mRNA using real-time PCR***

125 Total RNA was extracted from chicken tissues using Trizol reagent (15596-018, Invitrogen,  
126 Carlsbad, CA 92008). To study the expression of particular chick immune genes, real-time reverse  
127 transcription-polymerase chain reaction (RT-PCR) analysis was performed using an iCycler Real  
128 Time Detection System (Bio-Rad Laboratories, Hercules, CA 94547). The reverse transcription,  
129 amplification and detection methods used were as previously described (Takahashi *et al.*, 2008;  
130 Sato *et al.*, 2009). Primer sequences are shown in Table 1. At the end of each run, melting curve  
131 profiles were recorded. Analysis of the standard curve from each product allowed calculation of the  
132 mRNA levels of the respective genes. Results are presented as the ratio of each gene to ribosomal  
133 protein S9 (RPS9), to correct for differences in the amounts of template DNA used.

134

#### 135 ***Preparation of MNC suspensions from blood samples and spleens***

136 MNC were isolated from blood samples and spleens by density-gradient centrifugation. Collected  
137 spleens were pushed through mesh and suspended in RPMI-1640 medium (Invitrogen, Corp.,  
138 Carlsbad, CA) supplemented with 100U/mL penicillin and 100µg/mL streptomycin (Invitrogen,  
139 Corp., Carlsbad, CA). The MNC suspensions from blood and spleens were gently added to  
140 Histopaque-1077 tubes (Sigma, St. Lois, MO). Centrifugation was performed at 400×g for 50min at  
141 15°C. The boundary layers between the medium with blood or spleen cells and Histopaque-1077  
142 were collected as MNC, and the resulting MNC were washed three times with RPMI-1640 medium.

143

#### 144 ***Determination of splenic MNC proliferation***

145 The isolated splenic MNC were suspended in RPMI-1640 containing 10% fetal bovine serum.  
146 Aliquots of 100  $\mu$ L of cells per well, at a concentration of  $2.5 \times 10^6$  cells/mL, were added to  
147 flat-bottom 96-well culture plates and incubated at 39°C in a humidified 5% CO<sub>2</sub> atmosphere. T cell  
148 proliferation was induced by Con A (40  $\mu$ g/mL) stimulation for 48h (Takahashi *et al.*, 2010). Cell  
149 proliferation was measured using a Cell Counting Kit-8 (Dojindo, Osaka, Japan) according to the  
150 manufacturer's guidelines. Results were expressed as an arbitrary unit on the basis of absorbance  
151 values (A450nm - A655nm of cells cultured with Con A / A450nm - A655nm of cells cultured  
152 without Con A) of the MNC.

153

#### 154 ***Analysis of blood MNC phagocytosis***

155 The isolated blood MNCs were diluted with Hank's Balanced Salt Solution with 20mM HEPES  
156 (pH7.4) to a concentration of  $2.5 \times 10^6$  cells/mL and preincubated at 37°C for 2 min. Then, the cells  
157 were incubated with luminol-bound microbeads (Catalog No. KTS405; Kamakura Techno-Science  
158 Inc, Kanagawa, Japan). Chemiluminescence from cells that ingested the beads was measured on a  
159 TD-20/20 luminometer (Pormega) for 15 s every 1 min, up to 15 min. These data indicate the rate  
160 of phagocytosis in the cells.

161

#### 162 ***Measurement of plasma Cer and TBARS concentrations***

163 Plasma Cer concentration was determined by the procedure of Sunderman and Nomoto (1970)  
164 with slight modifications as described in our previous study (Takahashi *et al.*, 2008). TBARS  
165 content was analyzed using a commercially-available TBARS assay kit (Cayman Chemical,  
166 Michigan, USA) according to the method of Aoki *et al.* (2008).

167

#### 168 ***Statistical analysis***

169 The SPSS applications software package was used for statistical calculations (PASW Statistics

170 18.0, IBM, NY 10504). The group data for multiple comparisons were analyzed by ANOVA using a  
171 general linear model procedure followed by Tukey's test. Results are expressed as mean  $\pm$  standard  
172 deviation (SD). Statistical significance was interpreted as values of  $P < 0.05$ .

173

## 174 **RESULTS**

### 175 *Effect of MGN-3-supplemented diets on immune parameters of chickens (Experiment 1 and 2)*

176 Body weight gain, feed intake and tissue weight (spleen, thymus, and bursa fabricius) did not  
177 differ significantly in response to the MGN-3 dietary supplementation (data not shown). The level  
178 of CD3, IL-2, and IFN- $\gamma$  mRNA in the spleens of chickens fed a 100 ppm MGN-3 -supplemented  
179 diet was found to be significantly higher than that in control chickens, while there are no significant  
180 differences between chicken fed a 1,000 ppm MGN-3-supplemented diet and control chickens,  
181 except for IL-2 (Fig. 1). In contrast, the levels of those and TLRs mRNAs in the foregut was not  
182 affected by MGN-3-supplementation (Fig. 2). Bu-1 mRNA expression levels in bursa fabricius of  
183 chickens fed an MGN-3-supplemented diet were not significantly different from chicks fed a  
184 control diet (data not shown). The mitogen -induced proliferation of splenic MNC in chickens fed  
185 1,000 ppm MGN-3 supplemented diets was significantly higher than in chickens fed a basal diet  
186 (the control) (Fig. 3A). The rates of phagocytosis in blood MNC from chickens fed  
187 5-ALA-supplemented diets were significantly higher than those in chickens fed control diet (Fig.  
188 3B). Plasma TBARS concentrations gradually decreased with increasing MGN-3 concentration (Fig.  
189 4).

190

### 191 *Effect of 5-ALA dietary supplementation on the inflammatory responses of chickens during* 192 *LPS-induced immune stimulation (Experiment 3)*

193 Body weight gain, feed intake, and rectal temperature did not differ among the treatment groups  
194 24h after the LPS injections (data not shown). Fig. 5 shows the expression of CD3, IL-1 $\beta$ , IL-2,



195 IL-6, IFN- $\gamma$ , IL-1 $\beta$ , IL-6 and TL1A in the spleen of male broiler chickens fed  
196 MGN-3-supplemented diets at 2h after LPS injection. The expression of IL-2, IFN- $\gamma$ , and TL1A  
197 mRNA in chickens fed the 100 ppm MGN-3-supplemented diet significantly decreased 2h after  
198 LPS injection compared to control group. The levels of TLR4 and TLR7 mRNAs in the foregut of  
199 chickens fed MGN-3 supplemented diets were lower than those in the control chickens at 2h after  
200 injection of LPS (Fig. 6). Plasma Cer concentration in chickens fed the MGN-3-supplemented diet  
201 was significantly lower than that in control group at 24h after injection of LPS (Fig. 7).

202

## 203 **DISCUSSION**

204 Chicken immune systems have the species-specific difference compared to mammals. The major  
205 difference is TNF- $\alpha$ , which is a major pro-inflammatory cytokine and regulates host responses to  
206 infection, immune responses, inflammation and trauma in mammals (Dinarello, 2000). We recently  
207 reported that TL1A plays an important role as a pro-inflammatory cytokine instead of TNF- $\alpha$  in  
208 chickens (Takimoto *et al.*, 2008). Then, the response of immunomodulators, which improve the  
209 immune systems, may be difference between mammals and chickens. It has been reported that  
210 MGN-3 plays the immunomodulator, including the activation of NK cells (Ghoneum and Gollapudi,  
211 2003), IFN- $\gamma$  and TNF- $\alpha$  (Badr El-din *et al.*, 2008) in mammalian species. In addition, we have  
212 previously reported that dietary supplementation with nutrients enhances immunological function in  
213 chickens (Takahashi *et al.*, 1999, 2000) and immunobiotic lactic acid bacteria, i.e. *L. jensenii*  
214 TL2937 and *L. gasseri* TL2919, are appropriate immunomodulators to stimulate the gut-associated  
215 immune system in chicks (Sato *et al.*, 2009). These studies demonstrated up-regulations of  
216 immune-related gene expression; i.e. T-cell related gene (CD3, IL-2 and IFN- $\gamma$ ) in spleens and/or  
217 foreguts, concluding the immunomodulator. Moreover, the mitogen-induced MNC proliferation in  
218 spleen is useful for estimating the effect of nutritional status and nutrients on the immune system of  
219 chickens (Takahashi *et al.*, 1999; 2000). Here, we provide evidence that supplementation of the

220 diets of chickens with MGN-3, especially at a concentration of 100 ppm, enhances the expression of  
221 CD3, IL-2 and IFN- $\gamma$  mRNA in the spleen (Fig. 1), and enhances mitogen (Con A)-induced  
222 proliferation of splenic MNC (Fig. 3A). These results clearly show that MGN-3 stimulates the  
223 T-cell immune system in the spleen, suggesting that dietary supplementation with MGN-3  
224 modulates the immune system particularly targeting cellular immunity. Then, MGN-3 showed  
225 similar effects, as the immunomodulator, between mammals and birds, although their immune  
226 systems have the species-specific difference.

227 The supplementation of MGN-3 in the diet improves the antioxygenic potential and protect against  
228 oxidative stress in mice (Noaman *et al.*, 2008). We, therefore, measured plasma TBARS  
229 concentration as the marker of low lipid peroxide in chickens fed an arabonoxylan supplemented  
230 diet. Plasma TBARS concentrations gradually decreased with increasing MGN-3 concentration (Fig.  
231 4), suggesting that MGN-3 has the antioxidant activity in chicken as similar to mammals.

232 Colibacillosis is one of serious problem in poultry production (Oh *et al.*, 2011). Then, we have  
233 investigated inflammatory responses in chickens fed MGN-3-supplemented diets during  
234 LPS-induced immune stimulation. We have previously reported that dietary glycine  
235 supplementation modulates the inflammatory response, lowering expression levels of splenic  
236 pro-inflammatory cytokine, such as TL1A, IL-1,IL-6 and IFN- $\gamma$ , and foregut TLRs, and low plasma  
237 Cer concentration, during immune stimulation by LPS injection, with the result that chickens fed a  
238 glycine supplemented diet exhibited greater rates of growth than control chickens (Takahashi *et al.*,  
239 2008). Then, low expression levels of pro-inflammatory cytokine and TLRs, and low plasma Cer  
240 concentration during immune stimulation by LPS injection provide the improvement of immune  
241 status in chickens. In present study, the expression of splenic IL-2, IFN- $\gamma$  and TL1A mRNAs and  
242 foregut TLR4 and TLR7 mRNAs at 2h after LPS injection in the chickens fed the 100 ppm  
243 MGN-3-supplemented diet significantly decreased compared to the control chickens (Fig. 5). In  
244 addition, the plasma Cer concentration in the chickens fed the 100 ppm MGN-3-supplemented diet

245 was significantly lower than that in the control chickens (Fig. 7). These results suggest that the  
246 immune systems of chickens fed an MGN-3-supplemented diet rapidly return to normal levels at 2  
247 or 24h after LPS injection relative to the control group. It is, therefore, possible that  
248 supplementation of poultry diets with MGN-3 may prevent the catabolic changes induced by  
249 immunological stimulation under the LPS trials.

250 The evidence of a possible mechanism underlying the immunomodulation associated with MGN-3  
251 supplementation was not found in the present study. It has been reported that MGN-3 is partially  
252 absorbed into the blood through the intestinal wall and directly associate with immune functions in  
253 mice (Badr El-din *et al.*, 2008). In addition, there are some *in vitro* studies that MGN-3 is a potent  
254 direct inducer of immunefunctions in NK, T- and B-cells (Ghoneum and Gollapudi, 2003; Ghoneum  
255 and Matsuura, 2004). Then, the present results in chickens may be associated with the direct effect  
256 of MGN-3 on immune systems. Further experiments involving direct *in vitro* supplementation with  
257 MGN-3 in immune cell cultures of broiler chickens may help to elucidate the mechanism  
258 underlying the immunomodulation associated with MGN-3 supplementation. However, the present  
259 results provide the first evidence of the use of dietary MGN-3 to improve the immune systems in  
260 growing chickens.

261 The levels of T-cell related mRNA in the spleens of chickens fed a 100 ppm MGN-3  
262 -supplemented diet were found to be significantly higher than that in control chickens, while there  
263 **were** no significant differences between chicken fed a 1,000 ppm MGN-3-supplemented diet and  
264 control chickens, except for IL-2 (Fig. 1). These results suggest that the supplementation of MGN-3  
265 at the concentration of 1,000 ppm is too high as the immunemodulator. Although mitogen-induced  
266 proliferation of splenic MNC and phagocytosis in blood MNC in the chickens fed the 1,000 ppm  
267 MGN-3-supplemented diets were significantly greater than in the chickens fed the control diets, the  
268 expression levels of pro-inflammatory cytokines were modulated in the chickens fed the 100 ppm  
269 supplemented diet during LPS stimulation. Therefore, to induce an immune response sufficient to

270 protect from diseases without the decreasing growth performance, 100 ppm  
271 MGN-3-supplementation may be appropriate.

272 In conclusion, the modified arabinoxylan rice bran (MGN-3) dietary supplementation used in this  
273 study, particularly at the concentration of 100 ppm, enhanced immune system function in growing  
274 chickens, demonstrating that MGN-3 may behave as an immunomodulator to enhance immune  
275 system activity, protecting chickens from disease without reducing growth performance.

276

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360



361 **Figure legend**

362

363 Fig. 1

364 The effects of dietary modified arabinoxylan rice bran supplementation on cluster of differentiation  
365 3 (CD3; A), interleukin (IL)-2(B) and interferon (IFN)- $\gamma$  (C) mRNA expressions in the spleen of  
366 broiler chickens

367 The expression of each gene was determined by real-time RT-PCR (iCycler iQ Real Time Detection  
368 System, Bio-Rad Laboratories, Hercules, CA) as described in the Materials and Methods, and was  
369 expressed as a ratio to RPS9 levels. Bars indicate the SD of the mean (n = 6). Different superscripts  
370 indicate significant differences,  $P < 0.05$ .

371

372 Fig. 2

373 The effects of dietary modified arabinoxylan rice bran supplementation on cluster of  
374 differentiation 3 (CD3; A), interleukin (IL)-2(B) interferon (IFN)- $\gamma$  (C) Toll-like receptor  
375 (TLR)-2(D), -4(E), and -7(F) mRNA expressions in the foregut of broiler chickens

376 The expression of each gene was determined by real-time RT-PCR (iCycler iQ Real Time Detection  
377 System, Bio-Rad Laboratories, Hercules, CA) as described in the Materials and Methods, and was  
378 expressed as a ratio to RPS9 levels. Bars indicate the SD of the mean (n = 6). Different superscripts  
379 indicate significant differences,  $P < 0.05$ .

380

381 Fig. 3

382 The effects of dietary modified arabinoxylan rice bran supplementation on mitogen (concanavalin  
383 A)-induced proliferation of mononuclear cells in spleen (A), and macrophages of blood mononuclear  
384 cells (B) in broiler chickens.

385 Bars indicate the SD of the mean (n = 6). Different superscripts indicate significant differences,  $P <$

386 0.05. S.I.: stimulation index, AU: arbitrary unit.

387

388 Fig. 4

389 The effects of dietary modified arabinoxylan rice bran supplementation on plasma thiobarbituric  
390 acid reactive substances (TBARS) concentrations in chickens.

391 Bars indicate the SD of the mean (n = 6). Different superscripts indicate significant differences, P <

392 0.05.

393

394 Fig. 5

395 The effects of dietary modified arabinoxylan rice bran supplementation on mRNA expression of  
396 substances related to the inflammatory response in the spleen of broiler chickens following an  
397 intraperitoneal injection of lipopolysaccharide (LPS).

398 CD3, cluster of differentiation 3; IL, interleukin; IFN, interferon; TL, tumor necrosis factor-like  
399 ligand.

400 The expression of each gene was determined by real-time RT-PCR (iCycler iQ Real Time Detection  
401 System, Bio-Rad Laboratories, Hercules, CA) as described in the Materials and Methods, and was  
402 expressed as a ratio to RPS9 levels. Bars indicate the SD of the mean (n = 6). Different superscripts  
403 indicate significant differences, P < 0.05.

404

405 Fig. 6

406 The effects of dietary modified arabinoxylan rice bran supplementation on mRNA expression of  
407 substances related to the inflammatory response in the foregut of broiler chickens following an  
408 intraperitoneal injection of lipopolysaccharide (LPS).

409 CD3, cluster of differentiation 3; IL, interleukin; IFN, interferon; TLR, Toll-like receptor.

410 The expression of each gene was determined by real-time RT-PCR (iCycler iQ Real Time Detection

411 System, Bio-Rad Laboratories, Hercules, CA) as described in the Materials and Methods, and was  
412 expressed as a ratio to RPS9 levels. Bars indicate the SD of the mean (n = 6). Different superscripts  
413 indicate significant differences,  $P < 0.05$ .

414

415 Fig. 7

416 The effects of dietary 5-aminolevulinic acid (5-ALA) supplementation on plasma caruloplasmin  
417 concentration (mg/L) in broiler chickens at 24h after an intraperitoneal injection of  
418 lipopolysaccharide (LPS).

419 Bars indicate the SD of the mean (n = 6). Different superscripts indicate significant differences,  $P <$   
420 0.05.

421

422 日本語抄録

423

424 米ぬか由来アラビノキシランの飼料添加はブロイラーの免疫応答を調節する

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426

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430

431 本研究では、飼料への米ぬか由来アラビノキシラン(MGN-3)添加がブロイラーの免疫シス  
432 テムと免疫応答におよぼす影響を検討した。脾臓におけるCD3、インターロイキン(IL)-2お  
433 よびインターフェロン $\gamma$  (IFN- $\gamma$ ) mRNA発現は、100 ppmアラビノキシラン添加飼料給与区  
434 で対照区に比べ上昇した。一方、腸管前部におけるこれらの発現には変化が認められなか  
435 った。アラビノキシラン添加飼料を給与した鶏の脾臓単核球の幼若反応は、対照区の反応  
436 に比べ有意に上昇した。これらの結果は、アラビノキシランの飼料への添加が鶏におけるT  
437 細胞の免疫を賦活化したことをはじめて明らかにした。大腸菌リポ多糖を投与した2時間後  
438 の脾臓における前炎症性サイトカイン、すなわちIL-2、IFN- $\gamma$ およびTL1A mRNA発現は、ア  
439 ラビノキシラン添加飼料を給与した鶏で、対照区に比べ有意に低下した。アラビノキシラ  
440 ン添加飼料を給与した鶏のLPS投与後の腸管前部におけるToll様受容体4および7の発現は、  
441 対照区に比べ低い値を示した。LPS投与24時間後の血漿中セルロプラスミン濃度は、アラ  
442 ビノキシラン添加飼料給与により有意に減少した。以上の結果から、米ぬか由来アラビノ  
443 キシランは、成長中のブロイラーにおいてT細胞を賦活化し、大腸菌症などの疾病から鶏を  
444 守る体重減少を伴わない免疫改善因子として使用できる可能性が示唆された。

Table 1 Oligonucleotide sequences of sense and antisense primers for real-time PCR products determined

Gene		Primer sequences	Accession number <sup>1</sup>	Product size (bp)
CD3	sense	5'-CAGGGATTGTGGTCCGAGAT-3'	AJ250458	164
	anti-sense	5'-TACTGTCCATCATTCCGCTCAC-3'		
IL-2	sense	5'-ACTGCCATGATGTGCAAAGTACTGATCT-3',	AF017645	428
	anti-sense	5'-ATTTTTGGCCAAGATATCTCACAAAGTTGGT -3'		
IFN- $\gamma$	sense	5'-ACTGAGCCAGATTGTTTCGATGT-3'	X99774	288
	anti-sense	5'-TGCCATTAGCAATTGCATCTCCT-3'		
TLR2	sense	5'-CATTACCATGAGGCAGGGATAG-3'	AB046533	157
	anti-sense	5'-GGTGCAGATCAAGGACACTAGGA-3'		
TLR4	sense	5'-TTCAGAACGGACTCTTGAGTGG-3'	AY064697	131
	anti-sense	5'-CAACCGAATAGTGGTGACGTTG-3'		
TLR7	sense	5'-TTGCTGCTGTTGTCTTGAGTGAG -3'	AJ627563	182
	antisense	5'-AACAAACAGTGCATTTGACGTCCT-3'		
IL-1 $\beta$	sense	5'-ATGGCGTTCGTTCCCGACCTGGACGTGCTG -3'	Y15006	795
	antisense	5'-ACTTAGCTTGTAGGTGGCGATGTTGACCTG -3'		
IL-6	sense	5'-CAGCTGCAGGACGAGATGTGCAA -3'	AJ309540	238
	antisense	5'-GCACAG GACTCGACGTTCTGCT -3'		
TL1A	sense	5'-CCTGAGTTATTCCAGCAACGCA-3'	AB194710	131
	antisense	5'-CTTGTCATCTCTTGTTCCTGTA-3'		
Bu-1	sense	5'-GGCTGTTGTGTCCTCACTCATCT-3'	X92865	106
	antisense	5'-CACCACCGACATTGTTATTCCAT-3'		
RPS9	sense	5'-TGCGAAGTTTTGTGACTGAAACA-3'	XM416921	175
	antisense	5'- ATTCTTGGAGCATTTCAGCCTTTC-3'		

<sup>1</sup>Accession number refers to NCBI.

CD, cluster of differentiation; IL, interleukin; IFN, interferon; TLR, toll-like receptor; TL, tumor necrosis factors like ligand; RPS, ribosomal protein S.

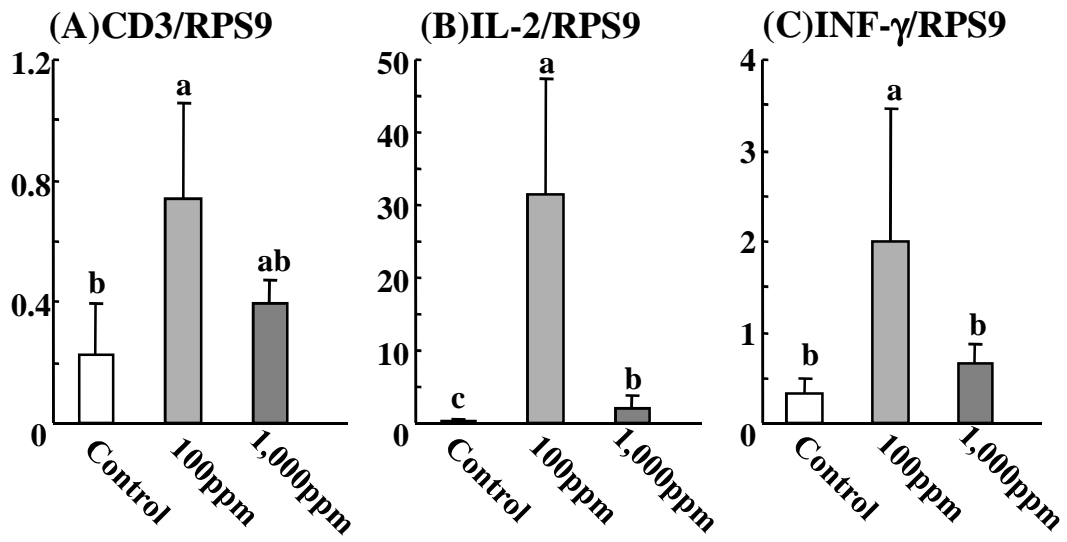


Fig.1 Sato et al.

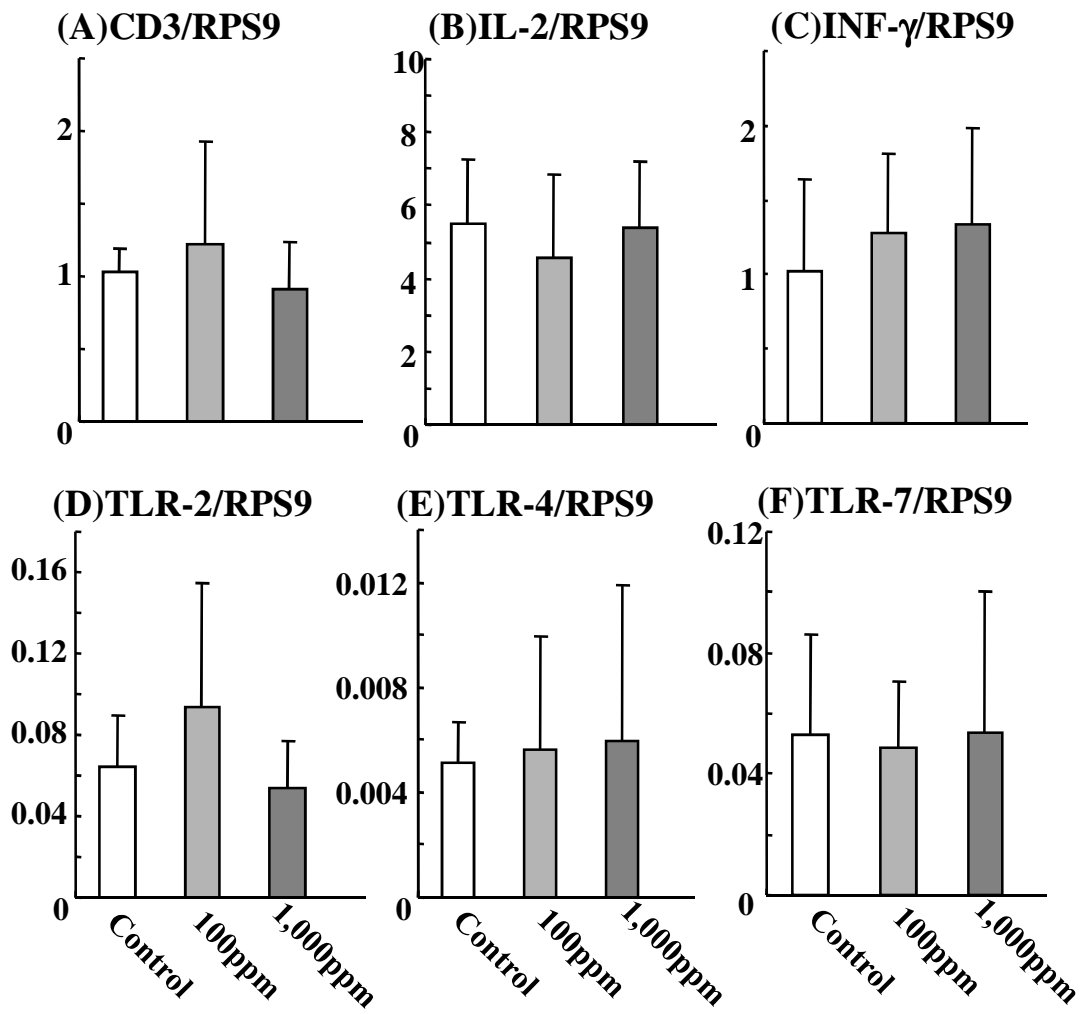


Fig.2 Sato et al.

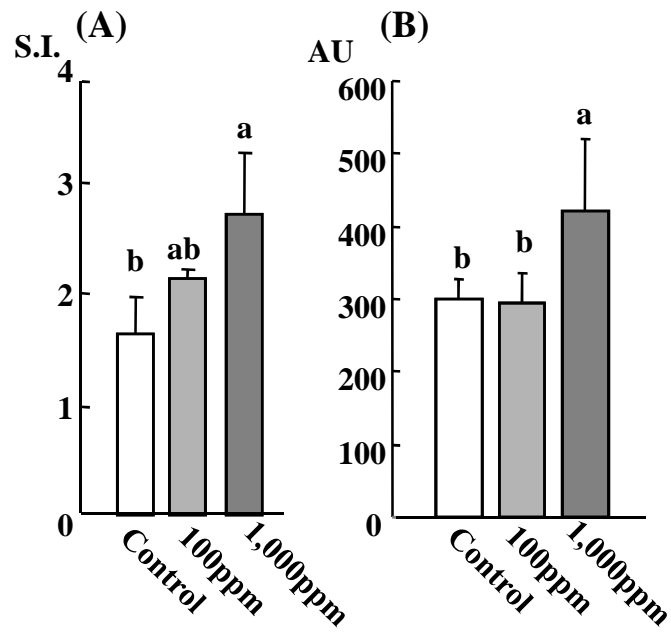


Fig.3 Sato et al.



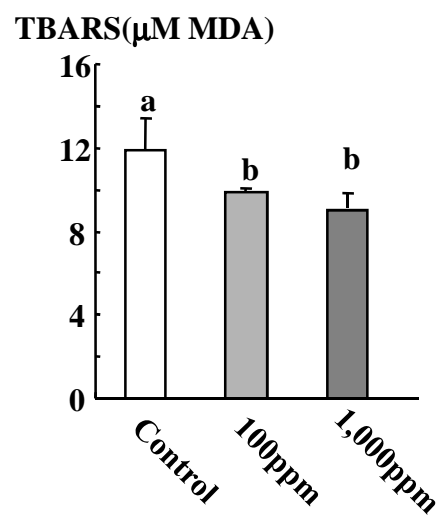


Fig.4 Sato et al.

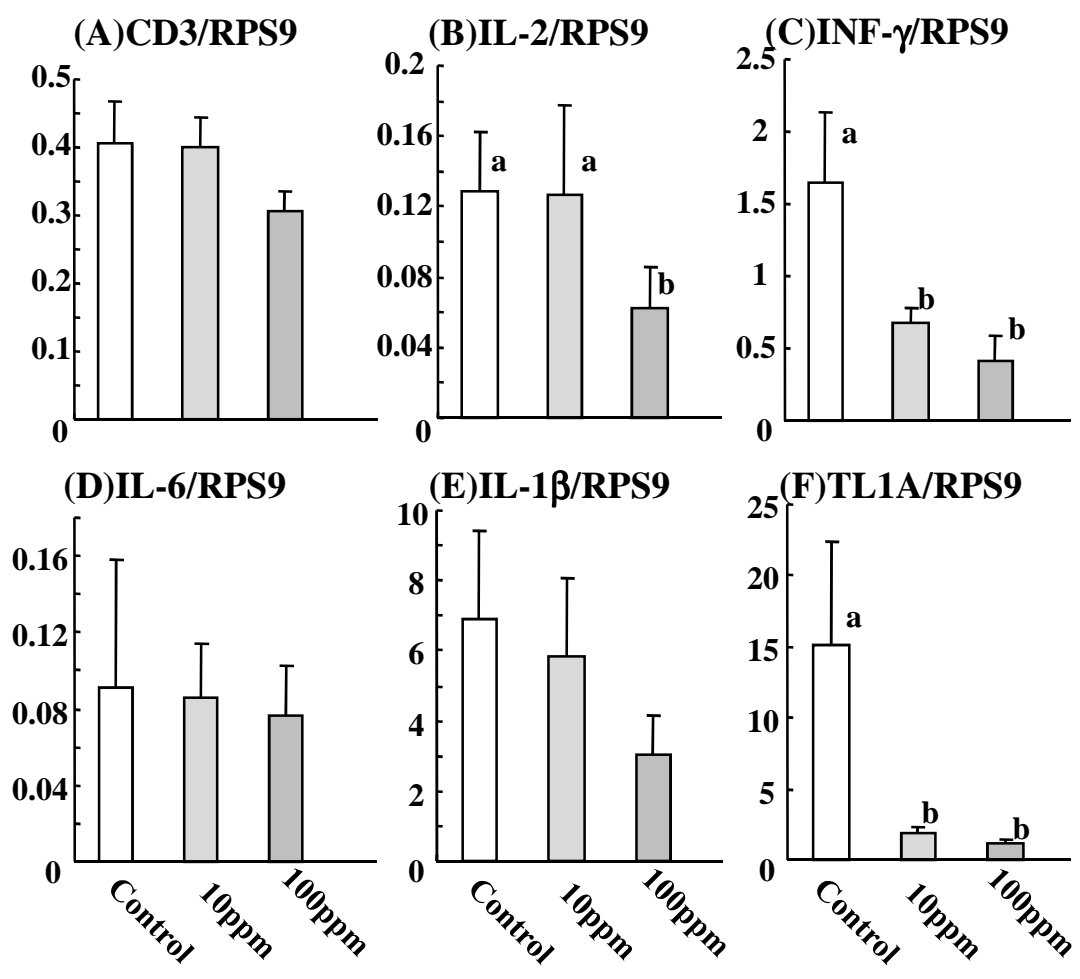


Fig.5 Sato et al.

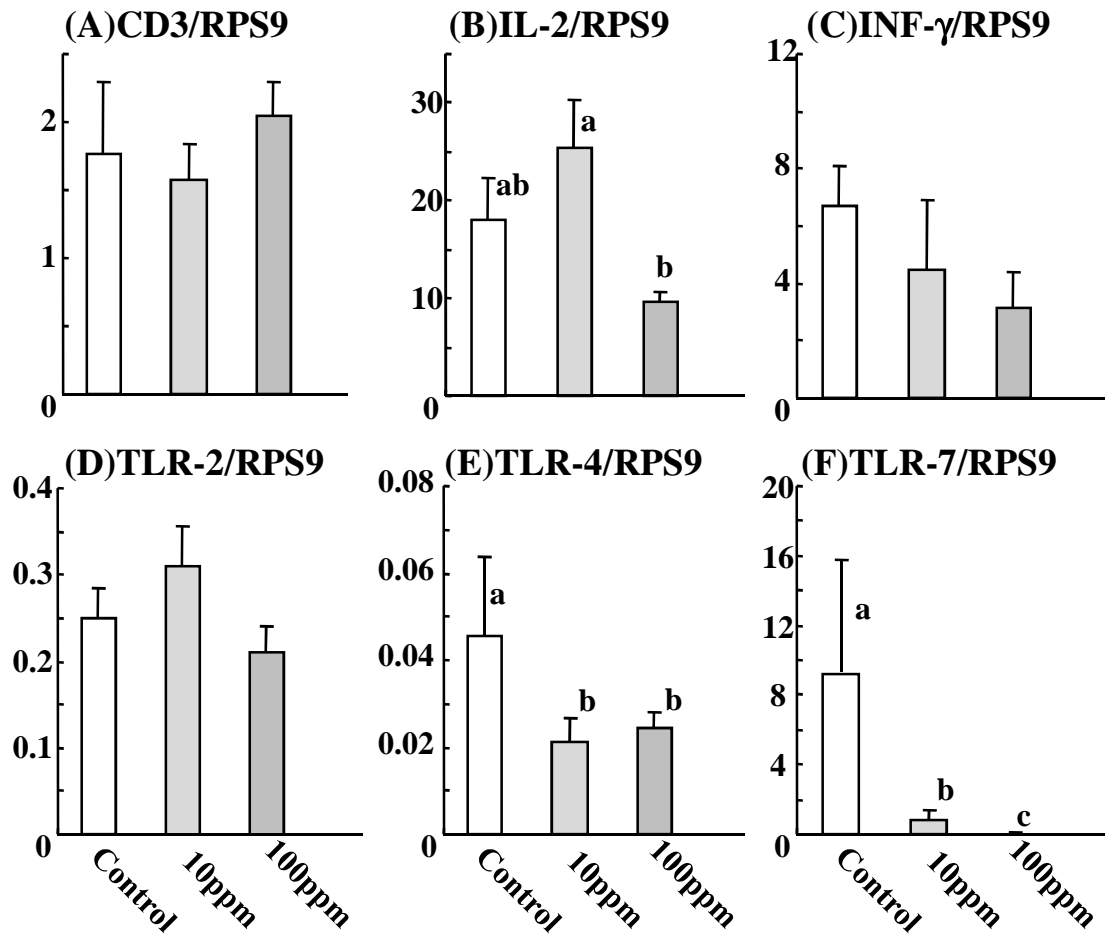


Fig.6 Sato et al.

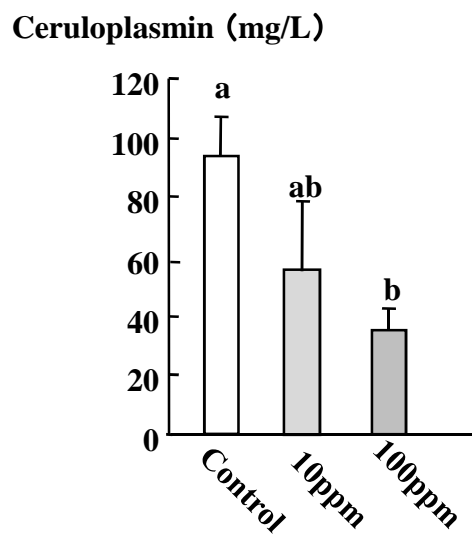


Fig.7 Sato et al.