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

Appropriate Scientific Section: feed and Nutrition

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Running Head: Immunomodulation by arabioxylan rice bran

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ABSTRACT

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

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

INTRODUCTION

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

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

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

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

MATERIALS AND METHODS

Animals, diet, blood sampling and lipopolysaccharide treatment

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

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

of three experimental diets for 14d *ad libitum*. The experimental diets were prepared by simply supplementation of basal diet with MGN-3 at 0 (Control), 100 or 1,000 ppm. Diet and water were freely provided. At age 28d, the chickens were then sacrificed by cervical dislocation, and the foregut (about 1g, from the end of the duodenum to the middle section of the jejunum) spleen and bursa fabricius samples were collected. Tissue samples were frozen in liquid N_2 and were stored at −80°C until analysis.

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

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

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

Quantitation of mRNA using real-time PCR

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

Preparation of MNC suspensions from blood samples and spleens

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

Determination of splenic MNC proliferation

The isolated splenic MNC were suspended in RPMI-1640 containing 10% fetal bovine serum. Aliquots of 100 µL of cells per well, at a concentration of 2.5×10^6 cells/mL, were added to flat-bottom 96-well culture plates and incubated at 39°C in a humidified 5% CO_2 atmosphere. T cell proliferation was induced by Con A (40 µg/mL) stimulation for 48h (Takahashi *et al*., 2010). Cell proliferation was measured using a Cell Counting Kit-8 (Dojindo, Osaka, Japan) according to the manufacturer's guidelines. Results were expressed as an arbitrary unit on the basis of absorbance values (A450nm - A655nm of cells cultured with Con A / A450nm - A655nm of cells cultured without Con A) of the MNC.

Analysis of blood MNC phagocytosis

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

Measurement of plasma Cer and TBARS concentrations

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

Statistical analysis

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

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

RESULTS

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

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

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

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

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

DISCUSSION

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

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

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

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

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

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

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Figure legend

Fig. 1

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

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

Fig. 2

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

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

Fig. 3

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

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 hiobarbituric

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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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γ(IFN-γ) 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-γ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[1]	Product size (bp)
CD3	sense	5’-CAGGGATTGTGGTCGCAGAT-3’	AJ250458	164
	anti-sense	5’-TACTGTCCATCATTCCGCTCAC-3’		
IL-2	sense	5’-ACTGCCATGATGTGCAAAGTACTGATCT-3’,	AF017645	428
	anti-sense	5’’-ATTTTTGGCCAAGATATCTCACAAAGTTGGT -3’		
IFN-γ	sense	5’-ACTGAGCCAGATTGTTTCGATGT-3’	X99774	288
	anti-sense	5’’-TGCCATTAGCAATTGCATCTCCT-3’		
TLR2	sense	5’-CATTCACCATGAGGCAGGGATAG-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’-AACAACAGTGCATTTGACGTCCT-3’		
IL-1β	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’-CTTGTCCATCTCTTGTCCTGTA-3’		
Bu-1	sense	5’-GGCTGTTGTGTCCTCACTCATCT-3’	X92865	106
	antisense	5’-CACCACCGACATTGTTATTCCAT-3’		
RPS9	sense	5’-TGCGAAGTTTTGTGACTGAAACA-3’	XM416921	175
	antisense	5’- ATTCTTGGAGCATTCAGCCTTTC-3’		

[1]Accession number refers to NCBI.

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

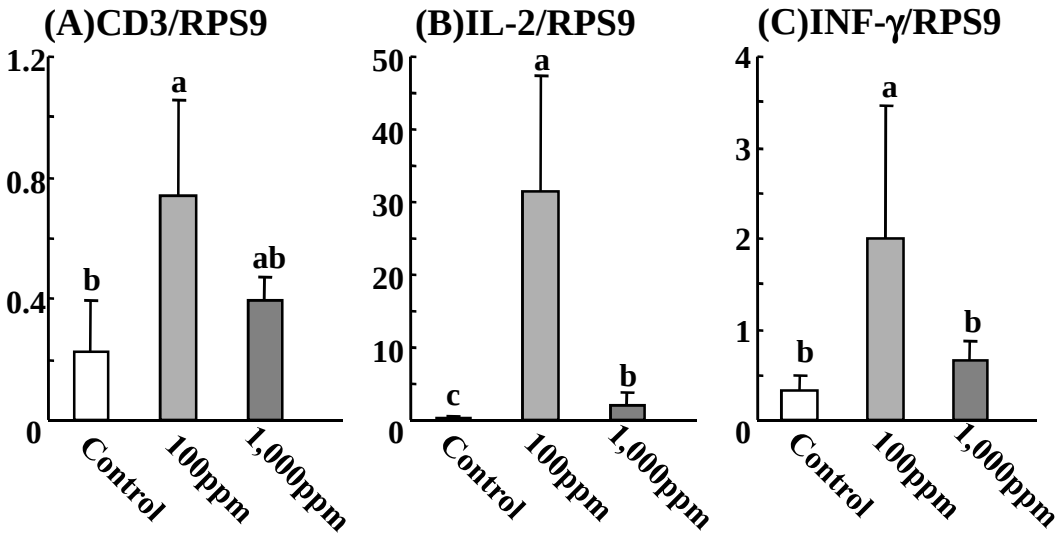

Fig.1 Sato et al.

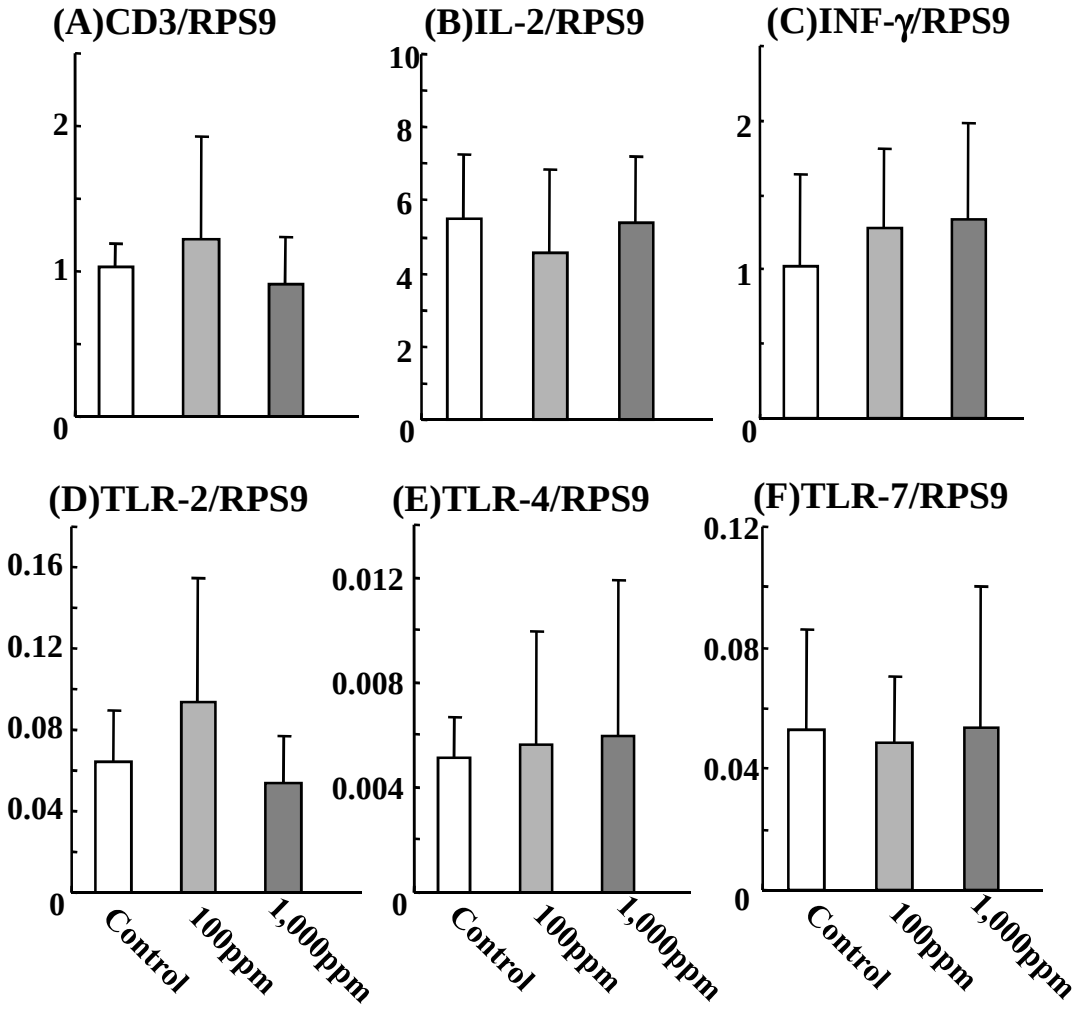

Fig.2 Sato et al.

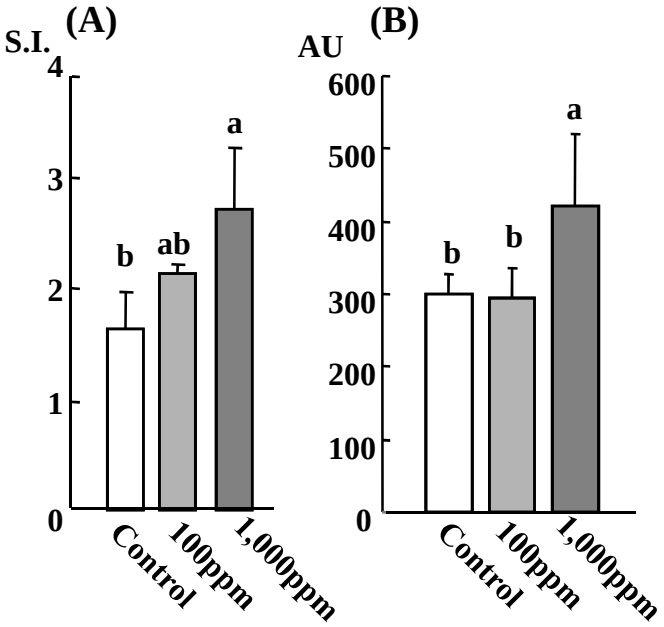

Fig.3 Sato et al.

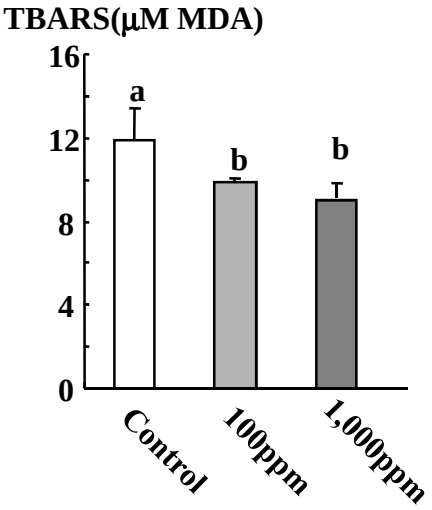

Fig.4 Sato et al.

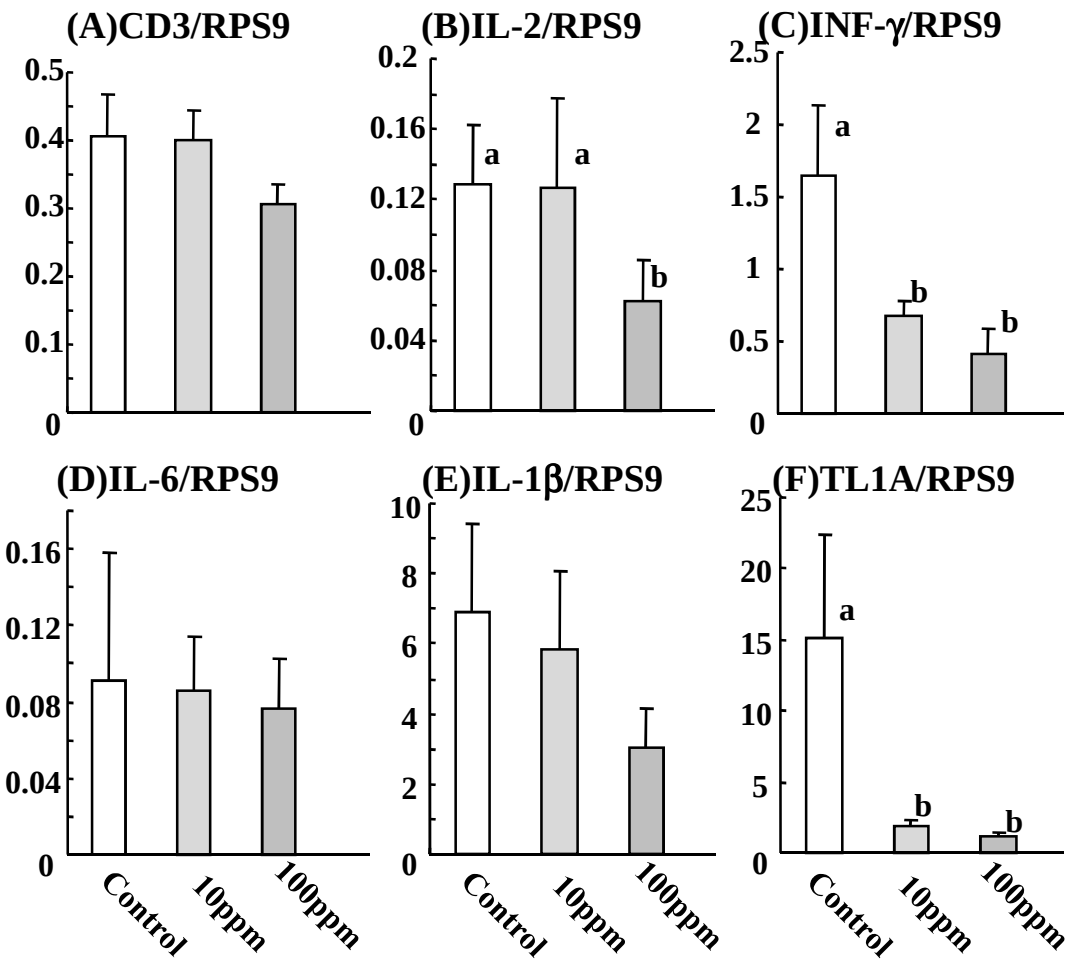

Fig.5 Sato et al.

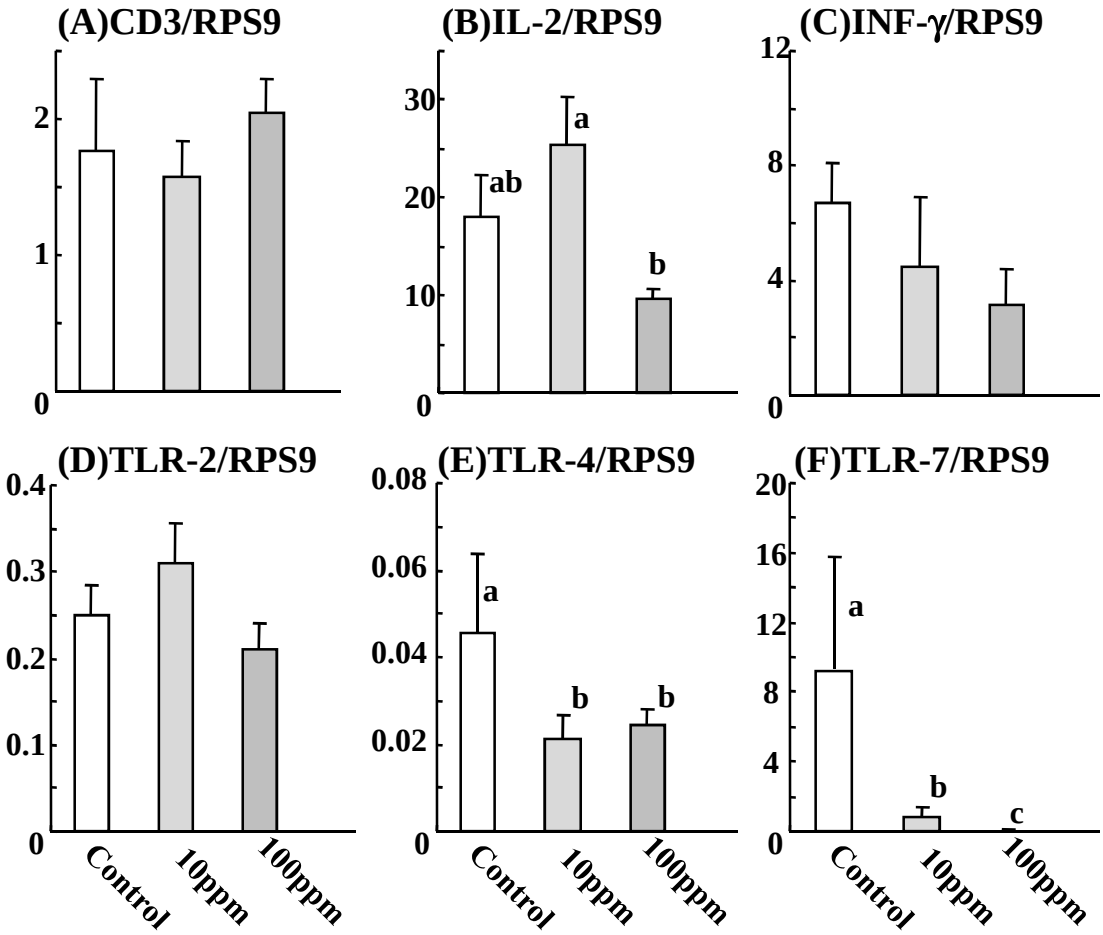

Fig.6 Sato et al.

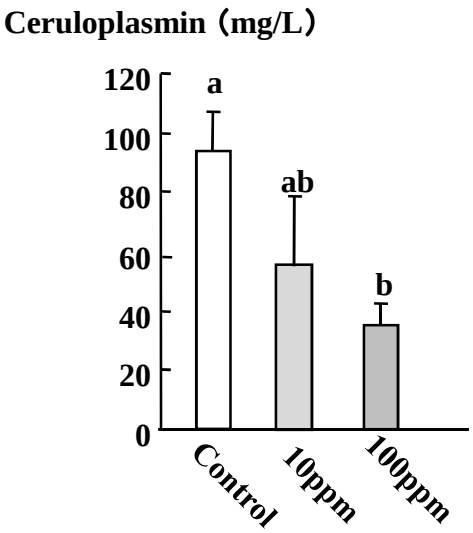

Fig.7 Sato et al.