Effect of dietary β-1,3-glucan supplementation and heat stress on growth performance, nutrient digestibility, meat quality, organ weight, ileum microbiota, and immunity in broilers

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ABSTRACT The objective of this study was to determine the effect of dietary β-1,3-glucan supplementation and heat stress (HS) on growth performance, nutrient digestibility, meat quality, organ weight, ileum microbiota, and immunity in broiler. A total of 1,440 1-day-old Ross 308 male chicks with an average initial BW of 43.06 ± 1.94 g were sorted into 6 (2×3) treatments, 14 replications per treatment. This trail included 2 factors: the dosage of β-1,3-glucan (0, 100 g/T, and 200 g/T) and feeding condition (HS and normal). During the whole trial, significant impacts were observed in BW gain, feed intake, feed conversion rate, and the digestibility of DM and energy between normal treatments and HS treatments (P < 0.05). From day 21 to 35, HS-challenged birds fed the diet with 200 g/T β-1,3-glucan had a lower feed conversion rate than those fed the diet with 0 or 100 g/T β-1,3-glucan (P < 0.05). Moreover, the HS-exposed birds that fed the diet with β-1,3-glucan indicated a greater energy digestibility than those fed the nontreatment diet (P < 0.05). Besides, β-1,3-glucan supplementation could elevate meat pH of all birds and decrease cooking loss significantly of HS-exposed birds (P < 0.05). The HS birds fed the β-1,3-glucan diet obtained a reduced amount of Escherichia coli in the ileum than those fed the nontreatment diet (P < 0.05). Besides, β-1,3-glucan supplementation lowered the level of tumor necrosis factor-α in HS-exposed birds significantly (P < 0.05). These results indicated 100 and 200 g/T β-1,3-glucan supplementation, under HS condition or not, can increase growth performance without a negative response on immunity. Under HS condition, the addition of β-1,3-glucan at dosage from 100 to 200 g/T in the diet can increase energy digestibility, decrease cooking loss, reduce E. coli mount in the ileum, and the tumor necrosis factor-α concentration.

Key words: β-1,3-glucan, growth performance, ileum microbiota, immunity, broiler

INTRODUCTION

As the requirement of animal protein increase, the broiler industry becomes one of the rapid rapidly growing industries in the world in which heat stress (HS) not only always challenges practical management of broilers but also results in enormous economic loss. And, it seems that HS challenge would be more and more serious. There are 2 reasons: On the one hand, the global climate has changed to warmer than before, so the tropical and subtropical region will be impacted more frequently by high ambient temperature, especially opening farms that completely depend on natural ventilation (Smith and Gregory, 2013). On the other hand, continuous gene selection for fast growth and improved feed efficiency has caused current broiler genotypes more sensitive to HS than ever before (Deeb and Cahaner, 2002). It has been reported that HS can decrease the feed intake (FI), BW gain (BWG), and feed efficiency (Onderci et al., 2004; Habibian et al., 2016); impair meat quality (Zhang et al., 2012a,b); and inhibit immune response (Lara and Rostagno, 2013). In essence, the increased levels of reactive oxygen species in mitochondria leads to the disturbance of balance between the oxidation and antioxidant defense systems, resulting in oxidative damages (Lin et al., 2006). Those conditions impair the immune system (Pamok et al., 2009). It is very difficult for heat-stressed birds to resist the challenges of disease. Besides, HS in chickens
has been reported to selectively suppress the immune system, leading to vaccination failures and immune organ involution (Shini et al., 2008, 2010) or to result in excessive immunization, leading to undergrowth performance. Therefore, regulation immunity must be a wise choice for solving HS problems.

β-glucans are structural components of cell walls in many different sources including bacteria, fungi, yeast, and algae. Some β-glucans have been shown to improve gut health (Shao et al., 2013) in poultry subjected to a bacterial challenge, to increase macrophage function (Qureshi, 2003), to increase antibody titers after a vaccination (Le et al., 2011), and to function as an anti-inflammatory immunomodulator (Cox et al., 2010a). However, molecular weight and branching degree can influence the immune response of β-glucan via the production of cytokines and the enhancement of phagocytic activity and antibactericidal activity. (Jacob and Pescatore, 2014).

Numerous investigators have detailed the effects of yeast cell wall β-1,3/1,6-glucan and their effects on broiler performance and as an immune modulator and growth promoter (Rathgeber et al., 2008; Morales-López et al., 2009). Meanwhile, β-glucans from various sources can cause divergent responses in relation with their molecular weight, three-dimensional structures or a ratio of β-1,3-glucan to β-1,6-glucan. The β-1,3-glucan derived from Euglena gracilis has similar molecular weight, structure, and size (1–3 mm). Besides, those β-1,3-glucan granules are not bound to other cell structures, and the cell is surrounded with a digestible proteinaceous pellicle instead of a thick cell wall (Barsanti et al., 2001). Furthermore, 1 possible advantage of linear β-glucan is that the immune receptors such as dectin-1 prefer linear form of β-1,3-glucan (Angelina S. Palma et al., 2006). Therefore, we hypothesized pure β-1,3-glucan might be an effectiveness of alternative sources of β-glucan mixer derived from the yeast. However, few studies were carried to evaluate effect of the β-1,3-glucan in broilers. No published data were found on the effect of β-1,3-glucan in broilers under HS. Hence, we conduct the experiment to test effect of dietary β-1,3-glucan supplementation on growth performance, nutrient digestibility, meat quality, organ weight, ileum microbiota, and immunity in broilers under HS.

**MATERIALS AND METHODS**

The experimental protocols describing the management and care of animals were reviewed and approved by the Animal Care and Use Committee of Dankook University, South Korea.

**Preparation of β-1,3-Glucan**

β-1,3-Glucan derived from algae (Euglena gracilis) was provided by Kemin Industries. The product content was 54.9 to 55.9% β-1,3-glucan.

**Animals, Diets, and Housing**

A total of 1,440 1-day-old Ross 308 male broiler chicks with an average initial BW of 43.06 ± 1.94 g were used. The chicks were sorted into 6 treatments, 14 replications per treatment, 17 birds in the first to 12th replication (pen) and 18 birds in the 13th and 14th replication. Dietary treatments included 1) TRT1, basal diet + normal temperature; 2) TRT2, basal diet +100 g/T β-1,3-glucan + normal temperature; 3) TRT3, basal diet +200 g/T β-1,3-glucan + normal temperature; 4) TRT4, basal diet + high temperature; 5) TRT5, basal diet +100 g/T β-1,3-glucan + high temperature; and 6) TRT6, basal diet +200 g/T β-1,3-glucan + high temperature.

Broilers were fed in an environmentally controlled house and caged in a three-tiered stainless-steel battery cage (124 cm width × 164 cm length × 40 cm height), which allowed ad libitum access to water and feed during the experimental period. Broiler were fed with starter (0–7 D), grower (8–21 D), and finisher (22–35 D) diets in a pelleted form. The replicates were equally distributed into the upper and lower cages to minimize the effect of cage level. The normal environmental temperature was maintained at 33°C ± 1°C for the first 3 D and then gradually reduced to 22°C until the end of the experiment. The chronic HS condition was maintained 32°C for 24 h from the fourth day of trial. All diets were formulated to meet or exceed the nutritional requirements of broiler as recommended by the NRC (1994, National Academy Press, Washington, DC). The initial and final diet compositions are shown in Table 1.

**Growth Performance**

BW and FI was measured by treatment on 7 D, 21 D, and on the last day (35 D). Based on each pen, BWG and FI corrected by mortality were calculated for each period, and feed conversion ratio (FCR) was also calculated by FI corrected/BWG.

**Nutrient Digestibility**

Nutrient digestibility rate was obtained by adding chromium oxide (Cr2O3) to the feed in the last 7 D of the experiment (35 D). The collected powder was dried for 72 h in a 60°C dryer and then pulverized with a Wiley mill for analysis. Chromium mixed with general components of the feed and labeling were analyzed as per the method of AOAC (2000).

**Meat Quality, Organ Weight, and Ileal Microbiota**

At the end of the experiment (35 D), 60 birds (10 birds per treatment and 1 bird per pen from first to 10th replicate) were killed to test meat quality, organ weight, and ileal microbiota. The liver, spleen, bursa of Fabricius, breast meat, abdominal fat, and gizzard were then removed and weighed by a trained personnel. To avoid variation in the cutting procedures, the same operator...
was employed. Organ weight was indicated as a percentage of BW. Meat color characteristics, tested using a Minolta colorimeter (CR-300, Tokyo, Japan), were calibrated by a standard white plate (L* = 93.5, a* = 0.3132, and b* = 0.3198). After 10 g of finely homogenized samples was mixed with 90 mL of double-distilled water, the pH value of raw breast meat was determined using a digital pH meter (NWKbinar pH, Landberg, Germany). Drip loss rate (%) was calculated on 1 cm² × 5 cm × 5 cm fillets, which was taken from the breast, weighed, and suspended in a zipper bag at 4°C for 7 D. The initial and final weights of each sample were used to calculate the drip loss. Its water-holding capacity was measured by the method described by Jin et al. (2011). Five grams of meat sample in a water bath was heated to 100°C for 30 min. Then, the sample was cooled with ice and centrifuged at 5°C at 1,000 g for 10 min subsequently. The water-holding capacity was calculated as the ratio of the weight of the liquid after centrifugation to that of the original liquid (%). Lactobacillus, Escherichia coli, Salmonella, and Bacillus bifidus were excreted fresh during the same time period, and then, Lactobacillus used MRS agar (Difco), E. coli used MacConkey agar (Difco), Salmonella used SS agar (Difco), and Bacillus bifidus used BSM agar (Sigma-Aldrich). Lactobacillus and E. coli were incubated at 37°C to 38°C for 24 h and Bacillus bifidus was incubated through the anaerobic system (VS-5600A, Vision, Korea) for 15 h before colony counting.

### Statistical Analysis

Data were analyzed as a completely randomized design, with a 2 × 3 factorial arrangement, temperature, and β-1,3-glucan supplementation, using GLM procedure of SAS (2003, SAS Institute Inc., Cary, NC). Variability in the data was expressed as the pooled SE, and P < 0.05 was considered statistically significant.

### RESULTS

#### Growth Performance and Nutrient Digestibility

The data of growth performance and nutrient digestibility are shown in Table 2 and Table 3, respectively. During 2,135 D and whole trial, significant impacts were observed in BWG, FI, and FCR between normal treatments and HS treatments (P < 0.05). And, β-1,3-glucan supplementation could increase BWG as well as decrease FCR statistically (P < 0.05). From day 21 to 35, HS-challenged birds fed the diet with 200 g/T β-1,3-glucan had a lower FCR than those fed diet with 0 or 100 g/T β-1,3-glucan (P < 0.05). Furthermore, there was an interaction of FCR between HS status and β-1,3-glucan supplementation (P < 0.05).

Heat stress significantly reduced the digestibility of DM and energy (P < 0.05), and β-1,3-glucan supplementation could raise energy digestibility of birds (P < 0.05). Moreover, the HS-exposed birds fed diet with 100 or 200 g/T β-1,3-glucan indicated a greater energy digestibility than those fed diet without β-1,3-glucan (P < 0.05).

#### Meat Quality and Organ Weight

The data of meat quality and organ weight are shown in Table 4. Several significant differences were observed in meat quality such as pH, lightness, water holding capacity (WHC), and day-7 drip loss, as well as organ weight including that of the breast and bursa of...
**Table 2.** The effect of β-glucan supplementation on growth performance in broilers1.

| Items | NT | HT | P-value | SEM |
|-------|----|----|---------|-----|
|       | 0 g/T | 100 g/T | 200 g/T | 0 g/T | 100 g/T | 200 g/T |
| Day 1–7 |     |       |         |     |       |         |
| BWG, g | 108 | 110 | 109 | 106 | 108 | 107 | 2 | 0.282 | 0.681 | 0.644 | 0.512 | 0.831 |
| FL, g  | 129 | 128 | 130 | 125 | 126 | 128 | 3 | 0.163 | 0.253 | 0.701 | 0.744 | 0.407 |
| FCR   | 1.198 | 1.169 | 1.206 | 1.177 | 1.173 | 1.197 | 0.033 | 0.746 | 0.432 | 0.742 | 0.617 | 0.395 |
| Day 7–21 |     |       |         |     |       |         |
| BWG, g | 638 | 640 | 644 | 626 | 629 | 631 | 7 | 0.060 | 0.622 | 0.401 | 0.323 | 0.681 |
| FL, g  | 963 | 968 | 965 | 949 | 953 | 950 | 9 | 0.065 | 0.373 | 0.619 | 0.108 | 0.847 |
| FCR   | 1.512 | 1.514 | 1.500 | 1.518 | 1.518 | 1.507 | 0.022 | 0.803 | 0.555 | 0.584 | 0.722 | 0.620 |
| Day 21–35 |     |       |         |     |       |         |
| BWG, g | 932b | 942ab | 960b | 865b | 874b | 890a | 13 | <0.001 | <0.001 | 0.790 | 0.010 | 0.033 |
| FL, g  | 1,540 | 1,541 | 1,537 | 1,512 | 1,517 | 1,514 | 14 | 0.036 | 0.400 | 0.205 | 0.830 | 0.682 |
| FCR   | 1,655 | 1,639 | 1,609 | 1,752a | 1,740a | 1,705b | 0.023 | <0.001 | 0.018 | 0.029 | 0.373 | <0.001 |
| Overall |     |       |         |     |       |         |
| BWG, g | 1,678b | 1,692b | 1,713a | 1,597b | 1,611b | 1,628a | 16 | <0.001 | 0.038 | 0.257 | 0.022 | 0.024 |
| FL, g  | 2,632 | 2,637 | 2,632 | 2,585 | 2,596 | 2,592 | 17 | 0.003 | 0.859 | 0.663 | 0.643 | 0.316 |
| FCR   | 1,570a | 1,559a | 1,539b | 1,621a | 1,613a | 1,593b | 0.012 | <0.001 | <0.001 | 0.702 | 0.018 | <0.001 |

1 Abbreviations: NT, normal temperature; HT, high temperature; 0 g/T, 100 g/T, 200 g/T means the dosage of β-glucan in diet; TNF-α, tumor necrosis factor-α; IgG, immunoglobulin G. Means represent 10 birds per treatment. All the treatments are as follows: TRT1, basal diet + normal temperature; TRT2, basal diet + 100 g/T β-glucan + normal temperature; TRT3, basal diet + 200 g/T β-glucan + normal temperature; TRT4, basal diet + high temperature; TRT5, basal diet + 100 g/T β-glucan + high temperature; TRT6, basal diet + 200 g/T β-glucan + high temperature.

2 Abbreviations: BWG, BW gain; Diet, diet effect; FCR, feed conversion ratio; FI, feed intake; Tem, temperature effect; T X D, interactive effect between temperature and diet.

3 Contrast among the different dosage of β-glucan treatments within normal temperature or high temperature challenge group.

**Immunity Profiles**

The result of immunity profiles is exhibited in Table 6. The level of TNF-α, interferon gamma, and IL-1 were elevated by HS exposure (P < 0.05). Besides, β-1,3-glucan supplementation lowered the level of TNF-α in HS-exposed birds significantly (P < 0.05). Furthermore, there was an obvious interaction of TNF-α concentration between the 2 factors (P < 0.05).

**DISCUSSION**

**Growth Performance, Nutrient Digestibility, and Ileum Microbiota**

There are lots of reports on β-glucan as a growth promoter for poultry. In this study, no matter whether those birds were challenged by HS or not, supplementation of β-1,3-glucan between HS and normal treatments (P < 0.05). The birds fed β-1,3-glucan had a greater meat pH (9 h and 12 h) than those that fed nontreatment diet (P < 0.05). Besides, β-1,3-glucan supplementation could elevate meat pH (9 h and 12 h) of all birds as well as decrease cooking loss significantly of HS-exposed birds (P < 0.05). Furthermore, an interactive effect on cooking loss between HS and β-1,3-glucan supplementation was observed (P < 0.05). No significant change was detected in others organ weight, when bird was fed the β-1,3-glucan diet (P > 0.05).

**Ileal Microbiota**

The result of amount of ileum microbiota is exhibited in Table 5. The HS-challenged birds fed the β-1,3-glucan diet obtained a reduced amount of *E. coli* in the ileum than those fed the nontreatment diet (P < 0.05).

**Table 3.** The effect of β-glucan supplementation on nutrient digestibility in broilers1.

| Items | NT | HT | P-value | SEM |
|-------|----|----|---------|-----|
|       | - | 100 g/T | 200 g/T | - | 100 g/T | 200 g/T |
| DM    | 71.74 | 72.16 | 72.80 | 69.94 | 70.02 | 70.50 | 1.03 | 0.019 | 0.811 | 0.647 | 0.520 | 0.833 |
| Nitrogen | 69.11 | 70.00 | 70.98 | 67.39 | 68.49 | 68.22 | 1.42 | 0.094 | 0.636 | 0.307 | 0.256 | 0.492 |
| Energy | 71.75 | 72.25 | 73.14 | 70.54b | 71.30a | 71.82a | 0.61 | 0.029 | 0.030 | 0.682 | 0.482 | 0.0015 |

1 Abbreviations: NT, normal temperature; HT, high temperature; 0 g/T, 100 g/T, 200 g/T means the dosage of β-glucan in diet; TNF-α, tumor necrosis factor-α; IgG, immunoglobulin G. Means represent 10 birds per treatment. All the treatments are as follows: TRT1, basal diet + normal temperature; TRT2, basal diet + 100 g/T β-glucan + normal temperature; TRT3, basal diet + 200 g/T β-glucan + normal temperature; TRT4, basal diet + high temperature; TRT5, basal diet + 100 g/T β-glucan + high temperature; TRT6, basal diet + 200 g/T β-glucan + high temperature.

2 Abbreviations: Diet, diet effect; Tem, temperature effect; T X D, interactive effect between temperature and diet.

3 Contrast among the different dosage of β-glucan treatments within normal temperature or high temperature challenge group.
100 g/T, 200 g/T β-1,3-glucan could improve BWG of birds. In agreement with our work, our laboratory documented dietary yeast cell wall (86.1% β-1, 3/1,6-glucan) supplementation at the dosage of 1,000 g/T can boost growth and increase the BWG of nonchallenged birds. Similarly, Chae et al. (2006) found that broilers fed finisher diets supplemented with 200 g/T and 400 g/T β-glucan (derived from Saccharomyces cerevisiae containing more than 40% β-1,3/1,6-glucan) had greater BWG than the control group. However, our laboratory study (Zhang et al., 2012a,b) reported no significant improvement in growth was observed when bird fed 1,000 g/T β-glucan (derived from Agrobacterium sp. 86.1% β-1,3/1,6-glucan). Besides, Cox et al. (2010b) pointed out that there was no significant difference in the growth performance of broilers with or without an Eimeria challenge when the diet is supplemented with 200 g/T or 1,000 g/T β-glucans.

### Table 4. The effect of β-glucan supplementation on meat quality in broilers on day 351.

| Items                      | Treatment                          | P-value |
|----------------------------|------------------------------------|---------|
|                            | NT                                 | HT      |
|                            | - 100 g/T 200 g/T                  | - 100 g/T 200 g/T | SEM  |
| pH value                   |                                    |         |
| Initial                    | 7.65 7.68 7.68                      | 7.30 7.27 7.30 | 0.02 |
| 3 h                       | 7.61 7.65 7.64                      | 7.25 7.22 7.24 | 0.02 |
| 6 h                       | 7.59 7.61 7.61                      | 7.22 7.20 7.22 | 0.02 |
| 9 h                       | 7.31 7.41 7.52a                      | 6.95 7.00 7.10b | 0.01 |
| 12 h                      | 7.39b 7.47a 7.55a                    | 6.78 6.83b 6.97b | 0.03 |
| 24 h                      | 7.57 7.52 7.52                      | 7.04 7.06 7.01 | 0.02 |
| Water holding capacity, %  |                                    |         |
|                            | 56.89 58.08 58.58                    | 52.44 53.81 55.13 | 2.16 |
| Cooking loss               |                                    |         |
|                            | 21.41 21.80 20.56                    | 24.35b 22.50a 20.75b | 0.90 |

1. Abbreviations: NT, normal temperature; HT, high temperature; 0 g/T,100 g/T, 200 g/T means the dosage of β-glucan in diet; TNF-α, tumor necrosis factor-α; IgG, immunoglobulin G. Means represent 10 birds per treatment. All the treatments are as follow: TRT1, basal diet + normal temperature; TRT2, basal diet + 100 g/T β-glucan + normal temperature; TRT3, basal diet + 200 g/T β-glucan + normal temperature; TRT4, basal diet + high temperature; TRT5, basal diet + 100 g/T β-glucan + high temperature; TRT6, basal diet + 200 g/T β-glucan + high temperature.

2. Abbreviations: Diet, diet effect; Tem, temperature effect; T × D, interactive effect between temperature and diet.

### Table 5. The effect of β-glucan supplementation on ileum microbiota in broilers on day 351.

| Items         | Treatment                          | P-value |
|---------------|------------------------------------|---------|
|               | NT                                 | HT      |
|               | - 100 g/T 200 g/T                  | - 100 g/T 200 g/T | SEM  |
| Lactobacillus |                                    |         |
| Escherichia coli |                                      |         |
| Salmonella    |                                    |         |
| Bacillus      |                                    |         |

1. Abbreviations: NT, normal temperature; HT, high temperature; 0 g/T,100 g/T, 200 g/T means the dosage of β-glucan in diet; TNF-α, tumor necrosis factor-α; IgG, immunoglobulin G. Means represent 10 birds per treatment. All the treatments are as follow: TRT1, basal diet + normal temperature; TRT2, basal diet + 100 g/T β-glucan + normal temperature; TRT3, basal diet + 200 g/T β-glucan + normal temperature; TRT4, basal diet + high temperature; TRT5, basal diet + 100 g/T β-glucan + high temperature; TRT6, basal diet + 200 g/T β-glucan + high temperature.

2. Abbreviations: Diet, diet effect; Tem, temperature effect; T × D, interactive effect between temperature and diet.
(extracted from *S. cerevisiae*). The negative effects of β-glucan could be because of energy redistribution to immune development, resulting in inefficient nutrient use of growth, strains, species, purity, dosage, composition, analysis of β-glucan, and the presence of challenges lead to such different results (Huff et al., 2006). On the whole, the addition of 200 g/T of dietary β-1,3-glucan cannot affect over response of immune system, a waste of energy in a nonchallenging or challenging circumstance.

Interestingly, there was an interaction between HS and β-1,3-glucan supplementation. Meanwhile, feeding birds with β-1, 3-glucan could improve the FCR of stressed birds at age of 21 to 35 D. In other words, β-1,3-glucan supplementation had a more effective influence of FCR on heat-stressed birds than on nonchallenged birds. In general, there might be 3 conventional reasons for improved growth performance of stressed birds: nutrient intake, maintenance energy, and nutrient digestibility. In terms of nutrient intake, an increase in ambient temperature reduces FI, resulting in the lack of most, if not all, nutrients that are necessary for optimal performance. Feed intake is reduced by elevated ambient temperatures and creates deficiency of most, if not all, nutrients which are essential for optimum performance (Donkoh, 1989). However, our data did not reveal that the β-1, 3-glucan can directly increase FI to ensure adequate nutrient needs. Therefore, nutrient intake is likely not a factor for better FCR. In terms of maintenance energy, higher maintenance energy must be required for heat-stressed birds. On the one hand, stressed birds have an increasing maintenance energy to release excess heat aiming to maintain body temperature. On the other hand, immune system of stressed bird is stimulated to overcome challenges, whose response consumes maintenance energy. Therefore, our data exhibited the stressed birds fed β-1, 3-glucan had an appropriate immune response (reduction in procytokines), which could imply lower maintenance energy for lower FCR and greater BW. Our current data of energy digestibility must be the main reason for better growth performance. Exposure of chickens to HS decreases nutrients digestibility as a result of lowering blood flow to the digestive system. This would reduce enzymatic activities and nutrient absorption (Belay et al., 1993). It is very hard for our own data to support that the β-1,3-glucan has capacity to improve enzymatic activities. However, stress-induced disorder in the integrity of the intestinal epithelium depress the protective function by compromising its cellular contacts and loss of mucus-producing and absorptive enterocytes (Olsen et al., 2005), resulting in pathogen colonization (McDonald and Monteleone, 2005). Relevant studies have reported that β-glucan supplementation increases the number of goblet cell growth and villus maturation and cure of villus damage/loss caused by pathogen challenges in the ileum (de Los Santos et al., 2007; Morales-López et al., 2009; Shao et al., 2013). Consequently, our results showed the stressed birds fed β-1,3-glucan had a reduction E. coli amount in the ileum, which might be an indirect evidence of better gut health for higher digestibility and another factor for FCR improvement of stressed birds. Consistent with current data, Stuyven et al. (2009) considered that piglets fed the β-glucan diet had a decrease in fecal excretion of F4+ E. coli and reduction in F4-specific antibody response comparing with control group. The author believed β-1,3-glucan might be have potential capacity to activate the enterocytes releasing certain antimicrobial peptides, which can indirectly impede E. coli colonization.

### Meat Quality and Organ Weight

It is reported that seasonal heat exposure can lead to undesirable changes in meat characteristics of turkeys that produce pale, soft, and exudative meat (McCurdy et al., 1996; McKee and Sams, 1997). The hyperthermia-associated myopathy is resulted in increased plasma activity of skeletal muscle–derived isoenzyme creatine kinase, reflecting Ca^{2+}-mediated alterations in muscle membrane integrity (Mitchell and Sandercock, 1997). Heat-stress–induced antemortem changes in muscle membrane permeability and concomitant changes in muscle metabolism in broilers may affect postmortem meat quality (Sandercock et al.,

| Items                      | NT          | 100 g/T     | 200 g/T     | SEM         | TEM       | DIET       | T X D       | P-value     |
|----------------------------|-------------|-------------|-------------|-------------|-----------|------------|-------------|-------------|
| TNF-α, pg/mL               | 513.32      | 489.90      | 508.56      | 619.44      | 548.23    | 521.70     | 31.95       | 0.038       |
| Interferon gamma, pg/mL    | 18.39       | 17.48       | 15.17       | 20.25       | 20.26     | 19.97      | 1.41        | 0.015       |
| IL 1, pg/mL                | 148.69      | 150.10      | 173.49      | 175.00      | 171.98    | 159.21     | 0.16        | 0.002       |
| IgG, mg/dL                 | 3.8         | 4.3         | 5.8         | 2.8         | 4.5       | 4.8        | 1.0         | 0.185       |
| SEM                       | 2.34        | 2.65        | 2.43        | 2.59        | 2.67      | 2.72       | 0.19        | 0.464       |
| P-value                    | 0.63        | 0.38        | 0.46        | 0.10        | 0.10      | 0.10       | 0.03        | 0.06        |

### Table 6. The effect of β-glucan supplementation on immune profiles in broilers on day 35.

| Main effect 2 | CON vs. Diet 3 |
|---------------|----------------|
| Tem          | Diet          |
| 0.038         | 0.003         |
| 0.386         | <0.001        |
| 0.831         | 0.426         |
| 0.560         | 0.173         |

**Table 6. The effect of β-glucan supplementation on immune profiles in broilers on day 35.**

# Abbreviations
- **NT**: Normal temperature
- **HT**: High temperature
- **0 g/T, 100 g/T, 200 g/T**: Mean the dosage of β-glucan in diet; **TNF-α**: Tumor necrosis factor-α; **IgG**: Immunoglobulin G; **SEM**: Standard error of the mean.

**Table 6. The effect of β-glucan supplementation on immune profiles in broilers on day 35.**

| Items                      | NT          | 100 g/T     | 200 g/T     | SEM         | TEM       | DIET       | T X D       | P-value     |
|----------------------------|-------------|-------------|-------------|-------------|-----------|------------|-------------|-------------|
| TNF-α, pg/mL               | 513.32      | 489.90      | 508.56      | 619.44      | 548.23    | 521.70     | 31.95       | 0.038       |
| Interferon gamma, pg/mL    | 18.39       | 17.48       | 15.17       | 20.25       | 20.26     | 19.97      | 1.41        | 0.015       |
| IL 1, pg/mL                | 148.69      | 150.10      | 173.49      | 175.00      | 171.98    | 159.21     | 0.16        | 0.002       |
| IgG, mg/dL                 | 3.8         | 4.3         | 5.8         | 2.8         | 4.5       | 4.8        | 1.0         | 0.185       |
| SEM                       | 2.34        | 2.65        | 2.43        | 2.59        | 2.67      | 2.72       | 0.19        | 0.464       |
| P-value                    | 0.63        | 0.38        | 0.46        | 0.10        | 0.10      | 0.10       | 0.03        | 0.06        |

**Table 6. The effect of β-glucan supplementation on immune profiles in broilers on day 35.**

**Abbreviations**: **NT**: Normal temperature; **HT**: High temperature; **0 g/T, 100 g/T, 200 g/T**: Mean the dosage of β-glucan in diet; **TNF-α**: Tumor necrosis factor-α; **IgG**: Immunoglobulin G.

**Main effect 2**: **CON vs. Diet 3**: Means in the same row with different superscripts differ (*P* < 0.05).

1. **Abbreviations**: **NT**: Normal temperature; **HT**: High temperature; **0 g/T, 100 g/T, 200 g/T**: Mean the dosage of β-glucan in diet; **TNF-α**: Tumor necrosis factor-α; **IgG**: Immunoglobulin G.

2. **Abbreviations**: **Diet**: Diet effect; **Tem**: Temperature effect; **T X D**: Interactive effect between temperature and diet.

3. **Contrast among the different dosage of β-glucan treatments within the normal temperature or high temperature challenge groups.**
In this study, lower meat pH, lighter meat color and lower WHC, and greater cooking loss were presented in HS treatment than in normal treatment. First, physiological changes of the muscle caused by HS including cell membrane or radicals could accelerate the anoxic respiration of muscle glycogen or cellular content oxidation, resulting in more lactic acid or lower meat pH. After that, as the meat pH decrease, parapeptone (myoglobinulin and actin) would be denaturalized, leading to a decrease in protein hydrophilic ability. The lower hydrophilic ability of the protein could be the reason for lower WHC and bigger drip loss. Then, with the water loss of muscle, water-soluble protein would be inactivated. The change of myoglobinulin in three-dimensional structure might result in a decreased light absorption capacity of meat and a brighter meat color. However, what is inconsistent with our results is that Moon et al. (2016) observed that there was no significant difference in the pH value of chicken breast meat between the dietary β-1,3 glucan treatment groups. To our best knowledge, no article has reported that β-glucan has radical scavenging ability to affect meat oxidation status directly. The report of Thondre et al. (2011) revealed that presence of polyphenol in the commercial β-glucan samples might contribute to the free radical scavenging ability. And, we speculate the difference might result from bird stress status, a decreased proinflammation cytokines concentration. The cooking loss and TNF-α reflecting an interaction between HS and β-1,3 glucan supplementation may confirm our assumption.

It is known that the bursa of Fabricius organ is not only the primary lymphoid organ in birds that produces immunologically competent cells but also the secondary or peripheral lymphoid organ that produces antibodies (Tsuji and Miyoshi, 2001). In this study, the bursa of Fabricius was atrophied in heat-stressed broilers. This phenomenon suggested that the abnormality of the bursa of Fabricious might be due to the oxidative damage during HS (Pamok et al., 2009). The β-1,3-glucan tends to increase weight of the bursa of Fabricius of stressed birds. In fact, the β-1,3-glucan, as an immune modulator, alleviate the HS impacts, which might be positive for meat quality, and bursa of Fabricius health.

**Immune Profile**

Proinflammation cytokines (TNF-α, interferon gamma, and IL-1) are the basic proteins of immunity and have been considered as endogenous signal molecules that mediate the cellular defense system against inflammatory response induced by high ambient temperature (Hietbrink et al., 2006). Meanwhile, IL-1, interferon gamma, and TNF-α may serve as signals to enhance the immune response, including activating cytotoxic macrophages, T-helper and natural killer cells, as well as promotion T cell proliferation and differentiation (Jacob and Pescatore., 2017). In agreement with our results, the increased levels of proinflammation cytokines of heat-exposed birds have been documented by previous works (Chang, 1993; Camus et al., 1998; Ostrowski et al., 1998; Moldoveanu et al., 2000; Suzuki K., 2000). The possible mechanism may be that the hyperthermia caused by passive heat exposure promotes the leakage of endotoxin from the intestine to the systemic circulation. The result is excessive activation of leukocytes and endothelial cells, characterized by the release of proinflammatory and anti-inflammatory cytokines (Bouchama and Knochel., 2002). Furthermore, HS leads to the activation of the hypothalamic–pituitary–adrenal axis, which eventually leads to the release of glucocorticoids. Glucocorticoids, in normal pulsatile release, promotes the release of proinflammatory cytokine. However, chronic increases in glucocorticoid levels have an inhibitory effect on most immune cytokines. Glucocorticoid acts in various ways, inhibiting the release of cytokine (Inbaraj et al., 2016). Shini et al. (2010) reported that chronic treatment of corticosterone downregulated proinflammatory cytokines and chemokines, indicating that the delayed effect of chronic stress can depress the immune response. In addition, the article by Quinterio-Filho et al. (2017) showed that chronic 31°C HS activated the hypothalamic–pituitary–adrenal axis in Salmonella-infected chicks from 35 to 41 D after birth, which increased corticosterone serum levels, resulting in a decrease in the plasmatic levels of proinflammatory cytokines. Whatever, an imbalance between inflammatory and anti-inflammatory cytokines may result in either inflammation-associated injury or refractory immunosuppression.

Interestingly, adding β-1,3-glucan into the diet decreased the levels of TNF-α of stressed birds in this article. Similarly, supplementation of β-1,3,1,6-glucan may also reduce the release of proinflammatory cytokines (Tzianabos, 2000), suggesting that animals may use more nutrients to boost tissue growth, and decrease the expense of immune response when stimulated with different levels of β-glucan supplementation. The hypothesis was confirmed by improving FCR data of this article as well. Zhang et al. (2008) suggested that stimulus resistance could be a possible explanation, including promoting the secretion of cytokine receptor (Poutsiaika et al., 1993), inhibiting synthesis of cytokines (Hogaboam et al., 1998), and the response to soluble β-glucan to release macrophage arachidonic acid metabolites (Castro et al., 1994). Intestinal integrity and gut health could be another explanation. β-glucan supplementation increases goblet numbers and villus height (de Los Santos et al., 2007; Morales-López et al., 2009), as well as raise tight junction protein expression (Shao et al., 2013). It seems that those innate immune enhancement of enteric physical barrier (enterocyte) and enteric chemical barrier (mucosal secretion) may prevent the partial leakage of endotoxin from the intestine to the systemic circulation, alleviate the challenge of HS, and thus reduce the proinflammatory cytokine released by the immune system.

Finally, based on 3 interactions including FCR, cooking loss, and TNF-α concentration between HS and β-1,3-glucan supplementation, it might be assumed...
that β-1,3-glucan supplementation can decrease alleviate HS status as result of the decreased level of proinflammation cytokines (TNF-α). The lower degree of stress status not only leads to a more stable meat quality (less cooking loss) but also decreases the immune system nutrient consumption, meaning lower maintenance energy, which will result in a lower FCR.

CONCLUSION

These results indicated that 100 and 200 g/T β-1,3-glucan supplementation, whether under HS condition or not, can increase growth performance without a negative response on immunity. Under HS conditions, the addition of β-1,3-glucan at dosage from 100 to 200 g/T in diet can increase energy digestibility, decrease cooking loss, and reduce the amount of ileum E. coli and the concentration of TNF-α.

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