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## Evaluation of an Algal Beta-1,3-Glucan on Broiler Growth Performance and Immune Response

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**Abstract:** The objective of the current study was to investigate the effects of an algal  $\beta$ -1,3-glucan (ABG) product on broiler performance, oocyst output following an *Eimeria* challenge and antibody titer levels following a Newcastle Disease vaccination program. Three experiments were conducted evaluating four dietary treatments: a control diet and three levels of ABG (100, 250 and 750 g/MT). Experiment 1 evaluated the effects of ABG on performance parameters and relative organ weight in a 42 d grow-out study. Ten replicates per treatment each contained 35 live-oocyst vaccinated broilers. Inclusion of ABG at 750 g/MT increased d 14 BW compared to the control while inclusion of ABG at 250 g/MT improved feed conversion ratio (FCR) compared to the control diet during the starter phase of production. At the conclusion of the trial, no differences in BW, FCR, or relative organ weights were observed. Experiment 2 consisted of 70 broilers per dietary treatment placed in battery pens and challenged with a 100 x dose of vaccine strain *Eimeria* oocysts on d 10. Fecal samples were collected on 6, 7, 8 and 9 d post inoculation to determine oocyst output. Inclusion of ABG at 100 and 250 g/MT numerically reduced oocyst/g of fecal material on d 6 post-challenge compared to the control. The inclusion of ABG at 250 and 750 g/MT reduced cumulative FCR through d 20 compared to the challenged control broilers. Experiment 3 consisted of 120 male broilers with 5 birds randomly placed in each of 6 replicate battery pens per treatment. Newcastle vaccine was administered on d 1 of age and a boost was administered on d 18 of age. Blood samples were collected 7 d post boost to evaluate Newcastle specific antibody titers. Greater antibody titers were observed on d 25 in birds fed ABG at 250 g/MT compared to the control group. Combined, these data demonstrate the ability of ABG to stimulate broiler immune response and improve early broiler performance during coccidial vaccine challenge.

**Key words:**  $\beta$ -glucan, broiler, antibody titer, oocyst output

### INTRODUCTION

Demand for limited and reduced use of antibiotics in poultry production has increased in the use of alternative supplements to benefit immune function and growth performance. The rise in antibiotic-resistant bacteria has led to a need for new forms of disease prevention and mitigation (Cox and Dalloul, 2010). One possible alternative that has been extensively investigated is the inclusion of  $\beta$ -1,3-glucans in poultry diets. Beta-1,3/1,6-glucans are a structural component of the cell wall of many bacteria, fungi, yeasts and algae (Jorgensen and Robertsen, 1995). Variations in  $\beta$ -glucan source, structure, molecular weight, degree of branching, degree of polymerization and intermolecular association can result in different physiological functions (Wagner *et al.*, 1988; Jamas *et al.*, 1991; Bohn and BeMiller, 1995; Kulicke *et al.*, 1997; Pins *et al.*, 2005a, b; Yoshitomi *et al.*, 2005; Leung *et al.*, 2006; Volman *et al.*, 2008).

Beta glucans are classified as biological response modifiers due to their ability to stimulate the immune

system (Cox and Dalloul, 2010). Immune modulation has been attributed to  $\beta$ -glucan because of its highly branched structure and insolubility (Vetvicka and Vetvickova, 2007; Zhang *et al.*, 2008; Cox *et al.*, 2010b) and its ability to activate macrophage (Sakurai *et al.*, 1992; Guo *et al.*, 2003) and immune regulatory cells. In particular, yeast derived  $\beta$ -glucans have been suggested to modulate both specific and non-specific immune responses in animals (Chae *et al.*, 2006). Nearly 15% of the cell wall of yeast is comprised of branched  $\beta$ -1,3/1,6-glucan of high molecular weight (Manners *et al.*, 1973), which is considered to be effective as an immune regulator due to its highly branched structure (Vetvicka and Vetvickova, 2007; Harada and Ohno, 2008).

Inconsistencies have been reported in the literature that indicate  $\beta$ -glucans can improve performance through increased BW (An *et al.*, 2008; Rathgeber *et al.*, 2008) and reduced feed conversion ratio (FCR) (An *et al.*, 2008; Rathgeber *et al.*, 2008; Morales-Lopez *et al.*, 2009), decrease BW of non-challenged birds (Huff *et al.*, 2006),

or have no effect on broiler performance (Cox *et al.*, 2010a; Chae *et al.*, 2006; Rathgeber *et al.*, 2008; Zhang *et al.*, 2012) in challenge and non-challenge settings. It has been suggested that the immune status of the animal and environmental challenge presented to the animal may play a role in performance responses observed in broilers fed  $\beta$ -glucan products (Chae *et al.*, 2006; Cox *et al.*, 2010a). Another major factor is the bioavailability of the  $\beta$ -glucan present in yeast-based  $\beta$ -glucan feed ingredients. Since the  $\beta$ -1,3/1,6-glucan in yeast is bound to other cell wall components, such as chitin and mannoprotein, it is not available to be taken up by gut-associated lymphoid tissues (GALT, commonly referred to as Peyer's patches). Various manufacturers have developed mechanical and chemical extraction techniques, but there is a large amount of variability in the final products (Vetvicka and Vetvicka, 2007).

Numerous investigators have detailed the effects of yeast cell wall  $\beta$ -glucans (YBG) and their effects on broiler performance and as an immune modulator when compared to antibiotic growth promoters, serving as an alternative form of disease prevention (Chae *et al.*, 2006; Rathgeber *et al.*, 2008; Morales-Lopez *et al.*, 2009; Cho *et al.*, 2013). Coccidiosis and Newcastle Disease are diseases of interest in poultry production. *Eimeria* species are known to invade the intestinal tract of animals, disrupting digestion, impeding absorption and causing performance losses to the host. Evidence of an *Eimeria* infection is left in the intestinal tract in the form of lesions (Brake *et al.*, 1997). The use of a vaccination program to combat *Eimeria* was reported by Lee *et al.* (2011) to impact broiler performance by reducing BW and increasing FCR. These observations were most important early, between d 13 and 17 (Lee *et al.*, 2011). Yeast  $\beta$ -glucans have been observed to improve immune function and performance in broilers (Zhang *et al.*, 2012). Beta glucans have been observed to improve immune function in *Eimeria* challenged broilers, decreasing fecal oocyst counts (Shanmugasundaram *et al.*, 2013) and reducing intestinal lesions and severity (Cox *et al.*, 2010b). The use of live-virus vaccines to combat Newcastle Disease is known to increase IgA, IgG and IgM antibodies in d old broilers (Meulmanns, 1988; Russell and Ezeifka, 1995). An *et al.* (2008) reported an increase in Newcastle disease specific antibody titers in Newcastle vaccinated broilers fed  $\beta$ -glucans in the diet. An increase in size of primary and secondary lymphoid organs has also been associated with  $\beta$ -glucan supplementation in feed (Guo *et al.*, 2003; Zhang *et al.*, 2008).

With numerous inconsistencies in published results with yeast derived  $\beta$ -glucan, further investigation is needed to determine the effectiveness of alternative sources of  $\beta$ -glucan. This study utilized a novel  $\beta$ -1,3-

glucan product derived from the microalgae *Euglena gracilis*. In contrast to yeast, this organism accumulates linear  $\beta$ -1,3-glucan up to about 50% by mass in its cytoplasm in the form of 1-3 mm granules. This particular  $\beta$ -glucan contains a high concentration of  $\beta$ -1,3-glycosidic linkages and does not contain the  $\beta$ -1,6 branches which are characteristic of yeast  $\beta$ -glucan products. A review of literature indicated no previous experiments evaluating an algal derived  $\beta$ -glucan product on poultry performance and immunological effects. The objective of this series of experiments was to investigate the effects of an algal  $\beta$ -1,3-glucan (ABG) product (Algamune™ ZPC, Algal Scientific Corporation) on broiler performance, oocyst output following an *Eimeria* challenge and antibody titers following a Newcastle Disease vaccination program in a series of three experiments.

## MATERIALS AND METHODS

**Experimental design:** A series of three experiments were conducted to evaluate the effect of ABG on broiler growth performance during a 42 d grow-out, oocyst output and performance during an *Eimeria* challenge and Newcastle Disease specific antibody titers following a Newcastle Disease vaccination program. One large basal diet was manufactured for each dietary phase and experiment, with ABG supplemented prior to pelleting at levels of 0, 100, 250 and 750 g/MT. This ABG product is available in the form of a dry algae powder containing ~50%  $\beta$ -1,3-glucan or a Zinc Polysaccharide Complex (ZPC) containing ~50%  $\beta$ -1,3-glucan and 2 to 10% zinc. The ZPC version containing 10% zinc was used in this study. Broilers were provided age appropriate heating and given access to feed and water *ad libitum*. All broilers followed an industry type dietary program (Table 1). All experiments were conducted in accordance with a Texas A&M University approved animal use protocol (IACUC).

**Experiment 1:** A 42 d grow-out experiment was conducted to evaluate the effects of dietary inclusion of ABG on broiler growth performance. On d of hatch, 1400 Cobb 500 males were weighed and randomly allotted to floor pens and dietary treatments based on body weight. Thirty-five broilers were placed per replicate pen, with 10 replicate pens per treatment for a total of 40 replicate pens. Stocking density was set to 1.00 sq. ft./bird. Pens contained recycled litter top dressed with fresh pine shavings. All broilers were vaccinated with a live oocyst vaccine upon arrival at the experimental location. The dietary program consisted of a starter (d 1-14), grower (d 14-28) and finisher (d 28-42). Broilers and feed were weighed at the end of each dietary phase on d 14, 28 and 42 to calculate average body weight and mortality corrected feed conversion ratio (FCR). On d 42, 15 birds

Table 1: Dietary formulation and calculated nutrient content of diets, based on percentages, for male broilers fed Algamune ZPC<sup>1</sup> at increasing levels

Ingredient	Starter	Grower	Finisher
Corn	58.19	63.36	68.47
Dehulled soybean meal (48%)	34.57	29.62	24.69
DL-Methionine (99%)	0.23	0.20	0.16
Lysine HCL	0.16	0.14	0.12
Fat, A/V blend	2.90	2.90	2.92
Limestone	1.57	1.58	1.56
Monocalcium phosphate	1.56	1.40	1.29
Sodium chloride	0.51	0.49	0.49
Vitamin premix <sup>2</sup>	0.25	0.25	0.25
Trace minerals <sup>3</sup>	0.05	0.05	0.05
<b>Calculated nutrient content</b>			
Protein	22.00	20.00	18.00
Lysine	1.30	1.15	1.00
Methionine	0.56	0.51	0.44
TSAA	0.92	0.84	0.75
Threonine	0.82	0.75	0.67
Calcium	0.95	0.92	0.88
Total phosphorus	0.70	0.66	0.63
Available phosphorus	0.45	0.41	0.38
Sodium	0.22	0.21	0.21
Metabolizable energy (kcal/kg)	3050	3100	3150

<sup>1</sup>Algamune ZPC is a zinc metal polysaccharide complex that contains beta-1,3-glucan from algae as part of the complex. This product is intended to provide a nutritional form of zinc metal that provides additional bioavailability when used with other nutritional forms of zinc. This product meets the AAFCO definition of metal polysaccharide. (Algal Scientific, Plymouth, MI, USA)

<sup>2</sup>Vitamin premix added at this rate yields 11,023 IU vitamin A, 3,858 IU vitamin D<sub>3</sub>, 46 IU vitamin E, 0.0165 mg B<sub>12</sub>, 5.845 mg riboflavin, 45.93 mg niacin, 20.21 mg d-pantothenic acid, 477.67 mg choline, 1.47 mg menadione, 1.75 mg folic acid, 7.17 mg pyroxidine, 2.94 mg thiamine, 0.55 mg biotin per kg diet. The carrier is ground rice hulls

<sup>3</sup>Trace mineral premix added at this rate yields 149.6 mg manganese, 125.1 mg zinc, 16.5 mg iron, 1.7 mg copper, 1.05 mg iodine, 0.25 mg selenium, a minimum of 6.27 mg calcium and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate and the premix contains less than 1% mineral oil

per dietary treatment were randomly selected, weighed and euthanized. Birds were necropsied and the liver, spleen and kidneys were removed and weighed to determine relative organ weight.

**Experiment 2:** Day old male broilers from the same hatch as males in Experiment 1 were placed in battery brooders for a 20 d experiment evaluating the effects of ABG during an *Eimeria* vaccine challenge setting. Starter feed manufactured for Experiment 1 was used for the duration of the experiment, with two separate treatment groups being fed the control diet. Ten replicate pens were used in conjunction with five dietary treatments, with seven male broilers placed per replicate pen, for a total of 350 male broilers used in the experiment. On d 10, all broilers fed ABG and one control treatment were challenged with a 100x dose of vaccine strain *Eimeria*

oocysts (Coccivac<sup>®</sup>-B) containing *Eimeria acervulina*, *E. maxima*, *E. mivati* and *E. tenella*; the remaining control group was not vaccinated and utilized as a non-challenged control group. Body weights and feed weights were recorded on d 10 (d of challenge), 17 (6 d post challenge) and 20 (termination of the experiment) for the determination of performance effects of ABG during an *Eimeria* challenge. On d 17, six d post-challenge, three broilers per replicate pen were euthanized and necropsied, with intestines removed for assessment of lesion development associated with the *Eimeria* vaccine challenge. Intestines were removed for evaluation of gross lesions associated with the challenge and given a value of 0 through 4, wherein 0 is normal and 1, 2, 3, or 4 indicate increasing severity of infection (Johnson and Reid, 1970). Sampling sites included the ascending and descending loops of the duodenum (upper intestine), five cm anterior and posterior of Meckel's diverticulum (middle intestine) and both ceca (lower intestine). Fecal material was collected from each replicate pen 6, 7, 8 and 9 d post challenge to quantify oocyst shedding associated with challenge using the method outlined by Oden *et al.* (2012). Fresh fecal droppings (minimum of 8 individual droppings per pen) on manure pans were collected and pooled for examination and quantification of oocysts present per gram of feces. Prior to analysis, each fecal sample was homogenized, weighed and diluted at a 3:1 ratio of water to fecal matter. Following agitation, fecal suspension was extracted and loaded into a hemacytometer to be observed microscopically for oocyst presence. A standard light microscope with 10 x eye-piece objective and a 20 x objective (200 x magnification) was used to quantify non-sporulated oocysts present in each sample for oocyst per gram of feces calculations.

**Experiment 3:** A third experiment was conducted to determine the effects of ABG on performance and Newcastle Virus specific antibody titers of broilers following a Newcastle/Bronchitis vaccination program. Experimental treatments remained consistent to the previous experiments. Five male broilers were placed in each replicate pen, with 6 replicate pens per treatment for a total of 120 Cobb 500 males used in the experiment. A starter feed consistent with the first two experiments was fed through d 18. A grower feed was manufactured without ABG inclusion and was fed from d 18 to 25. On d of hatch, each chick received 100 µl of Newcastle/Bronchitis vaccine diluted to the equivalent of a full dose; 50 µl was administered to each bird through intranasal and 50 µl intraocular. Broilers received a vaccine boost at d 18 consistent in dosage and administered through the same route as the initial vaccination. On d 25 (7 days post boost), blood was obtained from all broilers. Samples were allowed to clot overnight and centrifuged to obtain the serum. Serum

antibody titer levels were determined via ELISA using a commercially available kit (IDEXX Newcastle Disease Virus Antibody Test Kit). Body weights and feed weights were measured on d 7, 18 and 25 for the determination of performance effects of ABG during a Newcastle vaccination program on the basis of BW, average daily feed consumption and FCR.

**Statistical analysis:** Relative organ weights from Experiment 1, lesion scores and oocyst output from Experiment 2, antibody titer levels and feed consumption from Experiment 3 and body weight and FCR from all experiments were analyzed via Analysis of Variance (ANOVA) using the General Linear Model Procedure using SPSS V 18.0. (2010). Main effect means were deemed significantly different at  $p < 0.05$  and separated using Duncan's Multiple Range Test. The experimental unit for each parameter evaluated was replicate pen.

**RESULTS**

**Experiment 1:** On d 14, inclusion of ABG at 750 g/MT increased ( $p < 0.05$ ) BW compared to the control diet (Table 2). Inclusion of ABG at 100 g/MT negatively influenced BW ( $p < 0.05$ ) compared to the control diet on d 14 and 28. On d 28, inclusion of ABG at 250 and 750 g/MT did not impact BW compared to the control diet. At the conclusion of the experiment on d 42, all experimental treatments yielded similar body weights. With regards to FCR, inclusion of ABG at 100 g/MT significantly increased ( $p < 0.05$ ) FCR during the starter phase compared to the control diet. Inclusion of ABG at 250 g/MT resulted in a lower FCR during the starter phase compared to the control diet (Table 2). During the

grower phase, the inclusion of ABG diet had no impact on FCR compared to the control diet. The inclusion of ABG at 100 g/MT significantly reduced ( $p < 0.05$ ) FCR compared to the inclusion of ABG at 250 g/MT during the grower phase. Inclusion of ABG had no impact on FCR during the finisher phase compared to the control diet. Cumulative FCR was not impacted by ABG compared to the control diet from d 1-28 and at the conclusion of the experiment from d 1-42.

Inclusion of ABG diet had no impact on organ weight or relative weight of liver, spleen and kidney weights compared to the control diet at the conclusion of the trial (Table 3).

**Experiment 2:** On d 10 (day of challenge), inclusion of ABG at 250 g/MT increased ( $p < 0.05$ ) BW compared to control fed broilers while the 100 and 750 g/MT had similar BW to the control diet (Table 4). On d 17 and 20 (7 and 10 days post-challenge, respectively) inclusion of ABG at 250 and 750 g/MT increased ( $p < 0.05$ ) BW compared to the non-challenged control broilers. *Eimeria* challenge did not impact body weight gain during challenge in control fed broilers, as non-challenged and challenged broilers had similar weight gain. Broilers fed 100 g/MT of ABG had a reduced ( $p < 0.05$ ) rate of weight gain compared to control groups from d 10-17 and d 17-20. All other treatments experienced similar rates of body weight gain during the challenge period.

Inclusion of ABG at 250 and 750 g/MT reduced ( $p < 0.05$ ) pre-challenge (d 1-10) FCR compared to the control fed broilers (Table 4). While vaccine *Eimeria* challenge did not negatively impact body weight gain, FCR was

Table 2: Average BW, dietary phase mortality corrected FCR and cumulative mortality corrected FCR of male broilers fed increasing levels of ABG in a non-challenge setting (Experiment 1)

Treatment (g/MT ABG <sup>1</sup> )	Body weights			Dietary phase FCR			Cumulative FCR	
	Day 14 (g)	Day 28 (kg)	Day 42 (kg)	Starter	Grower	Finisher	Day 1-28	Day 1-42
0	383.5 <sup>b</sup>	1.524 <sup>a</sup>	3.049	1.336 <sup>b</sup>	1.534 <sup>ab</sup>	1.663	1.453	1.558
100	341.2 <sup>c</sup>	1.433 <sup>b</sup>	2.904	1.395 <sup>a</sup>	1.489 <sup>b</sup>	1.642	1.452	1.543
250	389.7 <sup>ab</sup>	1.539 <sup>a</sup>	3.062	1.290 <sup>c</sup>	1.591 <sup>a</sup>	1.668	1.472	1.563
750	395.4 <sup>a</sup>	1.513 <sup>a</sup>	2.974	1.321 <sup>b</sup>	1.538 <sup>ab</sup>	1.697	1.457	1.568
SEM	<0.1	0.010	0.026	0.012	0.011	0.014	0.007	0.007
p-value	<0.001	<0.001	0.294	<0.001	0.039	0.212	0.643	0.074

<sup>a-c</sup>Means in columns differ at  $p < 0.05$

<sup>1</sup>Algamune ZPC (ABG)-Algal Scientific, Plymouth, MI, USA

Table 3: Organ and relative organ weights of broilers fed increasing levels of ABG in a non-challenge setting (Experiment 1)

Treatment (g/MT ABG <sup>1</sup> )	Weights (g)				Relative organ weight (%)		
	Body WT	Liver	Spleen	Kidney	Liver	Spleen	Kidney
0	2900.0	71.9	3.4	13.8	2.48	0.12	0.47
100	2734.3	63.3	3.7	13.6	2.32	0.14	0.50
250	2941.9	69.2	4.1	14.5	2.35	0.14	0.50
750	2863.3	68.9	3.7	14.2	2.40	0.13	0.50
SEM	30.9	1.5	0.1	0.3	0.04	<0.01	0.01
p-value	0.094	0.217	0.290	0.808	0.592	0.264	0.870

<sup>1</sup>Algamune ZPC (ABG)-Algal Scientific, Plymouth, MI, USA

Table 4: Average BW, weight gain, phase mortality corrected FCR and cumulative mortality corrected FCR of broilers receiving a 100x dose of Coccivac®-B and fed increasing levels of ABG (Experiment 2)

Treatment (g/MT ABG <sup>1</sup> )	Body weights			Weight gain			Phase FCR			Cum. FCR
	Day 10 (g)	Day 17 (g)	Day 20 (g)	Day 10-17	Day 17-20	Day 1-10	Day 10-17	Day 17-20	Day 1-20	
0 (Non-challenged)	234.2 <sup>bc</sup>	530.2 <sup>bc</sup>	647.5 <sup>bc</sup>	310.7 <sup>a</sup>	445.4 <sup>a</sup>	1.184 <sup>a</sup>	1.283 <sup>b</sup>	1.691	1.299 <sup>bc</sup>	
0 (Challenged)		544.4 <sup>ab</sup>	672.8 <sup>ab</sup>	304.2 <sup>a</sup>	426.2 <sup>a</sup>		1.422 <sup>a</sup>	1.675	1.355 <sup>ab</sup>	
100	231.1 <sup>c</sup>	515.8 <sup>c</sup>	615.3 <sup>c</sup>	285.6 <sup>b</sup>	285.6 <sup>b</sup>	1.224 <sup>a</sup>	1.466 <sup>a</sup>	1.639	1.408 <sup>a</sup>	
250	251.0 <sup>a</sup>	562.2 <sup>a</sup>	700.2 <sup>a</sup>	311.3 <sup>a</sup>	444.9 <sup>a</sup>	1.111 <sup>b</sup>	1.363 <sup>ab</sup>	1.561	1.284 <sup>c</sup>	
750	244.4 <sup>ab</sup>	561.2 <sup>a</sup>	697.8 <sup>a</sup>	316.9 <sup>a</sup>	450.8 <sup>a</sup>	1.103 <sup>b</sup>	1.402 <sup>a</sup>	1.547	1.286 <sup>c</sup>	
SEM	2.7	5.3	6.9	3.7	5.4	0.015	0.015	0.037	0.013	
p-value	<0.001	<0.001	<0.001	0.007	<0.001	0.013	0.017	0.652	0.001	

<sup>a-c</sup>Means in columns differ at p<0.05

<sup>1</sup>Algamune ZPC (ABG)-Algal Scientific, Plymouth, MI, USA

Cum.: Cumulative

Table 5: Intestinal lesion scores of male broilers 7 d post challenge receiving a 100x dose of Coccivac® B and fed increasing levels of ABG (Experiment 2)

Treatment (g/MT ABG <sup>1</sup> )	Upper	Middle	Lower
0 (Non-challenged)	0.00 <sup>b</sup>	0.00 <sup>b</sup>	0.04 <sup>b</sup>
0 (Challenged)	1.10 <sup>a</sup>	0.00 <sup>b</sup>	0.34 <sup>ab</sup>
100	1.27 <sup>a</sup>	0.10 <sup>a</sup>	0.30 <sup>ab</sup>
250	1.07 <sup>a</sup>	0.00 <sup>b</sup>	0.50 <sup>a</sup>
750	1.03 <sup>a</sup>	0.00 <sup>b</sup>	0.13 <sup>bc</sup>
SEM	0.06	0.01	0.04
p-value	<0.001	0.021	0.001

<sup>a-c</sup>Means in columns differ at p<0.05

<sup>1</sup>Algamune ZPC (ABG)-Algal Scientific, Plymouth, MI, USA

increased (p<0.05) in control challenged broilers compared to non-challenged broilers (d 10-17). During the same period, inclusion of 250 g/MT of ABG reduced FCR to a level comparable to the non-challenge control fed broilers while inclusion at 100 and 750 g/MT was similar to challenge control fed broilers. When evaluating cumulative FCR (d 1-20), inclusion of ABG at 250 and 750 g/MT significantly reduced (p<0.05) FCR compared to the challenged control group to levels similar to the non-challenge control fed broilers.

Overall, lesion development associated with challenge was relatively low which may explain the lack of separation when evaluating BW gain. Inclusion of ABG had no impact on upper intestinal lesion scores of *Eimeria* challenged broilers compared to the challenged control group. All challenged broilers showed greater (p<0.05) average upper intestinal lesion scores compared to the non-challenged control group (Table 5). Lesion development in the mid portion of the small intestine was extremely low, however, broilers fed 100 g/MT ABG were observed to have greater (p<0.05) mid intestinal lesion scores compared to both control groups, although this represented only 2 broilers exhibiting lesions while no other treatments expressed any lesion development. Inclusion of ABG had no impact on average lower intestinal lesion scores compared to the challenged control group.

Regarding oocyst output, the inclusion of ABG had no impact on oocyst output 6, 7, 8 and 9 d post *Eimeria* challenge compared to the challenged control group (Table 6). With regards to the 4 d post challenge average oocyst output, inclusion of ABG had no impact on average oocyst output compared to the challenged control group. Inclusion of ABG at 250 g/MT lowered (p<0.05) 4 d average oocyst output compared to the inclusion of ABG at 750 g/MT. All challenged broilers showed significantly greater (p<0.05) oocyst output compared to the non-challenged control group 6, 7, 8 and 9 d post challenge and on a 4 d post challenge average oocyst output. There was a low level of oocyst shedding in one replicate pen of non-challenged control fed broilers indicating that horizontal transfer of the challenge organism did take place, however, broilers

Table 6: Oocyst output (oocysts/g of fecal material) of broilers receiving a 100x dose of Coccivac® B and fed increasing levels of ABG (Experiment 2)

Treatment (g/MT ABG <sup>1</sup> )	Days post challenge				Four day post challenge average
	Day 6	Day 7	Day 8	Day 9	
0 (Non-challenged)	563 <sup>a</sup>	750 <sup>b</sup>	188 <sup>b</sup>	188 <sup>b</sup>	422 <sup>a</sup>
0 (Challenged)	31.500 <sup>ab</sup>	16.050 <sup>a</sup>	4.050 <sup>a</sup>	4.200 <sup>a</sup>	13.950 <sup>ab</sup>
100	29850 <sup>b</sup>	13.950 <sup>a</sup>	5.550 <sup>a</sup>	5.550 <sup>a</sup>	13.750 <sup>ab</sup>
250	24.900 <sup>b</sup>	15.450 <sup>a</sup>	6.300 <sup>a</sup>	4.050 <sup>a</sup>	12.675 <sup>b</sup>
750	44.100 <sup>a</sup>	15.600 <sup>a</sup>	3.600 <sup>a</sup>	5.400 <sup>a</sup>	17.175 <sup>a</sup>
SEM	2656	1302	517	559	710
p-value	<0.001	0.001	0.001	0.012	<0.001

<sup>a,b</sup>Means in columns differ at p<0.05

<sup>1</sup>Algamune ZPC (ABG)-Algal Scientific, Plymouth, MI, USA

were reared in the same battery units as challenge organisms to allow for accurate evaluation and comparison of performance parameters to that of a non-challenge group.

**Experiment 3:** Inclusion of ABG had no impact on BW of Newcastle vaccinated broilers compared to the control group throughout the experiment (Table 7). Regarding FCR, the inclusion of ABG had no impact on FCR compared to the control group throughout the experiment, however, the inclusion of ABG at 750 g/MT reduced (p<0.05) FCR from d 7-18 compared to the inclusion of ABG at 250 g/MT (Table 7). Feed consumption (g/bird/day) was not effected with the inclusion of ABG compared to the control group throughout the experiment (Table 8).

Newcastle specific antibody titers (Log<sub>10</sub>) of Newcastle vaccinated broilers on d 25 were increased (p<0.05) in broilers fed ABG at 250 g/MT compared to control fed broilers (Table 7).

## DISCUSSION

In the present study, an algal β-1,3-glucan product (Algamune™ ZPC) was supplemented to non-challenged and challenged broilers to assess the effect of an algal derived β-glucan in three consecutive experiments. In Experiment 1, at d 14, inclusion of ABG at 750 g/MT increased (p<0.05) early BW from 383.5 g in the control diet to 395.4 g. Similar benefits to early BW gains were reported when feeding a derivation of a β-1,3/1,6-glucan from *Agrobacterium sp.* R259 KCTC 10197B by Cho *et al.* (2013). This group reported BW gain from weeks 0 to 3 in broilers fed 0.1% β-glucan in a non-challenge setting. However, Chae *et al.* (2006) reported no differences in BW on d 17 when feeding YBG, indicating inconsistencies in reports on early BW effects when feeding varying sources of β-glucans. Given the early impact on BW at d 14 in the 750 g/MT ABG group but the lack of statistically significant differences by the end of the 42 grow-out, these data suggest that ABG may be most beneficial in starter diets when chicks are experiencing stress (e.g., transport, finding feed and water, etc.) and developing their

immune system. Multiple publications have likewise indicated no effect on final BW in broilers fed YBG at varying levels in a non-challenge setting (Chae *et al.*, 2006; Morales-Lopez *et al.*, 2009; Cox *et al.*, 2010a; Zhang *et al.*, 2012).

The inclusion of ABG at 250 g/MT resulted in a lower FCR during the starter phase but did not show any significant differences during the grower and finisher phases compared to the control diet. This trend mirrors that observed for BW and suggests again that in a university pen-study with low environmental stress, ABG may be most beneficial in the starter diet. Unexpectedly, the use of ABG at 100 g/MT increased (p<0.05) FCR during the starter phase by nearly 6 points as compared to the control group. This impact on FCR can be explained by the smaller BW of broilers fed ABG at 100 g/MT at d 14, when feed consumption was not effected by treatment. Supplementation of ABG had no effect on dietary phase FCR and cumulative FCR compared to the control group for the remainder of the experiment, although decreasing ABG supplementation from 250 g/MT to 100 g/MT significantly reduced (p<0.05) FCR during the grower phase. The waning impact of ABG on FCR is similar to reports by Chae *et al.* (2006), Rathgeber *et al.* (2008), Morales-Lopez *et al.* (2009), Cox *et al.* (2010a) and Zhang *et al.* (2012) who observed no impact on FCR when feeding YBG. Relative organ weights of the liver, spleen and kidney were not impacted by feeding of ABG in this experiment, indicating this new feed ingredient is unlikely to be a source of toxicity or to adversely affect the functioning of these organs. Likewise, YBG was not found to impact relative liver weights by Morales-Lopez *et al.* (2009) and Zhang *et al.* (2012). Relative spleen weights have also been reported to be unaffected by YBG at levels as high as 0.1% by Rathgeber *et al.* (2008) and Cox *et al.* (2010). However, Zhang *et al.* (2012) reported a 3.9% increase in spleen weights in broilers fed YBG at similar levels. It was the conclusion of Chae *et al.* (2006) that the effects dietary supplementation of β-glucan to broilers on performance is dependent on use in a challenge vs. non-challenge setting. For this reason two consecutive experiments were conducted evaluating ABG in the

Table 7: Average BW, phase mortality corrected FCR, cumulative mortality corrected FCR and Newcastle Disease Virus antibody titers (Log<sub>10</sub>) of broilers receiving a full dose of Newcastle vaccine and fed increasing levels of ABG (Experiment 3)

Treatment (g/MT ABG <sup>1</sup> )	Body weights			Phase FCR			Cumulative FCR			Day 25 newcastle disease antibody titers
	Day 7 (g)	Day 18 (g)	Day 25 (g)	Day 1-7	Day 7-18	Day 18-25	Day 1-18	Day 1-25		
0	144.5	559.8	926.8	1.177	1.455 <sup>ab</sup>	1.692	1.395	1.517	1.833 <sup>b</sup>	
100	151.6	567.7	987.1	1.290	1.450 <sup>ab</sup>	1.605	1.417	1.497	1.834 <sup>b</sup>	
250	145.4	550.4	956.2	1.201	1.472 <sup>a</sup>	1.608	1.417	1.502	2.239 <sup>a</sup>	
750	142.3	575.9	968.2	1.228	1.382 <sup>b</sup>	1.637	1.355	1.473	2.051 <sup>ab</sup>	
SEM	2.0	8.0	10.3	0.026	0.014	0.024	0.012	0.014	0.060	
p-value	0.438	0.737	0.215	0.487	0.046	0.588	0.219	0.776	0.042	

<sup>a,b</sup>Means in columns differ at p<0.05

<sup>1</sup>Algamine ZPC (ABG)-Algal Scientific, Plymouth, MI, USA

presence of an *Eimeria* disease challenge and following a Newcastle Disease vaccination program. Beta glucan is thought to prime the immune system, allowing the bird to sustain growth with no losses in performance (Cox *et al.*, 2010b). For these reasons Experiment 2 explored the use of ABG during an *Eimeria* challenge setting. At 10 days of age, all broilers supplemented with ABG were challenged with 100X dose of vaccine strain *Eimeria* oocysts (Coccivac<sup>®</sup>-B) containing *Eimeria acervulina*, *E. maxima*, *E. mivati* and *E. tenella*. Half of the control group was also challenged, while the remaining half was kept as a non-challenged control group to allow for the evaluation of performance effects of challenge and influence of ABG. On d 10, prior to challenge, inclusion of ABG at 250 g/MT increased (p<0.05) BW by 17 g over the control group. These gains in early BW are similar to those observed in Experiment 1. Significant gains (p<0.05) in BW continued post-challenge to d 20 in broilers supplemented with ABG at 250 and 750 g/MT compared to the non-challenged control group. Inclusion of ABG at 250 and 750 g/MT also significantly reduced (p<0.05) d 10 FCR compared to the control groups and reduced cumulative FCR for the entire experiment compared to the challenged control group to levels similar to the non-challenge control group. These data suggest that ABG helped broilers better manage the challenge of the *Eimeria* parasite and maintain efficient growth. These data are corroborated by a report on the benefits of a whole yeast cell product provided to broilers by Shanmugasaundaram *et al.* (2013). On d 21 of the study, broilers were challenged with live coccidial oocysts. Broilers were supplemented with a whole yeast cell wall product as high as 0.2% of the diet, which increased BW gain and feed efficiency 12 d post coccidial challenge. Conversely to these findings, Cox *et al.* (2010b) observed no impact on performance when feeding YBG at levels up to 0.1% of the diet during an *Eimeria* challenge. The differences in the results between these two studies may be dose dependent since both are yeast derived β-glucan, or may be due to differences in manufacturing and the extent of bioavailability based on extraction efficiency. It could also be concluded that the differences in source of β-glucan impacted the differences in performance between these studies and the current experiment and whether in a challenge or non-challenge setting. The second experiment also evaluated the impact ABG during the *Eimeria* challenge on lesion scores and oocyst output. *Eimeria* disrupt intestinal cell linings such as the enterocyte layer, resulting in observable lesions. These lesions are associated with a reduction in nutrient absorption and reduced performance (Brake *et al.*, 1997). Lesion scores were observed and recorded in broilers supplemented with ABG 7 d post *Eimeria* challenge. Inclusion of ABG had no impact on average intestinal lesion score in the upper intestinal section



Table 8: Feed consumption (FC; grams of feed consumed per bird per day) of broilers receiving a full dose of Newcastle vaccine and fed increasing levels of ABG (Experiment 3)

Treatment	Phase FC			Cumulative FC	
	Day 1-7	Day 7-18	Day 18-25	Day 1-18	Day 1-25
0	16.7	86.2	88.0	51.1	53.2
100	19.4	86.3	95.5	52.1	55.3
250	17.7	85.3	93.0	51.5	54.5
750	17.4	85.8	91.5	51.6	54.5
SEM	0.5	1.7	1.24	1.0	0.9
p-value	0.247	0.997	0.189	0.990	0.869

<sup>1</sup>Algamune ZPC (ABG), Algal Scientific, Plymouth, MI, USA

compared to the challenged control group although overall lesion development was extremely low, which may have prohibited the separation between ABG and control treatments. While the challenged control group BW was not able to separate from the non-challenge control birds, the presence of *Eimeria* was sufficient to influence FCR, indicating significant stress associated with challenge. Feeding YBG at 0.1% has been reported to reduce upper and middle intestinal lesion scores by Cox *et al.* (2010b), although the same study found no differences in lower intestinal lesion scores in agreement with the current study. Lesions in the lower intestinal section and oocysts in the feces were detected in the non-challenged control group. Horizontal transfer of disease organism did take place in one replicate pen as treatments were randomly distributed in block design in stacked battery cages to allow for comparison of performance parameters to non-challenge control broilers. The current study also found no significant impact in oocyst output per gram of feces 6, 7, 8, or 9 d post challenge when broilers were supplemented with ABG, although numerical reductions were observed. However, supplementation of ABG at 250 g/MT significantly reduced the 4 d post challenge average of oocyst output compared to supplementation of ABG at 750 g/MT, though no impact by ABG was observed compared to the challenged control group. The effects of ABG on oocyst output is supported by Shanmugasundaram *et al.* (2013), who reported no effect on fecal oocyst output 5 and 12 d post *Eimeria* challenge when feeding whole yeast cell product at 0.1 and 0.2%. A reduction in fecal oocyst counts was reported by Shanmugasundaram *et al.* (2013) 7 d post challenge, indicating  $\beta$ -glucan can reduce oocyst output, but that source and dosage may play a role in the timing of oocyst reduction.

The third and final experiment evaluated the effects of ABG during a Newcastle vaccination program. Chicks were vaccinated at d of hatch and then fed ABG or a control in a starter diet to d 18. A vaccine boost was administered on d 18 and ABG was removed from the diet. Inclusion of ABG had no impact on performance parameters, similar to Experiments 1 and 2, although this may be associated with the lower number of replicates, total birds placed and even lower level of

environmental stress in cages compared to pens. When measured at d 25, inclusion of ABG at 250 g/MT increased ( $p < 0.05$ ) antibody titer levels compared to the control diet. The increase in antibody titers, even after a 7 d period of feeding without ABG, suggests that  $\beta$ -glucan primes the specific immune system and can have an effect for at least one week past feeding. These observations are supported by An *et al.* (2008) who also observed an increase in d 35 Newcastle virus antibody titers when feeding YBG at 0.05 and 0.1%, although not at 0.025%, suggesting dosage level of  $\beta$ -glucan impacted the increase in antibody titers.

These data confirm that an algal derived  $\beta$ -1,3-glucan can improve early performance parameters in a non-challenge setting, prevent growth performance reductions in *Eimeria* challenged broilers and increase Newcastle virus specific antibody titers. These data also confirm previous conclusions with YBG products that dosage level of  $\beta$ -glucan products at each dietary stage can play an important role in performance effects in both non-challenged and challenged settings. Results of these experiments confirm the statements by Chae *et al.* (2006) that variable effects of  $\beta$ -glucan on performance occur in a challenge vs. non-challenge setting. Inclusion rates and dosage of ABG to maximize observational effects seem to differ from YBG, which can likely be attributed to the difference in  $\beta$ -glucan structure and bioavailability. Our work suggests that lower doses of ABG compared to those used with YBG, between 100 and 250 g/MT, may be most beneficial. Future work should continue to investigate the effectiveness of ABG in broilers, with a focus on dosage in each dietary stage to maximize overall performance benefits.

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