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PERFORMANCE AND CARCASS TRAITS OF BROILER CHICKENS FED DIETS SUPPLEMENTED WITH AGED GARLIC EXTRACTOR HUMATES WITH PROBIOTICS AT DIFFERENT GROWTH STAGES

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ABSTRACT

This study was conducted to determine the effects of supplementing aged garlic extract (AGE) and humates with probiotics (HWP) at varying growth stages on the performance and carcass traits of broilers. The experimental study used 4x4 factorial in Completely Randomized Design, where the type of feed supplement as factor A (negative control, with AGE, with HWP, and positive control or antibacterial) and growth stage as factor B (brooder stage/0-12 d, starter stage/13-21 d, grower stage/22-28 d, and brooder to grower stages/0-28 d). A total of 320 male day-old Cobb broiler chicks were randomly distributed to 16 groups with four replicates. The supplements were given at 1g/kg inclusion rate. Significant interaction effects were observed between feed supplements and growth stages in terms of bodyweight, ADG and FCE. Results indicated that supplementation with either aged garlic extract (AGE) or humates with probiotics (HWP) was best done continuously at 0-28 d of age (brooder to grower stages). Supplementation with AGE or HWP at any stage of growth had no significant effect on carcass traits.

Key Words: aged garlic extract (AGE), broiler, humates, probiotics

INTRODUCTION

Intensive animal production has been achieved through the use of antibiotics and hormones. However, their residues in animal tissues have caused negative impacts on human health such as antimicrobial resistance and metabolic disorders. Chicken meat, one of the main sources of animal protein in the Filipino diet, is also conventionally produced with the use of said products. With the increasing demand for safer and healthier meat, chicken producers need to find the best alternatives to antibiotics and hormones for growth promotion and disease control.

There is a vast wealth of alternative growth promoters continuously being developed and discovered around the world, especially in countries where antibiotics have long been banned in animal production. Scientific testing of alternative products in the Philippine setting can help farmers in their search for alternative growth promotants that will give desired results in performance and will ensure profitability.

Aged garlic extract (AGE) contains thiosulfinate-cysteine compounds which are organosulfur bioactive substances derived only from garlic extract that has undergone a special fermentation process. S-allyl cysteine (SAC), S-methyl cysteine (SMC) and S-allyl mercaptocysteine (SAMC) are among the important thiosulfinate-cysteine compounds (Hossain *et al.*, 2014). They are considered to be amino-acid precursors, thus helping in the synthesis of proteins, enzymes, vitamins, and minerals in the body. Their activities also include anti-microbial, anti-oxidant, immuno-modulatory, and metabolism-, production-,

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and reproduction-enhancing properties (Hossain *et al.*, 2014).

Humates are considered as growth promoters and have been studied in Europe, North America and other parts of the world. They are derived from decayed organic matter in the soil, and may contain humus, humic acid, fulvic acid, ulmic acid and trace minerals in varying amounts. Several studies in food animals, including chickens, suggest that they improve growth, productivity and health (Mirnawati *et al.*, 2013; Ozturk and Coskun, 2006; Taklimi *et al.*, 2012a; Taklimi *et al.*, 2012b). Some of the purported effects of humate substances include improved metabolism of proteins and carbohydrates, adsorption and excretion of heavy metals, stimulation of natural immunity, and inhibition of pathogenic microorganisms (Islam *et al.*, 2005; Kucukersan *et al.*, 2005).

Probiotics include beneficial microorganisms that promote competitive exclusion of undesirable bacterial strains in the gut that cause gastrointestinal disorders or negatively affect bird performance or food safety (Amer *et al.*, 2013; Li *et al.*, 2014). They stimulate the immune system against pathogenic bacteria or exert an antagonistic action against pathogenic microorganisms through competition for nutrients and attachment sites, and/or production of bacteriocins or antibacterial substances against other bacterial species (Asha and Gayathri, 2012; Kizerwetter-Swida and Binek, 2009; Kral *et al.*, 2012). Among the most commonly used probiotics are strains of lactic acid bacteria, such as *Lactobacillus spp.,* which are normal components of healthy intestinal microflora.

This research aimed to determine the effects of dietary supplementation, at varying growth stages, of aged garlic extract (AGE) and humates with probiotics (HWP) on the performance and carcass traits of broilers. Specifically, the study aimed to: 1) determine the body weight, average daily gain, and feed conversion efficiency, and 2) evaluate the dressing percentage, abdominal fat percentage and relative organ weights (liver, spleen, and heart).

MATERIALS AND METHODS

This experimental study used 4x4 factorial in a Completely Randomized Design (CRD), using feed supplement and growth stage as the factors. A total of 320 male day-old chicks of Cobb strain, having a uniform average initial weight of 55 g, were distributed to 16 groups with four replicates each, and with five birds per replicate. For Factor A (feed supplement), the treatments were: A1) NC – basal diet; A2) with AGE – basal diet + aged garlic extract (AGE) product (1 g/kg feed); A3) with HWP – basal diet + probiotics and humic-fulvic acids (PHFA) product (1 g/kg feed); and A4) with ABP – basal diet + antibacterial product: tiamulin hydrogen fumarate and doxycycline hydrochloride (1 g/kg feed). For factor B (growth stage), the treatments were: B1) Brooder stage – 0 to 12 days; B2) Starter stage – 13 to 21 days; B3) Grower stage – 22 to 28 days; and B4) Brooder to Grower stages – 0 to 28 days. During the finisher stage (29-35 days), the birds received a uniform diet.

The experimental housing had a slatted floor made of plastic crates, and a monitor-type galvanized iron roof. The floor area was partitioned into 64 cages measuring 160 sq cm (5.25 sq ft) each using coconut lumber, bamboo and chicken wire. During the first

12 days, only 16 of the 64 units were used, but each of the 16 units was subdivided into four compartments. Brooder lamps and rice hull on old newspapers were provided in each pen. On day 13, the brooder was removed and the chicks were transferred to their permanent pens. Each pen was properly labeled for easy identification of each treatment group and replicate. Booster mash was given during the first twelve days, while starter crumble was given at days 13 to 21. Grower crumble was given on day 22 to day 28, and finisher feeds at day 29 to 35. Clean water and feeds were given *ad libitum* and were supplied twice a day, in the morning and in the afternoon. Shifting from one type of feeds to another was done gradually.

Commercial feeds were used for the study. The AGE product contained thiosulfinate-cysteine compounds that resulted from the special fermentation process of garlic with *Leukonostoc spp.* The HWP product contained *Lactobacillus spp.*, humic acid and fulvic acid. The feed supplements were mixed to the feeds prior to feeding at an inclusion rate of 1g/kg feed. The group A2B1 received the aged garlic extract (AGE) supplement during the brooder stage (0-12 days), A2B2 on starter stage (13-21 days), A2B3 on grower stage (22-28 days), and A2B4 on all three stages (0-28 days). Likewise, A3B1 received the humates with probiotics (HWP) supplement during the brooder stage (0-12 days), A3B2 on starter stage (13-21 days), A3B3 on grower stage (22-28 days), and A3B4 on all three stages (0-28 days). A1B1 to A1B4 (negative control) received no feed supplement at all, and A4B1 to A4B4 (positive control) received tiamulin-doxycycline feed additive during the first five days of life only. All birds had the same diet at 29-35 days of age.

After an overnight fasting, birds were weighed individually at 12, 21, 28 and 35 days of age. Average daily gain was calculated based on data on weight and days of production (also at day 12, 21, 28 and 35), while feed conversion efficiency was measured based on feed consumption of the birds divided by their average daily gain.

At day 35, four representative birds (1 per replicate) with body weight close to the group average were selected from each group and slaughtered for carcass characteristics. Birds were fasted for 12 hours then individually weighed, slaughtered, feathered, and eviscerated. Weights of carcass were recorded, and the dressing percentage was calculated as carcass weight divided by live body weight. The weights of the abdominal fat and edible visceral organs such as the liver, spleen, and heart were also recorded and calculated as percentage of carcass weight.

Results on performance data and carcass traits were analyzed using Analysis of Variance (ANOVA) of the Completely Randomized Design (CRD). Treatment means were compared using Duncan's Multiple Range Test (DMRT). The Statistical Tool for Agricultural Research (STAR), 2015 Version was used in the analysis.

RESULTS AND DISCUSSION

Results on performance at day 35 indicate that significant interaction effects were observed between diet and growth stages in terms of bodyweight, ADG and FCE (Table 1). Results generally indicated that supplementation with either aged garlic extract (AGE) or humates with probiotics (HWP) was best done continuously at 0-28 d of age

(brooder to grower stages). The best performance indices were obtained by the group supplemented with AGE at 0-28 d. These results concur with the studies of Hossain *et al.* (2014) wherein aged garlic extract supplemented at 1% and 2% produced significantly higher bodyweight, ADG and FCE compared to unsupplemented chickens. There are thiosulfinate-cysteine compounds in AGE that act as amino acid precursors, thus helping in the synthesis of proteins, enzymes, vitamins and minerals, that can produce an overall effect of improved performance. This is also in agreement with Lan *et al.* (2002) who reported that for supplements to be best utilized, they need to be given continuously for 2-7 weeks.

The results also implied that chickens supplemented with humates with probiotics (HWP), regardless of stage of growth, had better performance compared with the control. For humates, several studies on broiler showed increased ADG (Taklimi *et al.*, 2012a; Mirnawati *et al.*, 2013; Ozturk and Coskun, 2006) and FCE (Taklimi *et al.*, 2012a; Mirnawati *et al.*, 2013; Ozturk and Coskun, 2006), improved carcass percentage (Mirnawati *et al.*, 2013), and 4) increased SI villus height (Taklimi *et al.*, 2012b). Continuous supplementation of the broilers throughout the rearing period (brooder to grower-finisher stage, or 0-6

Table 1. Performance at 35th day of production of broilers fed diets supplemented with aged garlic extract or humates with probiotics at different growth stages.

Factor A	Factor B – Growth Stage of Birds			
Feed Supplement	B1 - Brooder (0 – 12 d)	B2 - Starter (13 – 21 d)	B3 - Grower (22 to 28 d)	B4 –Brooder to Grower (0 – 28 d)
Bodyweight (g)				
A1 – NC	1343.00 ^{g}	1342.50 ^{g}	1390.25 ^{fg}	1382.50 ^{fg}
A2 – AGE	1600.00 ^{bc}	1466.50 ^{de}	1434.75 ^{ef}	1707.50 ^{a}
A3 – HWP	1532.50 ^{cd}	1428.25 ^{ef}	1418.75 ^{ef}	1613.50 ^{b}
A4 – ABP	1602.25 ^{bc}	1589.00 ^{bc}	1603.25 ^{bc}	1614.25 ^{b}
Average Daily Gain (g)				
A1 – NC	36.80 ^{g}	36.78 ^{g}	38.15 ^{fg}	37.93 ^{fg}
A2 – AGE	44.14 ^{bc}	40.33 ^{de}	39.42 ^{ef}	47.22 ^{a}
A3 – HWP	42.21 ^{cd}	39.02 ^{ef}	38.96 ^{ef}	44.53 ^{b}
A4 – ABP	44.21 ^{bc}	43.83 ^{bc}	44.24 ^{b}	44.55 ^{b}
Feed Conversion Efficiency				
A1 – NC	1.58 ^{f}	1.58 ^{f}	1.52 ^{def}	1.53 ^{ef}
A2 – AGE	1.31 ^{b}	1.44 ^{cd}	1.47 ^{de}	1.23 ^{a}
A3 – HWP	1.38 ^{bc}	1.48 ^{de}	1.49 ^{de}	1.30 ^{ab}
A4 – ABP	1.31 ^{b}	1.33 ^{b}	1.31 ^{b}	1.30 ^{ab}

NC – negative control group (basal diet); **AGE** – basal diet + aged garlic extract (AGE) product (1 g/kg feed);**HWP** – basal diet + probiotics and humic-fulvic acids (PHFA) product (1g/kg feed); **ABP** – basal diet + tiamulin and doxycycline (1 g/kg feed). Data within the same 4x4 box with different superscripts are significantly different ($P<0.05$).

weeks age) was observed in these studies. However, it is difficult to compare the actual effects of humic acid preparations due to different sources and nature, as well as because rearing of animals in different regions of the world varies. Biological effects of humates depend on specifications (Islam *et al.*, 2005). For dietary probiotics, several studies reported improved performance on poultry. In the study of Král *et al.* (2012), probiotics containing *Lactobacillus spp.* had significant effect on the body weight of Cobb broilers at 5 weeks of age. Similarly, Taklimi *et al.* (2012a) and Amer *et al.* (2013) reported significant increases in weight gain, feed intake and feed efficiency in broilers continuously given probiotics until 6 weeks of age. Shabani *et al.* (2012) and Li *et al.* (2014) also reported improved weekly (21, 28, 35 and 42 days) data on ADG and FCR to broilers supplemented with probiotics continuously from brooding until 42 days old.

Supplementation with either AGE or HWP during the brooder stage was also shown to be more advantageous compared when done during the starter and grower stages. This might be because brooder stage is the most critical in the bird's life since they cannot yet thermoregulate their body, predisposing them to stress and lowered body resistance. The brooding period also highly influences the growth of the chicks. Broilers can increase their bodyweight up to four times during the brooding period. Chicks with poor growth during brooding will not achieve their full genetic potential (Lambio, 2010).

The results on carcass traits revealed no significant differences ($P>0.05$) on the carcass percentage and abdominal fat percentage among types of supplements and among growth stages (Table 2). These results agree with Kocabağli *et al.* (2002) and Ozturk and Coskun (2006) since they reported no difference in carcass yield and abdominal fat percentage due to feeding of humates. Researches on probiotics and garlic supplementation on broiler which were reviewed in this study did not examine their effects on carcass and abdominal fat percentages.

The results on relative organ weights (liver, spleen and heart) also revealed no significant differences among treatments ($P>0.05$). These findings agree with those of Ozturk and Coskun (2006), Rath *et al.* (2006) and Van Rensburg *et al.* (2006) who observed that supplementation with humates had no effect on the relative organ weights, interpreting that the humate products used had no adverse effect on the chickens' health. Reviewed researches on supplementation with probiotics and garlic did not also study their effects on relative organ weights.

Based on the results of this study, supplementation with AGE (aged garlic extract) or HWP (humates with probiotics) improved the bodyweight, ADG and FCE of broilers when given continuously at 0-28 d of age. However, supplementation with either product at any stage of growth had no effect on carcass traits.

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Table 2. Carcass traits of broiler chickens supplemented with aged garlic extract or humates with probiotics at different growth stages after 35 days of production.

Factor A	Factor B – Growth Stage of Birds			
– Feed Supplement	B1 - Brooder (0 – 12 d)	B2 - Starter (13 – 21 d)	B3 - Grower (22 to 28 d)	B4 –Brooder to Grower (0 – 28 d)
Carcass percentage (%)				
A1 – NC	71.62	73.28	73.67	73.97
A2 – AGE	73.79	72.20	73.42	73.77
	72.10	72.87	73.13	74.39
A3 – HWP	74.82	74.51	73.77	74.56
A4 – ABP	71.62	73.28	73.67	73.97
Abdominal fat percentage (%)				
A1 – NC	0.75	0.67	0.75	0.61
A2 – AGE	0.80	0.75	0.73	0.71
A3 – HWP	0.70	0.72	0.81	0.69
A4 – ABP	0.71	0.72	0.69	0.64
Liver percentage (%)				
A1 – NC	3.79	3.76	3.65	3.06
A2 – AGE	3.01	3.70	3.31	3.33
A3 – HWP	3.04	3.30	3.54	3.58
A4 – ABP	3.21	3.49	3.17	3.16
Spleen percentage (%)				
A1 – NC	0.22	0.14	0.17	0.16
A2 – AGE	0.18	0.17	0.14	0.22
A3 – HWP	0.17	0.18	0.14	0.16
A4 – ABP	0.18	0.19	0.17	0.14
Heart percentage (%)				
A1 – NC	0.81	0.89	0.75	0.76
A2 – AGE	0.78	0.81	0.76	0.80
A3 – HWP	0.81	0.84	0.78	0.66
A4 – ABP	0.73	0.75	0.85	0.86

NC – negative control group (basal diet); **HWP –** basal diet + probiotics and humic-fulvic acids (PHFA) product (1g/kg feed); **AGE –** basal diet + aged garlic extract (AGE) product (1 g/kg feed); **ABP –** basal diet + tiamulin and doxycycline (1 g/kg).

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