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# PERFORMANCE AND MEAT FATTY ACID PROFILE IN MIXED SEX BROILERS FED DIET SUPPLEMENTED WITH FERMENTED MEDICINAL PLANT COMBINATIONS

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## ABSTRACT

Efficacy of fermented *Salicornia herbacea* L. and *Houttuynia cordata* Thunb. (FSH) on performance and meat quality in broilers was assessed. Following completely randomized design 240 day old mixed sex Ross 308 broiler chicks were randomly allocated to five treatments with six replications with eight birds in each. Dietary treatments were: control (corn-soybean meal based basal diet); ABT (Basal diet + 0.05% oxytetracycline); and FSH1 (Basal diet + 0.5% FSH), FSH2 (Basal diet + 1.0% FSH) and FSH3 (Basal diet + 2.0% FSH). Dietary supplementation had no significant negative impact on performance and relative organ weight; however, mortality and abdominal fat was substantially reduced after FSH and ABT supplemented birds relative to control ($P<0.05$). Breast muscle crude protein content was higher whereas crude fat content was lower in FSH2 group in comparison to control ($P<0.05$). A substantial diminution was observed in the average meat TBARS value of FSH and ABT group compared to control ($P<0.05$). Among the breast meat fatty acids, sum of SFA content was down trended whereas sum of PUFA, omega-3 and omega-6 PUFA content was elevated in FSH2 compared to control ($P<0.05$). Overall, present findings indicated that FSH supplementation was beneficial regarding nutritional aspects of broiler meat where FSH2 found suitable.

**Key words:** Fermented *Salicornia herbacea* L. and *Houttuynia cordata* Thunb., Growth Performance, Meat fatty acids, Broilers.

## INTRODUCTION

Several alternative feed additives have recently been developed and used after worldwide concern against the antibiotic growth promoters, among them, natural substances and medicinal plants with excellent physiological activity are currently receiving attention from researchers (Hernandez *et al*., 2004). In the world there are around 297,326 species of plants among them many species have a wide range medicinal potentiality both in the human and animal kingdom (*Source:* The International Union for Conservation of Nature and Natural Resources (IUCN) 2007 Red List). Phytogenic feed additives are prepared from such types of medicinal plants derivatives which are used in animal feed to improve performance of agricultural livestock. These compounds are also called phytobiotics or botanicals, which helps to improve the productivity of livestock through amelioration of feed properties, and improve the quality of food derived from those animals (Windisch *et al*., 2008). *Salicornia herbacea* L. is a medicinal plant commonly known in Korea as humcho, tungtungmadi or glasswort under family Chenopodiaceae grown in the salty marshes and sea beaches and salty in taste (Im *et al*., 2003). It is an annual herb used in coastal areas and utilized as seasoned vegetables and fermented food; and as traditional medicine for diabetes, obesity, cancer and intestinal problems (Bang *et al*., 2002; Chung *et al*., 2005; Kim *et al*., 2011). The active component of *Salicornia herbacea* L. is tungtungmadic acid which is chlorogenic derivative; while chlorogenic acid is the ester of caffeic acid with quinic acid which mainly exhibits antioxidative functions (Chung *et al*., 2005; Bonita *et al*., 2007). It has been shown to have potential use as antioxidant, anti-inflammatory, immunomodulator, anti-cancerous and anti-heperglycemic (Lee *et al*., 2006; Kim *et al*., 2009) due to presence of several other bioactive components like sitosterol, uracil, stigmasterol, quercetin, caffeic acid, ferulic acid, procatechuric acid and isorhamnetic (Lee *et al*., 2005; Oh *et al*., 2007). In addition, *Houttuynia cordata* Thunb. (Family: *Sauruaceae*) is a well-known traditional Chinese medicinal material widely used in China, Japan and Korea and commonly known as eusungcho, fishmint or heartleaf. It possesses a variety of pharmacological functions including anti-bacterial, anti-microbial, anti-inflammatory and immunomodulatory activities (Sun *et al*., 2004; Meng *et al*., 2009), antioxidative, anti-cancerous and anti-mutagenic functions (Chang *et al*., 2001; Chen *et al*., 2003). The chemical constituents present in the *H. cordata* are houttuynum, decanoyl acetaldehyde, myrcene, undecanone, rutin, hyperoside, quercitrin, quercetin, acetic acid and pyridinamine (Liang *et al*., 2005; Lu *et al*., 2006; Xu *et al*., 2006; Ch *et al*., 2007).

It is widely accepted that, probiotics are beneficial microorganisms which maintain intestinal microbial balance and facilitate gut mucosa development, improving digestion and absorption rate; supplementation with 0.1 and 0.2% mixed probiotics containing *Lactobacillus acidophilus, Bacillus subtilis* and *Saccharomyces cerevisiae* improves performance and immunity in broilers (Jin *et al*., 1998; Kim *et al*., 2002). Medicinal plants along with probiotics also found effective in previous studies for improvement of performance and immunity in broilers (Hossain *et al*., 2012; Bostami *et al*., 2015). Including production and immunity, meat TBARS value is important parameters because it is the indicator of lipid oxidation inside muscle during storage period, if TBARS value is increased, quality of meat is decreased and considered that meat is being oxidized and spoiled through adverse changes in flavor, color, texture and nutritive value (Ura *et al*., 2008). In addition, animal nutritionists are searching alternative sources to manipulate meat fatty acid composition with increasing stability of storage meat for health conscious meat consumers through dietary supplementation of antioxidants (Hargis and Van Elswyk, 1993; Lopez-Ferrer *et al*., 2001). Medicinal plants are natural antioxidants, where fermented medicinal plants in single state with probiotics are ongoing research and tested beneficial for broilers performance and meat quality improvement (Cao *et al*., 2012; Hossain *et al*., 2012). Another option for animal scientists could be mixing of different plant materials and fermentation with single or multi-microbe probiotics for getting more efficacy. Kim *et al*. (2016) reported that combination of natural plant materials along with multi-microbe probiotics are helpful for improvement of performance, immunity and microbial balance; however, combination of medicinal plant materials with beneficial microorganism are still study interest for poultry nutritionist due to large diversity of these plant materials worldwide. *Salicornia herbacea* and *Houttuynia cordata* along with probiotics separately was tested not beneficial for the performance of broilers (Sarker *et al*., 2010; Sarker *et al*., 2011); however, for further detail analysis and for testing the efficacy on the functionality of meat, present experiment was set. Therefore, present study was conducted to assess the possibility of using *Salicornia herbacea* + *Houttuynia cordata* together with multi-microbe probiotics as broiler feed additives to obtain better efficacy on the aspects of meat fatty acid content as focus of consumer health aspects along with other parameters. To accomplish this, we evaluated effect of fermented *Salicornia herbacea* + *Houttuynia cordata* with multi-microbe probiotics (*Lactobacillus* spp., *Bacillus* spp. and *Saccharomyces* spp.) on growth performance, mortality, immunity, internal organ development, meat composition, meat oxidative stability and meat fatty acid profile of broilers.

## MATERIALS AND METHODS

***Birds, Diet and Experimental Design****:* Following completely randomized design a total of 240 day old mixed sex Ross 308 broiler chicks were allocated into five treatments with six replications of 8 birds per replications. The size of the cage where birds were reared was 100 centimeter long, 80 centimeter wide and 40 centimeter high and provided 1000cm$^2$/bird of floor space. The wire-floor caged-broiler house was arranged to provide sufficient favorable environment with 24 hours of daily light, well ventilation and closed system. Room temperature was maintained at 33°C through a supplemental heating system during first week, after that, temperature was gradually reduced at the rate of 3°C for every week up to 24ºC and then continue the similar temperature until end of experiment. Dietary treatments were: control (corn-soybean meal based basal diet); ABT (Basal diet + 0.05% oxytetracycline); and FSH1 (Basal diet + 0.5% FSH), FSH2 (Basal diet + 1.0% FSH) and FSH3 (Basal diet + 2.0% FSH). Feed and drinking water were provided *ad libitum.*

Experimental diets were divided into two phases, starter (0–3 weeks of age) and finisher (4–5 weeks of age). The diets were formulated according to NRC (1994). The fermented probiotics were prepared with Hamcho (*Salicornia herbacea)* and Eosungcho (*Houttuynia cordata)* along with multi-microbe probiotics (*Bacilli*, *Lactobacilli* and *Saccharomyces*). The chemical compositions of the diets were analyzed by following the detail guidelines of AOAC (2000) where the feed ingredients, chemical compositions were shown in Table 1.

**Fermentation of *Salicornia herbacea* and *Houttuynia cordata* (FSH):** After collection *Salicornia herbacea* and *Houttuynia cordata* from Southern seashore area of Republic of Korea was dried and finely grinded for proper mixing. Fermented *Salicornia herbacea* and *Houttuynia cordata* (FSH) was prepared by mixing 30% *Salicornia herbacea*, 30% *Houttuynia cordata* and 40% defatted rice bran (DRB). After taking weight and proper mixing beneficial microbes (*Lactobacillus acidophilus* (KCTC 3111), *Lactobacillus plantarum* (KCTC 3104), *Bacillus subtilis* (KCTC 3239), *Saccharomyces cerevisiae* (KCTC 7915)) was added and then fermented properly in two stage. Lactic acid bacteria and *Saccharomyces* spp. were mixed with the solid media containing the mixer and fermented at 40°C with rotation of 5 hours under anaerobic conditions and then for 3 hours under aerobic conditions for 48 hours in a commercial fermenter (W-1000; Wonbalhyo Industry Co., Incheon, South Korea). After that, *Bacillus* spp. was added with the fermented medium and again fermented for 72 hours with 5 hours and 3 hours standing and shaking, respectively to ensure proper fermentation and

mixing. Following fermentation and mixing, fermented material was dried in oven of forced air at 32 ºC for 2 days (Doori TEC, Doori TEC, FA, Co., Ltd.). The fermented product FSH was prepared and stored in air-tight bag for further utilization as feed additives. To determine the microbial concentration, the FSH sample of 1 g was taken and diluted with sterilized distilled water (10ml) in room temperature condition. After around 10 minutes, dilution of 10-fold was prepared with NaCl (0.85%) solution and then cultured in specific agar medium. The culture plates were then incubated for 24 to 48 hours at 37ºC temperature. Following incubation the plates were taken out and number of colonies were counted for each. The microbial strain content, population and chemical composition are shown in Table 2.

**Performance, Mortality and Immunity of Broilers:** Body weights were measured weekly from day 1 to the final day of the experiment. Feed intake was determined by measuring the feed residue on a weekly basis since the beginning of the experiment. Feed conversion ratio was obtained by dividing the feed intake to body weight gain. Dead birds were monitored and recorded on daily basis. At the end of the experimental period, three birds were randomly selected from each replicated pen in order to perform the immunological analyses. From selected birds' brachial vein blood samples were collected carefully, after which samples were quickly transferred into centrifuge tubes and care was taken to avoid any contamination or disturbance. Then the blood samples were centrifuged for 15 min at 1610× g in a cold chamber with temperature 4°C. Then the sera were carefully removed to the plastic vials and then stored at −20°C until the immunoglobulin (Ig) analysis was performed. Following the manufacturer's instructions concentrations of serum IgG, IgM and IgA were assayed by using the Chicken IgG (Cat. No. E30-104), IgM (Cat. No. E10-101) and IgA (Cat. No. E30-103) ELISA Quantitation Kits (Bethyl Laboratories Inc., Montgomery, TX, USA). Where each sample was run in duplicate. The absorbance of each well was then measured within 30 min by using micro-plate auto-reader (Thermo Lab Systems, Helsinki, Finland) at 450 nm. Finally serum immunoglobulin value was expressed as mg/ml.

**Measurement of Relative Organ Weight:** Relative organ development was measured by weighing the organs after slaughtering and separating the individual organs at the end of the experimental period. Relative organ weight was measured based on the final body weight before slaughter of the bird.

**Determination of Meat Composition:** At the end of the experimental period, 2 chickens from each replication were slaughtered and samples were collected from the equal mixing of breast and thigh muscle. The chemical composition of the broiler meat (equally mixed breast and thigh meat) was then determined according to the AOAC (2000) (Official methods of analysis of the association of Official analytical chemists, 17th ed., Gaithersburg, MD, USA). Determination of trace mineral contents were done using an Atomic Absorption Spectrophotometer (AA-6200, Korea). In brief, 2.5 g of sample was taken in a crucible and then dried at 105 ºC. After drying the sample was burned by placing in a muffle furnace where temperature was set at 550 ºC until it's color became grayish white, and then the crucible was allowed to cool by placing in the desiccator. After that, 1–2 drops of distilled water (DW) were added to the crucible including 10 ml of primary reagent (HCl: DW = 1:1). The crucible was then placed on a hot plate stirrer for evaporation, after which 10 ml of secondary solution (HCl:DW = 1:3) was added. After evaporation the 100 ml of sample was filtered with distilled water through using Whatman No. 6 filter paper. After diluting commercial standard solutions (1000 ppm) of Ca and Fe by 0.5, 1.0 and 2.0 ppm and magnesium (Mg) and sodium (Na) by 0.1, 0.2 and 0.4 ppm, the absorbance levels were then measured by comparison with the calibration curve and then the results expressed as mg/100 g of meat.

**Determination of Lipid Oxidation:** Lipid oxidation of broiler breast and thigh mix (equal amount) meat was determined according to the method described by Witte *et al*. (1970). A new extraction method for determining 2-thiobarbituric acid values of pork and beef during storage. Journal of food Science, 35(5), 582-585) with slight modification. For this analysis, 10 g of breast and thigh meat mixture (equal amount of each) were blended at full speed for 1.5 min in a chilled stainless watering blender cup with 25 ml of extracting solution containing 20% trichloroacetic acid (TCA) in 2M phosphoric acid. The resulting sediment was transferred quantitatively to a 50ml volumetric flask with 20ml distilled water, diluted by shaking and homogenized. After which a 25ml aliquot was filtered through Whatman No.1 filter paper, and then 5ml filtrate was transferred to a test tube and 5ml of 2-thiobarbituric acid (0.005 M in DW) was added. The solution was then subsequently shaken in a water bath at 80°C for 30 min. After cooling, color development was measured at 530 nm in a Libra S22 spectrophotometer (Biochrom Ltd., Cambridge, England). Thiobarbituric acid reactive substances (TBARS) values were expressed as micromoles of malondialdehyde (MDA) per hundred grams of meat.

**Determination of Fatty Acids Profile of Broiler Meat:** The fatty acids compositions of breast and thigh meat mix (equal amount) were determined by a direct method for fatty acid methyl ester (FAME) synthesis using little bit modification of the method described by O'Fallon *et al*. (2007) (O'fallon, J. V., J.R. Busboom, M.L. Nelson, and C.T. Gaskins (2007). A direct method for fatty acid

methyl ester synthesis: application to wet meat tissues, oils, and feedstuffs. *Journal of Animal Science*, *85*(6), 1511-1521.). In a short, 1 g of minced meat sample was placed into a 15 ml Falcon tube, after which 0.7 ml of 10 N KOH in water and 6.3 ml of methanol were added. Then the tube was incubated in a 55°C water bath for 1.5 h with vigorous hand-shaking for 10 s every 30 min to properly permeate, dissolve and hydrolyze the sample. Following cooling to below room temperature in a cold tap water bath, 0.58 ml of 24 N $H_2SO_4$ in water was added. Then the tube was mixed by inversion, after that $K_2SO_4$ was precipitated. The sample with the precipitate was incubated again in a 55°C water bath for 1.5 h with vigorous hand-shaking for 10 s every 30 min. After the synthesis of FAME, the tube was cooled in a cold water bath. Following that, 3 ml of hexane were added and the tube was vortexed for 5 min on a multitube vortexer. The tube was subsequently centrifuged for 5 min at 3000 × g (HANIL, Combi-514R, Korea), after which the top (hexane) layer containing the FAME was dehydrated through the anhydrous $Na_2SO_4$. Then the extracted and dehydrated hexane was concentrated to 1.5 ml and placed into a GC vial for analysis.

The composition of fatty acid of the FAME was determined using a Gas Chromatograph (Agilent, 7890A series, USA) equipped with a flame ionization detector and a Hewlett Packard HP-88 capillary column (J&W Scientific, USA) with a length of 60 m, a 0.52 mm internal diameter and a 0.20 µm polyethylene glycol-film thickness. Samples were injected using an auto-sampler (Agilent Technologies 7693, USA). Where the initial oven temperature was 125°C, which was held for 1 min, then increased to 145°C at 10°C/min, where it was held for 26 min, then further increased to 220°C at 2°C/min, where it was held for 2 min. Purified air and hydrogen were applied at a flow rate of 400 ml/min and 40 ml/min as the carrier gas, whereas helium was applied at 40 ml/min as the makeup gas. Where both the injector and detector temperature were set at 260°C and the split ratio was 30:1. Fatty acids were identified by comparison of their retention times with those of a standard FAME mixture (Supelco™ 37 Component FAME Mix, 10 mg/ml in $CH_2Cl_2$. Catalog Number 47885-U. Supelco, Bellefonte, PA 16823-0048, USA). The ratio and sum useful for evaluating the nutritional value and healthiness of the fatty acid profile were also determined; specifically, the sum of saturated fatty acids (ΣSFA), the monounsaturated fatty acids (ΣMUFA), the polyunsaturated fatty acids (ΣPUFA), the n-3 fatty acids (Σn-3), the n-6 fatty acids (Σn-6) and the ratios of MUFA to SFA (MUFA/SFA), PUFA to SFA (PUFA/SFA), n-6 to n-3 (n-3/n-6) and hypocholesterolaemic to hypercholesterolaemic (H/H) fatty acid ratio. The H/H ratio was determined as follows:

H/H = [(sum of C18:1 cis-9, C18:2 n-6, C20:4n-6, C18:3 n-3, C20:3n-6, C20:5 n-3, and C22:6 n-3)/(sum of C14:0 and C16:0)] (Santos-Silva *et al*., 2002).

**Statistical Analysis:** All data were analyzed using the General Linear Models (GLM) function of the Statistical Analysis System (SAS, 2003, Version 9.1, SAS Institute, Cary, NC, USA). Duncan's multiple range tests (DMRT) (1955) were used to identify significant differences between treatment means. Differences were considered significant at $P \leq 0.05$. Each cage was considered as the experimental unit for growth performance parameters (BW, ADG, ADFI and FCR), whereas an individual bird served as the experimental unit for blood, meat composition, meat fatty acid profile and meat oxidative stability.

## RESULTS AND DISCUSSION

**Growth Performance, Mortality and Immunity:** As shown in Table 3, ADG, ADFI and FCR of broiler chicks did not differ significantly among the dietary treatment groups. Thus, it was apparent from the present study that, owing to the addition of fermented *Salicornia herbacea* and *Houttuynia cordata* with multi-microbe probiotics (FSH) had no significant negative impact on the performance of broilers. Supporting to our study, Sarker *et al*. (2009) found no significant difference in response to the addition of 0.5% and 1.0% fermented green tea probiotics. Usually mixed probiotics are able to improve the feed efficiency in broilers; for example Kim *et al*. (2002) reported that 0.1 to 0.5% probiotics with *Lactobacillus* sp., *Bacillus* sp. and *Saccharmyces spp.* effectively improved feed efficiency in broilers. While *Salicornia* has the capability to reduce the body weight in dose dependent manner in mice study (Park *et al*., 2006). Thus for the present result it was postulated that the growth performance of broilers were not impacted due to negative and positive impact of plant and probiotics derived biochemical constituents and their interactions.

Dietary supplementation of FSH and ABT significantly reduced the mortality of birds relative to control group ($P<0.05$) (Table 3). In addition, the concentration of serum immunoglobulin was found improved in the FSH and ABT supplemented group; where IgG and IgM was significantly elevated in the FSH2 and IgA was significantly elevated in the FSH1 and FSH2 group compared to control ($P<0.05$) (Figure 1). Since the immunity of the birds were improved, the mortality also decrease after dietary treatments of FSH. Plant secondary metabolic compounds (sitosterol, uracil, stigmasterol, quercetin, caffeic acid, ferulic acid, procatechuric acid and isorhamnetic) and the organic compounds from multi-microbe probiotics (Sun *et al*., 2004; Lee *et al*., 2005; Lee *et al*., 2006; Oh *et al*., 2007; Kim *et al*., 2009; Meng *et al*., 2009) might be attributed to the potentially reduction of the mortality of birds in the present study. The presence of phytochemicals can

stimulate the cytokine production (TNF-α and IL-1β) and immunomodulators which consequently induce the activation of macrophages and improve the immunity (Im *et al*., 2003). In addition to that, dietary supplementation of FSH leads higher content of minerals, while it also contain betaine and choline which might help in improving immunity and health status of birds which consequently reduced bird's mortality (Min *et al*., 2002; Shin *et al*., 2002; Cho *et al*., 2008; Lim *et al*., 2013). Betaine is usually presents in the plants and microorganisms which helps to protect the cells through osmolytic activity; and acts as methyle donar in the metabolism of immune organ liver and kidney; and improve vascular risk factor which ultimately improve the health status of the individuals (Craig *et al*., 2004).

**Relative Organ Weight:** As shown in Table 4, no adverse effects were observed in response to the addition of medicinal plants mixed with probiotics in any of the internal organs; however, abdominal fat pad were significantly lower in broilers fed diets supplemented with FSH and ABT fed broilers relative to control ($P<0.05$). Supporting to our study, Cao *et al*. (2012) reported abdominal fat decrement in the fermented *Ginkgo biloba* supplemented group compared to control. Since *Salicornia* and *Houttuynia* composed of flavonoid compounds (which are biologically active), that might exerted lipolytic impact and influenced in the lowering of abdominal fat content in the FSH supplemented group of the present study (Nakagawa *et al*., 2004; Zarrouki *et al*., 2010). Mixture of probiotics organisms can also influenced in the reduction of abdominal fat in case of broilers (Anjum *et al*., 2005; Swain *et al*., 2012).

**Broiler Meat Composition:** The meat composition of broilers is shown in Table 5. The crude protein content was significantly increased while crude fat content was significantly decreased after dietary inoculation of FSH2 compared to control ($P<0.05$). Where there was found no significant differences between FSH and ABT regarding crude protein and crude fat content of meat. The moisture and crude ash in broiler meat did not affect due to the addition of FSH and ABT groups relative to the control ($P>0.05$). Although Anjum *et al*. (2005) reported that, multi-strain probiotic has no significant impact on the meat composition of broilers; in the current study the diminution of crude fat and elevation of crude protein in broiler meat of FSH2 group might be attributed to the effect of fermentation of medicinal plants along with multi-microbe probiotic bacteria. Combination of *Salicornia* and *Houttuynia* might increase the amino acid content (glutamic acid, aspartic acid, lysine, tyrosine, praline and taurine) as well the polyphenolic compounds of the diet which consequently resulted increment of meat crude protein content (Min *et al*., 2002; Wu *et al*., 2003; Jang *et al*., 2008). Consistent to our study regarding mixing of medicinal plant, Jang *et al*. (2008) observed insignificant improvement of crude protein content (21.97% to 25.29%) of raw breast meat in the 0.3% medicinal herb mix (mulberry, Japanese honeysuckle and goldthread) compared to control due to higher polyphenolic compounds. Usually fermentation can improve the nutritional quality of the fermented product specially the protein content, which might increase the availability of amino acids and consequently the crude protein content of broiler meat (Hong *et al*., 2004). On the other hand, it was reported that supplementation of microbial fermented product in broiler diets significantly reduced carcass fat deposition; because gastro-intestinal microbes can impaired the absorption of bile which might affect the crude fat content in the meat of broilers (Santoso *et al*., 2001). In addition, increment of the mineral content (Fe and Mg) might be attributed to the combination of *Salicornia* and *Houttuynia*, where especially *Salicornia* contain higher concentration of salts and minerals (Mg, Ca, K and Fe) (Cho *et al*., 2008; Min *et al*., 2002). The mineral content can play important role on the improvement of the production indices in chicken (Şara *et al*., 2008) which was concurred in the meat quality parameters.

**Lipid Oxidation of Meat:** The TBARS (thiobarbituric acid reactive substances) test is the most widely used method for quantifying lipid oxidation development in meat and meat products. As shown in the Figure 2, meat TBARS content was substantially reduced in the FSH and ABT supplemented groups relative to control ($P<0.05$). The TBARS test determines the amount of malondialdehyde (MDA), a major secondary byproduct of lipid oxidation, in oxidized lipids. Lipid oxidation causes loss of nutritional and sensory values, as well as the formation of potentially toxic compounds that compromise meat quality and reduce shelf life. Result of the current study is consistent with Aksu *et al*. (2005). *Salicornia herbacea* and *Houttuynia cordata* composed of quercetin, caffeic acid, acetic acid, ferulic acid, procatechuric acid, isorhamnetic, myrcene, undecanone, rutin, hyperoside, and pyridinamine (Liang *et al*., 2005; Lee *et al*., 2005; Lu *et al*., 2006; Xu *et al*., 2006; Ch *et al*., 2007; Oh *et al*., 2007). The anti-oxidative properties due to the presence of phytochemicals of *Salicornia herbacea* and *Houttuynia cordata* and multi-microbe probiotics observed in the current study agreed well with those reported in the previous studies (Chen *et al*., 2008; Nuengchamnong *et al*., 2009). Where Nuengchamnong *et al*., (2009) reported that antioxidants activity primarily exhibited due to the presence of chlorogenic acids and its derivatives, catechin and procyanidin. Sarker *et al*. (2009) also reported the reducing trend of lipid oxidation when broilers were fed medicinal plant (green tea) with the similar probiotic combination (*Lactobacilli*, *Bacilli* and *Saccharomyces*) and similar inclusion level (0.5%, 1.0% and 2.0%). Separate use of *Salicornia herbacea* and

*Houttuynia cordata* with probiotics did not show significant impact on meat TBARS value of longer storaged meat (Sarker *et al*., 2010; Sarker *et al*., 2011); however, combination of *Salicornia herbacea* and *Houttuynia cordata* along with multi-microbe probiotic exhibited better result of longer period storage meat and average value of meat TBARS value in the current study. Because the combination of the polyphenolic compounds of natural plants *Salicornia herbacea* and *Houttuynia cordata* might exhibited higher antioxidative activities thereby prolonged the shelf life and quality of meat and meat products (Farag *et al*., 1989; Botsoglou *et al*., 2002); where they produce stable product through reacting with the lipid and hydroxyl radicals (Yanishlieva-Maslarova, 2001).

**Fatty Acid Profile of Meat:** Table 6 shows the effects of FSH and ABT on meat fatty acids profile. The mixture of breast and thigh meat total saturated fatty acid (SFA) content was significantly down trended in the FSH2 relative to the control ($P<0.05$); which was mainly due to lower C16:0, C18:0 and C20:0 contents after FSH2 supplementation. The total MUFA content of FSH and ABT did not differ significantly compared to control; however, FSH2 did differ with FSH1 and FSH3 ($P<0.05$). The total omega-3 and omega-6 fatty acids content was significantly elevated after inoculation of FSH and ABT; where highest value of omega-3 and omega-6 fatty acids was exerted in the FSH1 and FSH2 group, respectively ($P<0.05$). The total PUFA content was significantly increased in the FSH and ABT group ($P<0.05$); where FSH2 and FSH3 also significantly differed ($P<0.05$). The ratio of MUFA and PUFA to SFA was significantly higher in the FSH2 group ($P<0.05$); while the ratio of omega-6 to omega-3 fatty acids significantly diminuted after FSH and ABT supplementation ($P<0.05$). The ratio of hypocholesterolaemic/hpercholeterolamic fatty acids (H/H) was significantly higher in the FSH and ABT supplemented group ($P<0.05$).

Since the lower SFA and higher PUFA content is desirable on the aspects of public health; the significant diminution of SFA and elevation of PUFA with both the omega-3 and omega-6 obtained after FSH supplementation was apparently beneficial for the broiler nutrition. Desirable changes in the fatty acids as well as in the ratio of fatty acids of the current study elucidated that, combination of natural plant materials along with multi-microbe was beneficial approach. The addition of medicinal plants (*Salicornia herbacea* and *Houttuynia cordata*) and multi-microbe probiotics (FSH) may have the potential to improve the fatty acids due to the plant phenolic compounds and organic acids; because they exerted the antioxidant potential and consequently prevent the oxidation of PUFA (Jahan *et al*., 2005; Jang *et al*., 2008). The anti-hyperlipidemic activity due to the phenolic acids of *Salicornia* and *Houttuynia* might be attributed in the improvement of the fatty acid content of broiler meat through the lipid homeostasis and fatty acid synthase enzyme (Park *et al*., 2006; Lin *et al*., 2013). Jung *et al*. (2011) reported that, combination of organic acid (gallic and linoleic acids) can significantly improve the fatty acid content of egg yolk of laying hen. Consistent to that, several researchers have suggested that it would be possible to modify the fatty acid contents in poultry meat through dietary manipulation of natural plant materials (Sarker *et al*., 2009; Ahmed *et al*., 2015). In addition, the higher mineral content (specially the Mg and Fe) can acts on the activities of enzyme (desaturase enzyme); where desaturase enzyme acts on the synthesis of different fatty acids (Cunnane and Wahle, 1981). While magnesium (Mg), ATP (Mg content regulate the ATP level) and CoA are essential for the formation of the true substrates of desaturase enzyme (Co-enzyme-A esters of fatty acids) (Burton, 1980; Fleckenstein, 1983). Magnesium and iron play a significant role on the functioning of desaturase which acts on conversion of SFA to MUFA and PUFA (especially stearic acid to other unsaturated fatty acids) (Rao *et al*., 1983; Seelig and Rosanoff, 2003). Since minerals are usually required a little amount but their function is important, therefore, the FSH2 (compared to other level) might contain proper amount and proportion of magnesium and iron content (due to combination of medicinal plants *Salicornia herbacea* and *Houttuynia cordata*, and multi-microbe probiotics) which properly join and function in the activation of desaturase enzyme, and ultimately triggers to reduce the total SFA and increase of total PUFA in the present study. Since the total SFA content was lower and MUFA and PUFA was higher in case of FSH2 compared to other FSH group, therefore the ratio MUFA/SFA and PUFA/SFA also differed among the FSH group in the current study. Finally, it was postulated that the Mg and Fe content in the FSH supplemented group (Table 6) additionally attributed in the higher desirable fatty acid synthesis in the meat of broilers.

Consumers are becoming more conscious of their health, and are particularly interested in reducing the risk of cardiovascular and other diseases by consuming more PUFA, especially omega-3 fatty acids (Gebauer *et al.*, 2006; von Schacky and Harris, 2007). The most important omega-3 fatty acids in human nutrition are eicosapentaenoic acid (EPA; 20:5n-3), docosahexaenoic acid (DHA; 22:6n-3), and α-linolenic acid (ALA; 18:3n-3), which serve as precursors for the synthesis of EPA and DHA (Burdge, 2004; Arterburn *et al.*, 2006). The EPA values were significantly higher in the FSH groups ($P<0.05$). The International Society for the Study of Fatty Acids and Lipids recommended a daily intake of 2,220 mg of ALA and 650 mg of EPA+DHA, with a minimum of 220 mg of both EPA and DHA (Simopoulos, *et al.*, 1999). The ratio of hypo to hyper cholesterolaemic fatty acid (H/H) is the indicator of the functionality of fatty

acids (Santos-Silva *et al*., 2002) which was significantly higher in the FSH group which is obviously another positive impact of the combination of natural plant materials and probiotics in the current study. In the present study, the ratio of omega-6 to omega-3 PUFA was downgraded in the FSH supplemented group compared to control which was also desirable effect of fermented product on the aspects of nutrition; because the ratio should be balanced and it is well known fact that the higher intake of omega-6 fatty acids relative to omega-3 fatty acids causes pathological changes in humans (Kromhout *et al*., 1985). Overall value of omega-6 to omega-3 fatty acids in meat in the current study was around 6:1. Kralik, *et al.* (2001) found the ratio of 5:1 in chicken meat. The recommended ratio of omega-6 to omega-3 fatty acids in the human diet should be around 10:1 to 5:1; however, in the United States and Europe this ratio is 25:1 to 50:1, and in Japan is 12:1 due to differences in the diet composition (Grashorn, 2007). Therefore, the ratio of the present study was in the acceptable moderate range.

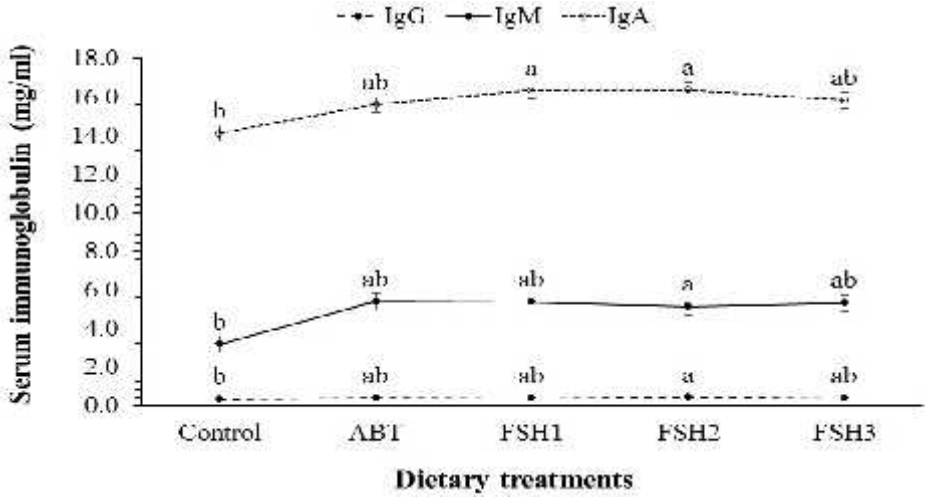


**Figure 1. Effect of fermented *Salicornia herbacea* and *Houttuynia cordata* (FSH) on serum concentrations of broilers (mg/ml).**

[a,b] Means with different superscripts within the same line are significantly different (P<0.05).

Fermented *Salicornia herbacea* and *Houttuynia cordata* (FSH);

Dietary treatments: control (corn-soybean meal based basal diet); ABT (Basal diet + 0.05% oxytetracycline); and FSH1 (Basal diet + 0.5% FSH), FSH2 (Basal diet + 1.0% FSH) and FSH3 (Basal diet + 2.0% FSH).

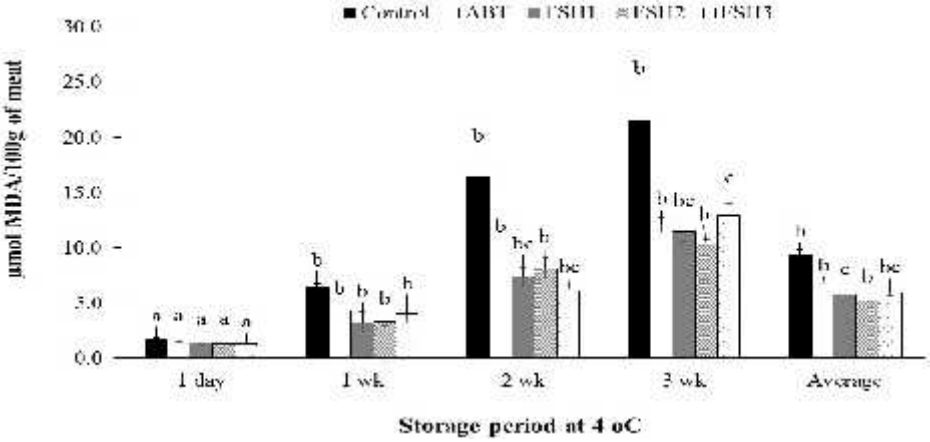


**Figure 2. Effect of fermented *Salicornia herbacea* and *Houttuynia cordata* (FSH) on TBARS value of broiler meat (µmol MDA/100g).**

MDA = Malondealdehyde; TBARS = Thiobarbituric acid reactive substances

[a,b,c] Means with different superscripts within the same bar are significantly different (P<0.05).

Fermented *Salicornia herbacea* and *Houttuynia cordata* (FSH);

Dietary treatments: control (corn-soybean meal based basal diet); ABT (Basal diet + 0.05% oxytetracycline); and FSH1 (Basal diet + 0.5% FSH), FSH2 (Basal diet + 1.0% FSH) and FSH3 (Basal diet + 2.0% FSH).

**Table 1: Feed ingredients and chemical compositions of the basal diets.**

| Items | Starter diet (0 to 3 weeks) | Finisher diet (4 to 5 weeks) |
|---|---|---|
| Ingredients (%, as fed basis) | | |
| Corn grain | 57.58 | 60.64 |
| Soybean meal | 26.80 | 24.90 |
| Corn gluten | 5.00 | 3.50 |
| Soybean oil | 2.20 | 2.20 |
| Animal fats | 4.50 | 5.00 |
| Common salt | 0.25 | 0.25 |
| Dicalcium phosphate | 2.14 | 2.00 |
| Limestone | 0.92 | 0.88 |
| Vitamin-mineral premix [1] | 0.30 | 0.30 |
| Choline | 0.08 | 0.07 |
| L-lysine HCl (78%) | 0.24 | 0.16 |
| DL-Methionine | 0.20 | 0.10 |
| Calculated composition (% DM) | | |
| ME (MJ/kg) | 13.03 | 13.27 |
| Moisture | 12.07 | 13.08 |
| Crude Protein | 20.89 | 19.12 |
| Ether extract | 4.65 | 2.43 |
| Crude Fiber | 4.42 | 3.71 |
| Crude Ash | 5.63 | 5.61 |
| Calcium | 1.05 | 0.81 |
| Available phosphorus | 0.55 | 0.45 |
| Lysine | 1.42 | 1.10 |
| Methionine | 0.49 | 0.45 |

[1]Vitamin-mineral mixture provided the following nutrients per kilogram of diet: vitamin A, 15,000 IU; vitamin $D_3$, 1,500 IU; vitamin E, 20.0 mg; vitamin $K_3$, 0.70 mg; vitamin $B_{12}$, 0.02 mg; niacin, 22.5 mg; thiamine, 5.0 mg; folic acid, 0.70 mg; pyridoxine, 1.3 mg; riboflavin, 5 mg; pantothenic acid, 25 mg; choline chloride, 175 mg; Mn, 60 mg; Zn, 45 mg; I, 1.25 mg; Se, 0.4 mg; Cu, 10.0 mg; Fe, 72 mg; Co, 2.5 mg (Bayer Korea Ltd., Dongjak-Ku, Seoul, Korea).

**Table 2. Microbial population and composition of fermented *Salicornia herbacea* and *Houttuynia cordata* (FSH).**

| Microflora and Strain | Concentration (cfu/g) |
|---|---|
| *Lactobacillus acidophilus* ( KCTC 3111) | $4.2 \times 10^7$ |
| *Lactobacillus plantarum* (KCTC 3104) | $5.8 \times 10^6$ |
| *Bacillus subtilis* (KCTC 3239) | $2.6 \times 10^7$ |
| *Saccharomyces cerevisiae* (KCTC 7915) | $6.2 \times 10^9$ |
| **Composition of FSH** | **(%)** |
| Moisture | 40.09 |
| Crude protein | 11.54 |
| Crude fat | 1.44 |
| Crude fiber | 12.50 |
| Crude ash | 10.81 |
| Nitrogen free extract | 25.74 |
| Na | 11.74 |
| K | 1.42 |
| Ca | 0.31 |
| Mg | 0.93 |

KCTC: Korean Collection for Type Cultures
Fermented *Salicornia herbacea* and *Houttuynia cordata* (FSH)

**Table 3. Effect of fermented *Salicornia herbacea* and *Houttuynia cordata* (FSH) on broiler growth performance.**

| Parameters | Dietary treatments | | | | | SEM | P-value |
|---|---|---|---|---|---|---|---|
| | Control | ABT | FSH1 | FSH2 | FSH3 | | |
| IBW (g/bird) | 45.16 | 45.29 | 45.26 | 45.33 | 45.22 | 0.46 | 0.999 |
| FBW (g/bird) | 1917.86 | 1921.23 | 1909.03 | 1927.77 | 1894.96 | 31.45 | 0.957 |
| ADG (g/bird) | 53.51 | 53.60 | 53.25 | 53.78 | 52.85 | 0.89 | 0.957 |
| ADFI (g/bird) | 83.50 | 83.30 | 83.05 | 83.18 | 83.01 | 0.89 | 0.996 |
| FCR | 1.56 | 1.55 | 1.56 | 1.55 | 1.57 | 0.03 | 0.986 |
| Mortality (%) | 7.77[a] | 2.88[b] | 3.11[b] | 2.21[b] | 2.49[b] | 0.91 | 0.008 |

[a,b] Means with different superscripts within the same row are significantly different (P<0.05).
FSH: Fermented *Salicornia herbacea* and *Houttuynia cordata*; IBW: Intial body weight; FBW: Final body weight; ADG: Average daily gain; ADFI: Average daily feed intake; FCR: Feed conversion ratio (feed to gain ratio);
Dietary treatments: control (corn-soybean meal based basal diet); ABT (Basal diet + 0.05% oxytetracycline); and FSH1 (Basal diet + 0.5% FSH), FSH2 (Basal diet + 1.0% FSH) and FSH3 (Basal diet + 2.0% FSH).

**Table 4. Effect of fermented *Salicornia herbacea* and *Houttuynia cordata* (FSH) on relative organ weight of boilers.**

| Internal organs | Dietary treatments | | | | | SEM | P-value |
|---|---|---|---|---|---|---|---|
| | Control | ABT | FSH1 | FSH2 | FSH3 | | |
| Crop | 0.36 | 0.33 | 0.32 | 0.32 | 0.32 | 0.03 | 0.873 |
| Proventriculus | 0.48 | 0.47 | 0.46 | 0.46 | 0.46 | 0.05 | 0.998 |
| Gizzard | 1.74 | 1.54 | 1.55 | 1.62 | 1.86 | 0.15 | 0.665 |
| Heart | 0.76 | 0.71 | 0.59 | 0.59 | 0.73 | 0.06 | 0.203 |
| Liver | 2.54 | 2.35 | 2.36 | 2.37 | 2.46 | 0.21 | 0.964 |
| Gall bladder | 0.10 | 0.12 | 0.09 | 0.11 | 0.10 | 0.01 | 0.540 |
| spleen | 0.09 | 0.08 | 0.09 | 0.08 | 0.08 | 0.01 | 0.601 |
| Pancreas | 0.25 | 0.23 | 0.21 | 0.24 | 0.22 | 0.02 | 0.581 |
| Small intestine | 3.23 | 3.11 | 3.01 | 2.99 | 2.99 | 0.23 | 0.924 |
| Large intestine | 0.17 | 0.20 | 0.19 | 0.21 | 0.26 | 0.03 | 0.290 |
| Cecum | 0.53 | 0.46 | 0.38 | 0.43 | 0.48 | 0.06 | 0.552 |
| Kidney | 0.83 | 0.70 | 0.64 | 0.68 | 0.76 | 0.04 | 0.105 |
| Abdominal fat | 1.78[a] | 1.34[b] | 1.30[b] | 1.24[b] | 1.37[b] | 0.08 | 0.002 |
| Bursa | 0.20 | 0.21 | 0.17 | 0.20 | 0.15 | 0.02 | 0.351 |

[a,b] Means with different superscripts within the same row are significantly different (P<0.05).
FSH: Fermented *Salicornia herbacea* and *Houttuynia cordata*;
Dietary treatments: control (corn-soybean meal based basal diet); ABT (Basal diet + 0.05% oxytetracycline); and FSH1 (Basal diet + 0.5% FSH), FSH2 (Basal diet + 1.0% FSH) and FSH3 (Basal diet + 2.0% FSH).

**Table 5. Effect of fermented *Salicornia herbacea* and *Houttuynia cordata* (FSH) on chemical composition of broiler meat.**

| Parameters | Dietary treatments | | | | | SEM | P-value |
|---|---|---|---|---|---|---|---|
| | Control | ABT | FSH1 | FSH2 | FSH3 | | |
| Moisture (%) | 74.49 | 73.51 | 73.46 | 73.22 | 73.27 | 0.52 | 0.476 |
| Crude protein % | 21.18[c] | 22.12[b] | 22.19[ab] | 22.98[a] | 22.37[ab] | 0.23 | 0.004 |
| Crude fat % | 4.08[a] | 3.81[ab] | 3.66[bc] | 3.38[c] | 3.56[bc] | 0.12 | 0.012 |
| Crude Ash (%) | 1.26 | 1.26 | 1.35 | 1.32 | 1.39 | 0.09 | 0.811 |
| Fe (mg/100g) | 2.80[b] | 2.71[b] | 4.55[a] | 4.70[a] | 4.47[a] | 0.44 | 0.032 |
| Ca (mg/100g) | 4.00 | 4.51 | 4.65 | 4.94 | 4.62 | 0.35 | 0.489 |
| Mg (mg/100g) | 2.75[b] | 2.87[b] | 4.72[a] | 4.70[a] | 4.47[ab] | 0.47 | 0.050 |
| Na (mg/100g) | 61.97 | 62.44 | 63.50 | 63.78 | 63.61 | 2.07 | 0.963 |

[a,b] Means with different superscripts within the same row are significantly different (P<0.05).
FSH: Fermented *Salicornia herbacea* and *Houttuynia cordata*;
Dietary treatments: control (corn-soybean meal based basal diet); ABT (Basal diet + 0.05% oxytetracycline); and FSH1 (Basal diet + 0.5% FSH), FSH2 (Basal diet + 1.0% FSH) and FSH3 (Basal diet + 2.0% FSH).

**Table 6. Effect of fermented *Salicornia herbacea* and *Houttuynia cordata* (FSH) on the fatty acids profile in broiler meat (g/100g fatty acids).**

| Parameters (g/100g of FA) | Dietary treatments | | | | | SEM | P-value |
|---|---|---|---|---|---|---|---|
| | Control | ABT | FSH1 | FSH2 | FSH3 | | |
| Myristic acid (C14:0) | 0.54 | 0.49 | 0.41 | 0.48 | 0.42 | 0.03 | 0.167 |
| Palmitic acid (C16:0) | 24.78$^{a}$ | 23.31$^{b}$ | 21.99$^{b}$ | 21.99$^{b}$ | 22.15$^{b}$ | 0.41 | 0.001 |
| Stearic acid (C18:0) | 10.36$^{a}$ | 9.66$^{ab}$ | 9.87$^{a}$ | 8.48$^{b}$ | 10.03$^{a}$ | 0.34 | 0.043 |
| Arachidic acid (C20:0) | 0.43$^{a}$ | 0.34$^{ab}$ | 0.23$^{b}$ | 0.30$^{b}$ | 0.31$^{ab}$ | 0.03 | 0.002 |
| Palmitoleic acid (C16:1) | 4.26 | 4.47 | 4.17 | 5.03 | 3.92 | 0.33 | 0.269 |
| Oleic acid (C18:1) | 36.04 | 35.78 | 34.66 | 35.89 | 35.00 | 0.52 | 0.350 |
| Eicosaenoic acid (C20:1) | 1.14 | 1.19 | 1.15 | 1.23 | 1.22 | 0.09 | 0.943 |
| Tetracosaenoic acid (C24:1) | 1.15 | 1.20 | 1.23 | 1.23 | 1.40 | 0.12 | 0.735 |
| α-Linolenic acid (C18:3n3) | 1.34$^{c}$ | 1.46$^{ab}$ | 1.40$^{bc}$ | 1.56$^{a}$ | 1.33$^{c}$ | 0.03 | 0.003 |
| Eicosapentanoic acid (C20:5n3) | 0.38$^{c}$ | 0.54$^{b}$ | 0.67$^{a}$ | 0.55$^{b}$ | 0.62$^{a}$ | 0.02 | <0.0001 |
| Docosahexanoic acid (C22:6n3) | 1.31$^{d}$ | 1.42$^{c}$ | 1.59$^{a}$ | 1.49$^{b}$ | 1.48$^{c}$ | 0.02 | <0.0001 |
| Linoleic acid (C18:2n6) | 14.75$^{b}$ | 15.77$^{a}$ | 15.47$^{a}$ | 15.78$^{a}$ | 15.44$^{a}$ | 0.16 | 0.008 |
| DGLA (C20:3n6) | 1.14$^{b}$ | 1.22$^{b}$ | 1.25$^{ab}$ | 1.36$^{a}$ | 1.20$^{b}$ | 0.04 | 0.015 |
| Arachidonic acid (C20:4n6) | 2.45 | 2.42 | 2.51 | 2.56 | 2.30 | 0.09 | 0.361 |
| ΣSFA | 37.11$^{ab}$ | 35.79$^{bc}$ | 36.49$^{ab}$ | 34.24$^{c}$ | 37.90$^{a}$ | 0.45 | 0.002 |
| ΣMUFA | 42.57$^{ab}$ | 42.64$^{ab}$ | 41.20$^{b}$ | 43.37$^{a}$ | 41.53$^{b}$ | 0.45 | 0.034 |
| ΣPUFA | 21.36$^{c}$ | 22.83$^{ab}$ | 22.88$^{ab}$ | 23.30$^{a}$ | 22.37$^{b}$ | 0.22 | <0.0001 |
| MUFA/SFA | 1.15$^{bc}$ | 1.19$^{ab}$ | 1.13$^{bc}$ | 1.27$^{a}$ | 1.10$^{c}$ | 0.02 | 0.003 |
| PUFA/SFA | 0.58$^{c}$ | 0.64$^{b}$ | 0.63$^{b}$ | 0.68$^{a}$ | 0.59$^{c}$ | 0.01 | <0.0001 |
| H/H | 2.27$^{b}$ | 2.46$^{a}$ | 2.57$^{a}$ | 2.64$^{a}$ | 2.54$^{a}$ | 0.05 | 0.003 |
| Σn-3 | 3.03c | 3.42b | 3.65a | 3.60a | 3.43b | 0.04 | <0.0001 |
| Σn-6 | 18.34$^{c}$ | 19.41$^{ab}$ | 19.22$^{ab}$ | 19.70$^{a}$ | 18.94$^{bc}$ | 0.20 | 0.004 |
| n-6/n-3 | 6.06$^{a}$ | 5.68$^{b}$ | 5.26$^{c}$ | 5.47$^{bc}$ | 5.53$^{b}$ | 0.07 | <.0001 |

$^{a,b}$ Means with different superscripts within the same row are significantly different (P<0.05).
FSH: Fermented *Salicornia herbacea* and *Houttuynia cordata*; Dietary treatments: control (corn-soybean meal based basal diet); ABT (Basal diet + 0.05% oxytetracycline); and FSH1 (Basal diet + 0.5% FSH), FSH2 (Basal diet + 1.0% FSH) and FSH3 (Basal diet + 2.0% FSH).DGLA = Dihomo-γ-linolenic acid is a 20-carbon ω−6 fatty acid. SFA: Saturated fatty acid, MUFA: Monounsaturated fatty acid, PUFA: Polyunsaturated fatty acid, USFA: Unsaturated fatty acid; ΣSFA = saturated fatty acid; ΣMUFA = mono-unsaturated fatty acid; ΣPUFA = poly-unsaturated fatty acid; Σn-3 = total omega 3 fatty acid; Σn-6 = total omega 6 fatty acid, H/H = hypocholesterolaemic to hypercholesterolaemic fatty acid ratio.

**Conclusions:** Result of the present study elucidated that, dietary inclusion of two medicinal plants (*Salicornia herbacea* L. and *Houttuynia cordata* Thunb.) and multi-microbe probiotics (*Lactobacillus* spp., *Bacilli* spp. and *Saccharomyces* spp.) (FSH) did not show any significant negative impact on the growth performance and relative organ weight of broilers; however, immunity was significantly improved, mortality and abdominal fat content was significantly down trended after dietary inclusion of FSH and ABT. Broiler meat crude protein content was significantly higher whereas crude fat content was significantly lower in the FSH groups compared to control. The thiobarbituric acid reactive substances (TBARS) value in meat was significantly lower after addition of FSH and ABT relative to the control (P<0.05); where FSH2 significantly differed with ABT as well. The total SFA content of breast meat of broiler was significantly diminuted while the total PUFA content was elevated after FSH2 supplementation. The total omega-3 and omega-6 PUFA was significantly elevated in the FSH and ABT group compared to control. The ratio of PUFA to SFA, and H/H was significantly upgraded; while omega-6 to omega-3 ratio was downgraded in the FSH and ABT group relative to control. Thus this study affirms that FSH could be added in the broiler diet as potential feed additives for nutritional enrichment as well as functionality of meat; while FSH2 could be the best choice since it exerted the best outcomes. Further detail research is required to test the detail mechanism of combined medicinal plant along with multi-microbe probiotics.

**Conflict of Interest:** The authors of this research and article declare that, there is no any conflict of interest regarding the publication and dissemination of knowledge.

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