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Evaluation of dietary fat sources on growth performance, excreta microbiology and noxious gas emissions in Ross broilers

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An experiment was conducted to evaluate fat sources on growth performance, excreta microbiology and noxious gas emissions in broilers. Experimental birds were reared for 5 weeks and divided into two periods: Starter (0 to 3 weeks) and finisher (4 to 5 weeks). A total of 150 one day old Ross 308 broiler chicks were randomly allocated into five treatments with five replications (six birds per replicate) following a completely randomized design. Dietary treatments included FT1 (corn-soybean meal based basal diet with soybean oil), FT2 (corn-soybean meal based basal diet with chicken fat), FT3 (corn-soybean meal based basal diet with tallow), FT4 (corn-soybean meal based basal diet with tallow and lard) and FT5 (corn-soybean meal based basal diet with pork fat/lard). Overall body weight gain and feed intake did not differ due to addition of different dietary fat sources in broiler diet; however, feed conversion ratio was better in FT1 fed group in comparison to other fat groups (P<0.10). The result of the serum immunoglobulins data indicated that, a significant elevation of serum immunoglobulin M (IgM) was observed after dietary addition of FT1, FT2 and FT5 relative to FT4 (P<0.05) Excreta pH did not differ, however, yeast and mold count was highest in FT4 and FT5 relative to FT1 and FT3 (P<0.05). Excreta noxious gas emissions (NH_3, H_2S and SO_2) were lower in FT1 and FT2 in comparison to other fat groups (P<0.05). Overall, the results of the present study suggested that FT1 and FT2 can be prioritized in the diet of broilers with positive influence on body weight gain and feed efficiency, and substantial reduction of noxious gas emissions. Further detail study could be conducted to investigate the single and combination of different dietary fats (with different ratio) on performance and meat quality indices.

Key words: Broilers, growth performance, immunity, noxious gas emissions.

INTRODUCTION

The global population is currently 7.4 billion (World Population, 2016), and according to the United Nations reports, population will be 11.2 billion by 2100 (United Nations Report, 2015). The demand of food as well as meat is increasing all over the world (FAO report, 2009; Tilman et al., 2011). Among different meat red meat is under criticism due to different health aspects of human, while chicken meat is mostly favoured due to its lower price and risk of claimable diseases like cardiovascular diseases and colon cancer. However, many more factors influence the performance and production of chicken meat from the farm to the consumers table. Dietary

sources are the concerns for the poultry nutritionist to test both the performance indices and the meat quality attributes. Most of the research are now-a-days focusing on the feed additives like prebiotics, probiotics, synbiotics, medicinal plants, fermented medicinal plant along with probiotics, extracts from natural plants to investigate the performance, microbiology and gaseous emissions (Bostami et al., 2015; Bostami et al., 2016; Kim et al., 2016; Sarker et al., 2016). However, the basal feed ingredients might also can affect the parameter indices and could be the basic research which can further helps to identify the proper amount and proportion of the feed additives. Most feed ingredients provided to animals are obtained from plant sources, which is generally accepted for the production purposes; however, there are some controversies regarding the use of genetically modified plants (GMP), because some plants are produced through animal gene transfer technology and they possess health and ethical issues (Kurien, 2002; Riaz and Chaudry, 2004).

To ensure different nutrients, several feed ingredients are provided to the poultry for growth performance, meat or egg production. Birds generally consume to fulfil their energy requirements; however, due to limitation of the gastrointestinal tract and parallel genetic improvement of the hybrid broilers, animal and plant fats are widely used as energy sources as well as source of essential fatty acids (Sanz et al., 2000). Deficiency of fatty acids in the diet cans results in metabolic disorders, depression of growth and immunity (Weiseman, 1984; Zollitsch et al., 1997). The amount and proportion of the fatty acids, especially the PUFA content varied between animal and plant fats and also within the similar source fats. However, replacement of animal fat by vegetable fat reported to be beneficial from the aspect of reducing abdominal fat pads in broilers (Newman et al., 2002). Moreover, better feed efficiency was reported for dietary plant oil than animal fat in broiler study (Newman et al., 2002). After ensuring the performance of broilers, it is also necessary to consider the effects of dietary fat sources on the animal excreta noxious gas emissions, which are the foremost research topics through dietary manipulation. Since the broiler industry is expanding tremendously, large gaseous emissions can greatly influence the health of both animals and humans (Myer and Bucklin, 2007). Research on feed additives (probiotics and natural plant materials) to minimize odorous gas emissions are under consideration and ongoing research all over the world (Bostami et al., 2015, 2016); where the basal feed ingredients can also influence the excreta microbial population and gas emissions. The gastrointestinal tract of poultry is the store house of microbiome (Pan and Yu, 2014). The type of fat source is likely to indirectly influence the intestinal microflora (Danicke et al., 1999), which can contribute to the gas emissions as well (Leek et al., 2004; Smith et al., 2004); because the microorganisms present in the gastrointestinal tract interact closely and densely with host and the ingested feed ingredients (Pan and Yu, 2014).

Although some researches has been conducted on fat sources to test the performance effect, however, the immunity, excreta microbiology and noxious gas emissions are the important task in the case of broiler industry. Due to the continuous genetic improvement in the broiler hybrids, the basic feed ingredients as well the feed additives research should be persistent. It was hypothesized that, different fat sources or their combinations could affect the growth performance, immunity, excreta microbiology and noxious gas emissions in broilers. Therefore, this study was conducted to investigate the dietary fat sources in broiler feed ingredients on the growth performance, immunity, excreta microbiology and noxious gas emissions as basic research for the further detail study on the other feed ingredients and additives.

MATERIALS AND METHODS

Experimental design, dietary treatments and birds husbandry

After collection of the broiler chicks from the local hatchery of Daejon, birds were reared at the experimental farm of Sunchon National University, Suncheon, Republic of Korea. A total of 150 one-day-old Ross 308 broiler chicks were randomly allocated into five treatments with six replications (six birds per replicate pen) in a completely randomized design. In Korea as well as other countries, different types of fats are being utilized based on the availability. Due to continuous genetic improvement of chicken, fats (energy and fatty acid sources) as major feed ingredients should be given paramount importance and to be tested for more efficacies. Based on availability and utilization of the fat sources in Korea, following fats were selected and divided into five treatments. Dietary treatments included FT1 (corn-soybean meal based basal diet with soybean oil), FT2 (corn-soybean meal based basal diet with chicken fat), FT3 (corn-soybean meal based basal diet with tallow), FT4 (corn-soybean meal based basal diet tallow and lard with 1:8 ratio) and FT5 (corn-soybean meal based basal diet with pork fat/lard). The basal diet was formulated to meet the Nutrient Requirements of Poultry (NRC, 1994; KFS, 2012). Feed was formulated and prepared for the period of 5 weeks dividing into two stages: Starter from 0 to 3 weeks, and finisher from 4 to 5 weeks. All feed ingredients were collected and were hand mixed based on feed formulation. The chemical composition of the experimental diet was analyzed in triplicate for moisture, ash, crude protein (CP), crude fiber (CF) and ether extract (EE), as described by AOAC (2000). The ingredients, chemical composition, and vitamin and mineral content of the basal diets are shown in Tables 1 and 2.

The plant oil and animal fat amended with the basal feed

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Table 1. Feed ingredients and chemical composition of the experiment diets (starter).

Ingredients (% DM as feed basis)	Starter diet (0 to 21 days)				
	FT1	FT2	FT3	FT4	FT5
Corn	50.00	50.00	50.00	50.00	50.00
Soybean meal	37.00	37.00	37.00	37.00	37.00
Corn gluten meal	0.50	0.50	0.50	0.50	0.50
Wheat-10%	6.00	6.00	6.00	6.00	6.00
Limestone-Small	2.03	2.03	2.03	2.03	2.03
Salt-Proc	0.25	0.25	0.25	0.25	0.25
DCP-18%	0.40	0.40	0.40	0.40	0.40
L-lys Sulfate 70%	0.30	0.30	0.30	0.30	0.30
Minemix	0.20	0.20	0.20	0.20	0.20
Vitamix	0.05	0.05	0.05	0.05	0.05
L-threonine-98%	-	-	-	-	-
MHA-Liquid	0.26	0.26	0.26	0.26	0.26
Sunphase5000FTU	0.01	0.01	0.01	0.01	0.01
Soybean oil	3.00	-	-	-	-
Chicken fat	-	3.00	-	-	-
Tallow		-	3.00	-	-
Tallow+Lard		-	-	3.00	-
Lard	-	-	-	-	3.00
Calculated composition					
ME (kcal/kg)	3,090.20	3,090.20	3,090.20	3,090.20	3,090.20
Crude protein (%)	22.04	22.04	22.04	22.04	22.04
Crude fat (%)	5.35	5.35	5.35	5.35	5.35
Crude ash (%)	5.71	5.71	5.71	5.71	5.71
Crude fiber (%)	2.59	2.59	2.59	2.59	2.59
Ca (%)	1.09	1.09	1.09	1.09	1.09
Phosphorus (%)	0.45	0.45	0.45	0.45	0.45
Lysine (%)	1.34	1.34	1.34	1.34	1.34
Methionine (%)	0.57	0.57	0.57	0.57	0.57

Dietary treatments: FT1: Basal feed with soybean oil); FT2: Basal feed with chicken fat; FT3: Basal diet with tallow; FT4: Basal diet with tallow and lard (1:8): FT5: Basal feed with pork fat/lard. Provided the following amount of ingredients: Na 11.0%; Cl 0.19%; Cu 73.02 ppm (starter) and 72.07 ppm (finisher); Mn 77.92 ppm (starter) and 75.80 ppm (finisher); Zn 73.75 ppm (starter) and 71.57 ppm (finisher); I 0.94 ppm; Se 0.30 ppm; Fe 148.63 ppm (starter) and 4141.21 ppm (finisher); Vit A 12,061.00 IU/k and 12,122.00 IU/k (finisher); Vit D_3 3,000.00 IU/k; Vit E 28.06 ppm (starter) and 29.35 ppm (finisher); VIt K 2.10 ppm (starter) and 2.11 ppm (finisher); Choline 1,329.10 ppm (starter) and 1,146.20 ppm (finisher).

ingredients were purchased from local markets of Republic of Korea. Soybean oil and lard was purchased from the Daejon city; where chicken and tallow were collected from abattoirs of Suwon and Daejon city of Republic of Korea. Chicken fat and tallow was collected from a slaughterhouse in which chickens and cattle were slaughtered according to halal rules and in which birds and animals were inspected by a veterinarian to ensure that they were disease free. After collection of raw fat, it was chopped and sliced into smaller chunks of equal size (25 mm), then was placed in a fry pan with a lid. Water was added at a 1:1 ratio (W/W), after which it was covered with a lid and boiled for 30 min, followed by an additional 30 min of boiling without the lid. When the boiled fat showed a reddish color indicating that the fatty liquid had been extracted from the solid portions of the fat, the heater was switched off. Following fully boiling off the water, a wire mesh strainer was used to remove the remnants and particles. After straining three times, the fat was poured into a heat-proof ceramic container and covered tightly, then stored for further use. After collection of the lard from the pig abattoir, it was processed in a similar fashion as chicken fat and tallow, and then stored in a separate container and location until further use. The fatty acid composition of the experimental fat sources was determined by following the direct method for fatty acid methyl ester (FAME) synthesis using a gas chromatograph (GC), with slight modification as described by O'Fallon et al. (2007).

Broilers were reared in a closed, ventilated, wire-floor caged broiler house (100 cm long × 90 cm wide × 40 cm high) with a floor space of 1,125 cm^2/bird. During the experiment, all guidelines for the care and use of animals in research set by the Korean Ministry for Food, Agriculture, Forestry and Fisheries (2008) were followed. The cages had a linear feeder in the front and a nipple drinker in the back to provide *ad libitum* feed intake and free access to water. The internal temperature of the broiler house was set and maintained at 34°C for the first week, after which it was gradually reduced to 23°C with decreasing at the rate of 3°C per week, where it was maintained until the end of the experimental period. The internal house relative humidity was maintained at around 50% throughout the experimental period. Continuous lighting was provided for the entire experimental period.

Table 2. Feed ingredients and chemical composition of the experiment diets (finisher).

Ingredients (% DM as feed basis)	Finisher diet (22 to 35 days)				
	FT1	FT2	FT3	FT4	FT5
Corn	56.00	56.00	56.00	56.00	56.00
Soybean meal	28.84	28.84	28.84	28.84	28.84
Corn gluten meal	1.00	1.00	1.00	1.00	1.00
Wheat-10%	7.00	7.00	7.00	7.00	7.00
Limestone-Small	1.92	1.92	1.92	1.92	1.92
Salt-Proc	0.25	0.25	0.25	0.25	0.25
DCP-18%	0.46	0.46	0.46	0.46	0.46
L-lys Sulfate 70%	0.18	0.18	0.18	0.18	0.18
Minemix	0.20	0.20	0.20	0.20	0.20
Vitamix	0.05	0.05	0.05	0.05	0.05
L-threonine-98%	0.01	0.01	0.01	0.01	0.01
MHA-Liquid	0.29	0.29	0.29	0.29	0.29
Sunphase5000FTU	0.01	0.01	0.01	0.01	0.01
Soybean oil	3.80	-	-	-	-
Chicken fat	-	3.80	-	-	-
Tallow	-	-	3.80	-	-
Tallow+Lard	-	-		3.80	-
Lard	-	-	-	-	3.80
Calculated composition					
ME (kcal/kg)	3,210.90	3,210.90	3,210.90	3,210.90	3,210.90
Crude protein (%)	19.00	19.00	19.00	19.00	19.00
Crude fat (%)	6.23	6.23	6.23	6.23	6.23
Crude ash (%)	5.26	5.26	5.26	5.26	5.26
Crude fiber (%)	2.39	2.39	2.39	2.39	2.39
Ca (%)	1.04	1.04	1.04	1.04	1.04
Phosphorus (%)	0.43	0.43	0.43	0.43	0.43
Lysine (%)	1.07	1.07	1.07	1.07	1.07
Methionine (%)	0.55	0.55	0.55	0.55	0.55

Dietary treatments: FT1: Basal feed with soybean oil); FT2: Basal feed with chicken fat; FT3: Basal diet with tallow; FT4: Basal diet with tallow and lard (1:8): FT5: Basal feed with pork fat/lard. Provided the following amount of ingredients: Na 11.0%; Cl 0.19%; Cu 73.02 ppm (starter) and 72.07 ppm (finisher); Mn 77.92 ppm (starter) and 75.80 ppm (finisher); Zn 73.75 ppm (starter) and 71.57 ppm (finisher); I 0.94 ppm; Se 0.30 ppm; Fe 148.63 ppm (starter) and 4141.21 ppm (finisher); Vit A 12,061.00 IU/k and 12,122.00 IU/k (finisher); Vit D_3 3,000.00 IU/k; Vit E 28.06 ppm (starter) and 29.35 ppm (finisher); VIt K 2.10 ppm (starter) and 2.11 ppm (finisher); Choline 1,329.10 ppm (starter) and 1,146.20 ppm (finisher).

Measurement of growth performance

Chicks were inspected daily and dead birds were removed following recording of the mortality (pen, date and body weight). No vaccination or medication program was followed during entire rearing period. Feed intake was measured based on residual feed deduction from the total feed provided to the birds. Body weight (BW) and feed intake were recorded weekly by replicate, and the feed intake (FI), body weight gain (BWG), and FCR (feed to gain ratio) per cage were then calculated by period and for the total experimental period.

Collection and analyses of blood samples for bird's immunity

At the termination of the experiment, two birds (based on mean body weight) were randomly selected from each replicated pen for blood sample collection. Blood samples were collected (10 mL) from the wing veins of the randomly selected birds into a 10-mL anticoagulant-free vacutainer tube (Greiner Bio-One GmbH, Kremsmunster, Austria). After collection of the blood samples, subsequently stored on ice during the period of collection and then immediately centrifuged to separate the serum (centrifugation for 15 min at 1,610 × *g* at 4°C). The serum samples were carefully transferred to plastic vials and stored at -20°C until immunoglobulin analysis was performed. The concentrations of serum IgG, IgM, and IgA were assayed using appropriately diluted samples by a sandwich ELISA with chicken-specific IgG (Cat. No. E30-104), IgM (Cat. No. E10-101), and IgA (Cat. No. E30-103) ELISA quantitation kits (Bethyl Laboratories Inc., Montgomery, TX) according to the manufacturer's instructions. For serum immunoglobulin test, each experiment was run in duplicate and the results represent the means of triplicate experiments. The absorbance of each well at 450 nm was measured within 30 min using a microplate autoreader (Thermo Lab Systems, Helsinki, Finland). The concentrations of immunoglobulin G (IgG), immunoglobulin M (IgM) and immunoglobulin A (IgA) were determined using standard curves constructed from the respective immunoglobulin standards. The

Table 3. Effect of dietary fat sources on growth performance in broiler.

Item	FT1	FT2	FT3	FT4	FT5	SEM	P-value
BW (g/bird)							
0 day	39.53	39.80	39.60	39.53	39.73	0.62	0.997
22 day	576.67^{b}	638.93^{a}	574.10^{b}	527.57^{b}	547.53^{b}	18.20	0.010
35 day	1,573.33	1,629.33	1,530.67	1,521.33	1478.00	45.09	0.272
0 to 3 weeks							
BWG (g/bird)	537.14^{b}	599.13^{a}	534.50^{b}	488.03^{b}	507.80^{b}	17.97	0.009
FI (g/bird)	757.11^{b}	860.43^{a}	791.39^{ab}	757.33^{b}	783.53^{ab}	24.76	0.063
FCR	1.42	1.44	1.48	1.55	1.54	0.03	0.111
4 to 5 weeks							
BWG (g/bird)	996.67	990.40	956.56	993.77	930.47	41.01	0.774
FI (g/bird)	1,573.25	1,644.20	1,582.00	1,573.97	1,500.63	47.88	0.414
FCR	1.59	1.66	1.66	1.58	1.62	0.03	0.198
0 to 5 weeks							
BWG (g/bird)	1,533.81	1,589.53	1,491.07	1,481.80	1,438.27	45.33	0.278
FI (g/bird)	2,330.36	2,504.63	2,373.39	2,331.29	2,284.16	58.86	0.168
FCR	1.52^{b}	1.58^{ab}	1.59^{a}	1.57^{ab}	1.59^{a}	0.02	0.063

^{a,b}Means with different superscripts within the same row are significantly different (P < 0.05). SEM: Standard error of the mean. BW: Body weight; ADG: Average daily gain; ADFI: Average daily feed intake; FCR: Feed conversion ratio (feed to gain ratio); Dietary treatments: FT1: Basal feed with soybean oil); FT2: Basal feed with chicken fat; FT3: Basal diet with tallow; FT4: Basal diet with tallow and lard (1:8): FT5: Basal feed with pork fat/lard.

result of the bird's serum immunoglobulins (IgG, IgM and IgA) were expressed as mg/ml of serum.

Collection and analyses of excreta microbiology and pH

Broiler excreta samples were carefully collected from the three replicated pens of each treatment for microbial analysis. Samples were diluted 1:10 in sterile saline solution (0.85% NaCl in distilled water). Next, 100 µl aliquots were plated in triplicate on MacConkey Sorbitol Agar, Salmonella Shigella Agar, Lactobacilli MRS (Mann, Rogosa and Sharpe) Agar, and Potato Dextrose Agar were used to screen for *E. coli*, *Salmonella*, *Lactobacillus*, and yeast and mold, respectively. Samples were incubated under anaerobic conditions at 37°C for 24 h (*E. coli* and *Salmonella)* and 48 h (*Lactobacillus*, and yeast and mold). Following enumeration of microbial colonies, microbial counts were expressed as $\log_{10}$CFU/ml.

Excreta of broilers from each replicated pen were collected carefully by mixing well in falcon tubes after carefully removing any foreign materials. The pH of the excreta samples was determined by blending 2 g of excreta with 18 ml of distilled water for 1.5 min in a homogenizer. The pH values were measured using a standardized electrode attached to a digital pH meter (Docu-pH + meter, Sartorius, USA).

Collection of excreta sample and noxious gas measurements

At the end of experiment, 35th day, excreta samples (mixtures of feces and urine) were collected from each replicate pen of all treatments into plastic bags and stored immediately at -20°C until use. The total sampled manure from each pen was then thawed and homogenized, after which 500 g subsamples were placed in 2 L plastic boxes in triplicate to measure the NH_3, H_2S and SO_2 emissions. Each plastic box was equipped with a cover containing a hole to allow insertion of a gas measuring tube that was sealed inside with adhesive plaster. The samples were allowed to ferment for a period of 3 h at room temperature (24 to 28°C), after which the gas concentration was measured using a Gastec AP-20 gas sampling pump (Gastec Corp., Kitagawa, Japan) and Gastec detector tubes (No. 3M and 3LA for NH_3; 4LT and 4L for H_2S; and 5La and 5Lb for SO_2). For analysis, the adhesive plaster was punctured and 100 mL of headspace air was collected from approximately 2.0 cm above the sample surface. After sampling, the tubes were again sealed with adhesive plaster and incubated at room temperature. Additional gas samples were collected at 6, 12, 24 and 48 h. The concentration of NH_3, H_2S and SO_2 was expressed as ppm of excreta.

Statistical analyses

All data were subjected to ANOVA using the General Linear Models (GLM) function of the Statistical Analysis System (SAS, 2003, Version 9.1, SAS Institute, Cary, NC, USA). Each cage was considered as the experimental unit for growth performance parameters (BW, BWG, FI and FCR), excreta microbiology, pH and gaseous emissions. A probability level of P<0.05 was considered statistically significant and a level of P<0.10 was considered as a statistical tendency.

RESULTS

Growth performance

As shown in Table 3, a higher final body weight was exhibited in the FT2 group during 22 day of experimental period (P<0.05); however, although a higher numerical body weight in case of FT2 during 0 and 35 day there

Table 4. Effect of dietary fat sources on serum immunoglobulins in broiler.

Serum immunoglobulins (mg/ml)	FT1	FT2	FT3	FT4	FT5	SEM	P-value
Immunoglobulin G (IgG)	3.62	3.73	3.89	3.96	4.20	0.16	0.124
Immunoglobulin M (IgM)	1.94[a]	1.99[a]	1.69[ab]	1.38[b]	1.78[a]	0.12	0.013
Immunoglobulin A (IgA)	5.01	5.01	4.68	5.10	5.21	0.41	0.924

[a,b]Means with different superscripts within the same row are significantly different (P < 0.05). SEM: Standard error of the mean. Dietary treatments: FT1: Basal feed with soybean oil); FT2: Basal feed with chicken fat; FT3: Basal diet with tallow; FT4: Basal diet with tallow and lard (1:8): FT5: Basal feed with pork fat/lard.

Table 5. Effect of dietary fat sources on excreta microbiology and pH in broiler.

Excreta microbiology (log_{10}CFU/g)	FT1	FT2	FT3	FT4	FT5	SEM	P-value
Lactobacillus	8.68[b]	8.94[a]	8.77[ab]	8.96[a]	8.93[a]	0.07	0.053
Bacillus	8.18	8.11	8.50	8.48	8.74	0.22	0.296
Yeast and mold	8.20[b]	8.39[ab]	8.24[b]	8.49[a]	8.48[a]	0.06	0.013
E. coli	6.49	6.89	6.21	6.66	6.13	0.46	0.771
Salmonella	6.80	7.04	6.24	6.34	6.22	0.22	0.070
Excreta pH	7.18	7.10	7.38	7.00	6.96	0.28	0.846

[a,b]Means with different superscripts within the same row are significantly different (P < 0.05). SEM: Standard error of the mean. Dietary treatments: FT1: Basal feed with soybean oil); FT2: Basal feed with chicken fat; FT3: Basal diet with tallow; FT4: Basal diet with tallow and lard (1:8): FT5: Basal feed with pork fat/lard.

was no differences among fat treatments. During 0 to 3 weeks, the body weight gain (BWG) was highest in FT2 fed birds followed by the FT1, FT3, FT5 and FT4 fed birds (P<0.05). While there was found no significant differences among fat treatments during 4 to 5 weeks and overall period. During 4 to 5 weeks, the feed intake (FI) was tended to be lower in FT1 followed by FT4, FT5, FT3 and FT2 fed birds. Moreover, during overall period, the feed conversion ratio (FCR) was tended to be lower in FT1 compared to FT3 and FT5 group which indicated the better efficiency (P<0.10).

Immunity of broilers

The result of the serum immunoglobulins data indicated that, a significant elevation of serum immunoglobulin M (IgM) was observed after the dietary addition of FT1, FT2 and FT5 relative to FT4 (P<0.05) (Table 4). However, no significant impact was found on immunoglobulin G (IgG) and immunoglobulin A (IgA) after different fat addition in the broiler diet.

Excreta microbiology and pH

Table 5 shows that, among the broiler excreta microfloral population, a tendency of lower *Lactobacillus* spp. was exhibited in FT1 group than that of FT2, FT4 and FT5 group (P<0.10). The yeast and mold count was substantially elevated in FT4 and FT5 in comparison to the FT1 and FT3 (P<0.05). In addition to that, excreta pH did not differ among the fat treatments groups (P>0.05).

Excreta noxious gas emissions

The result of excreta noxious gas emissions was shown in Figure 1, the average NH_3, H_2S and SO_2 gas emissions was found lowest in FT1 relative to the emission from other fat groups (P<0.05). The highest NH_3 and H_2S emission was exhibited in FT4 and lowest in FT1 group (P<0.05). The NH_3 emission was suppressed in FT1 and FT2 than FT3 and FT4 group (P<0.05). Where, the H_2S emission was depressed in FT1, FT2 and FT5 relative to FT3 and FT4 (P<0.05). The SO_2 emission was found highest in FT5 and lowest in FT1 group (P<0.05). The SO_2 emission was also diminished in FT1 than that of the FT2 group (P<0.05); while both in FT3 and FT4 it was lessen relative to FT5 (P<0.05).

DISCUSSION

Growth performance of birds

Appreciable quantities of dietary fat could be utilized by birds as an energy source, which can reduce the rate of passage of the digesta and ensure better utilization of nutrients (Peebles et al., 2000; Baião and Lara, 2005;

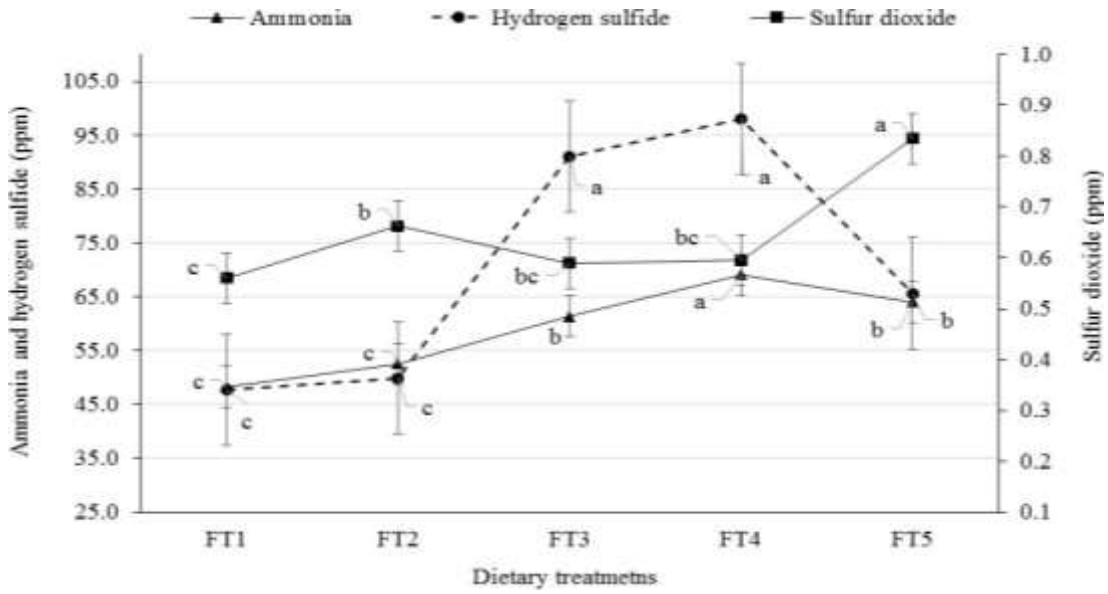

Figure 1. Effect of dietary fat sources on excreta noxious gas emissions in broiler. [a,b,c]Means with different superscripts within the same line are significantly different (P < 0.05). Error bar indicate standard error of the mean. Dietary treatments: FT1: Basal feed with soybean oil); FT2: Basal feed with chicken fat; FT3: Basal diet with tallow; FT4: Basal diet with tallow and lard (1:8): FT5: Basal feed with pork fat/lard.

Latshaw, 2008). The polyunsaturated fatty acid (PUFA) content is generally higher in FT1 followed by FT2, FT5, FT4 and FT3. The major PUFA content in FT1 is linoleic acid (C18:2n-6), while others group contained low to moderate amount. The feedstuffs dominated with unsaturated fatty acids are competent to improve the digestion process; where digestion process can be promoted by the secretion of bile and formation of micelle, consequently intensify the utilization of saturated fatty acids, and finally can impact on the performance augmentation of birds (Fascina et al., 2009). The effects of dietary fat on weight gain are influenced by the age of the birds and the amount of fat added to the diet (Zelenka et al., 1997). It was reported that, addition of fat usually beneficial after the first week of age, because young birds are less able to digest the saturated fatty acids (Leeson and Summer, 1997). In the present study, however, the body weight gain was found higher in case of FT2 group compared to other group during 0 to 3 weeks of experimental period. According to Hulan et al. (1984) after addition of fat in the diet the body weight differences were significant during 28 day but not during 48 day of experimental period, which supports our study. Fatty acid pattern and composition is responsible for the growth performance of birds, where PUFA content reported to be more beneficial than the SFA. Although the PUFA content of FT1 group is higher than the other groups, the FT2 group exhibited higher growth, the reason is not clear to us. The possible explanation could be like this, FT2 might be utilized efficiently or there might be other factors responsible for the growth enhancement in case of dietary fats, warrants further detail study to discover the mechanism.

The degree of unsaturation of the dietary fat can influence the secretion and function of hormones and enzymes (Shall et al., 1989; Guimbaud et al., 1997), and digestibility (Zollitsch et al., 1997). Therefore, growth hormones might be linked with the dietary fat and fatty acids in case of broilers. Manilla et al. (1999) reported higher body weight in broilers those are fed vegetable oils relative to the birds fed animal fats. It has been reported that feed intake can be decreased after addition of dietary fat in broiler chickens (Firman et al., 2010). In the current study, feed consumption tended to be lower in the FT1 and FT4 group than the FT2 group. There was found no significant differences among the animal fat sources on body weight gain, feed intake and feed efficiency during 4 to 5 weeks of experimental period. Supporting to the present study, no significant effect of animal fat was reported by Crespo and Esteve-Garcia (2002) in broiler study. Other studies also reported no significant differences between various fat sources while added to the diet of young and finishing broilers (Pesti et al., 2002; Firman et al., 2008). In the present study, during overall period (0 to 5 weeks), weight gain and feed intake did not differ among fat treatments (although higher numerical value was exhibited in the FT1 and FT2), whereas a tendency of better feed efficiency was observed in the FT1 group than the FT3 and FT5 group, which might be attributable to the presence of more unsaturated fatty acids in the plant fats. Because fats from vegetable origin contain higher unsaturated fats relative to the fats from

animal origin (Waldroup et al., 1995). It was reported that, plant fat such as corn oil, seed oil, or palm oil fed at the rate of 0.5 to 1.0% of the diet improved growth performance and feed efficiency in rats, mice and pigs (Dugan et al., 1997; Ostrowska et al., 1999). These findings are consistent with those of a previous study that showed feed efficiency could be improved in the birds fed diet with plant oil relative to those that received animal fat (Newman et al., 2002).

Although in the current study, the function of hormone and enzyme in the digestive physiology was not tested, however, it could be helpful explanation regarding the improvement feed efficiency. The secretion of the hormone cholecystokinin and prolongation of the gastrointestinal transit time of feed and therefore the presence of enzyme in the digestion and absorption canals may be another mechanism through which fats helps to improve feed efficiency (Hulan et al., 1984; Scheideier and Baughman, 1989). Fatty acids can stimulate the secretion of cholecystokinin hormone secretion (McLaughlin et al., 1998), which stimulates secretion of pancreatic enzymes into the small intestine for further feed utilization into the gastro-intestinal tract. Furthermore, unsaturated fatty acids are more influential to the stimulation of cholecystokinin than saturated fatty acids (Bradford et al., 2008). Since FT1 contains higher levels of unsaturated fatty acids than FT5, the FT1 group might potentially ameliorate the feed efficiency in birds in the current study. The interaction of saturated and unsaturated fatty acid is one of the most important factor which determines the function of combination of fatty acids (Ketels and DeGroote, 1989; Leeson and Summers, 2001). It was stated that interaction of saturated and unsaturated fatty acid is balanced while animal fat and vegetable oils are well mixed (Dvorin et al., 1998). However, in the current study, combination of two animal fat did not exhibit beneficial impact on the performance of broilers.

Immunity of birds

Now-a-days commercial broilers are marketed at 5 weeks of age due to fast growth, which resulting immunosuppression of birds and exposing to different stressors and pathogens under the farm environment (Cheema et al., 2003; Shira et al., 2005). Triggered by the present situation dietary intervention to modulate immunity of birds is important consideration to the animal scientists (Calder, 2001; Klasing, 2007). Immunity of birds in general can be governed by the nutrition, through modulation and regulation of the immune process, cellular activation and movement, anatomical development of lymphoid tissues, intracellular killing of pathogens, synthesis of immunologically active substances (Butcher and Miles, 2002). Immune response of chicken can be significantly affected by dietary fat sources with their fatty acid components (Weiseman, 1984; Fritsche et al., 1991). Different dietary fat sources can affect the immune response of chicken through influencing the lymphocyte proliferation and antibody production, where concanavalin, lipopolysaccharide and pokeweed mitogen is affected by the fatty acids, and consequently the immune system (Fritsche et al., 1991). The combination of animal fats (tallow and lard) in the present study indicated that, due to interaction of fatty acids there might negatively impacted on the serum immunity of the broilers, therefore, the IgM value was lower in FT4 than that of FT1, FT2 and FT5. Fatty acids act as immunomodulatory molecules which shaped on the mediation of cellular communication, elaboration of second messenger and membranal fluidity (Klasing, 1997; Watkins, 1991). Among fatty acids, the n-3 PUFA and n-6 PUFA acts as booster for the immune system (Butcher and Miles, 2002). Oleic acid has anti-inflammatory functions shaped on the activation of different pathways of immune competent cells (Carrillo et al., 2012); linoleic acid can enhance the antibody production in the broilers thereby can influence the immunity of birds (Friedman and Sklan, 1995). It was reported that, conjugated linoleic acid can induce the immune system in chicken and rat (Cook et al., 1993; Wong et al., 1996). After all, the differences in the fatty acids among fats might be attributable to the variation in immunity, however, further detail study could ensure the mechanism.

Excreta microbiology and pH

There is a close interaction between the gastrointestinal tract (GIT) of a host and ingested diet. The gut microbiota benefits the host through providing nutrients from the dietary substrates and thereby modulates the function of the digestive system. Dietary protein or fat can influence the gut microbiota (Knarreborg et al., 2002; Drew et al., 2004). Microbial population can be elevated in the birds those are fed animal fat compared to those received plant fat (Knarreborg et al., 2002). Fat originated from the plant has been reported to diminished the diversity of bacteria (De Wit et al., 2012) through its impact on lipid metabolism-related genes in the distal small intestine and changes in the composition of the bile acid of the host in response to the diet (Devkota et al., 2012; Huang et al., 2013). Consistent to that, in the current study, a tendency of lower *Lactobacillus* count was exhibited in the FT1 relative to FT2, FT4 and FT5 group. Latour et al. (1994) observed in their study that, addition of lard in the diet of broiler can increase the fat content in excreta, which can increase the chance of growing the microbes in the excreta, since the microbial diversity depends on the water activity, nutrient content, oxygen tension and pH of the matrix (Maciorowski et al., 2007). The tendency of difference in the bacterial population might also be affected by the type of fat and fatty acid content, which is

supported by Knarreborg et al. (2002), who reported that, dietary fat sources can affect the ileal microbial population in the chicken. The lower transition time of the intestinal digesta can be influential on the microbial population into the gastrointestinal tract (Rinttilä and Apajalahti, 2013).

The rate of digesta passage is usually reduced by the addition of fat in the diet of chicken; however, due to mixing of two animal fats, the rate of passage might be affected (Peebles et al., 2000; Latshaw, 2008), opined to be influential on the microbial population of the current study. The type of fat indirectly can influence the intestinal microflora through its persuading on viscosity of the digesta, intestinal transit time, and digestion in the small intestine (Danicke et al., 1999). The yeast and mold count difference of FT1 with FT4 and FT5 might be due to the difference in the source of fat (plant and animal); however, difference of FT3 with FT4 and FT5 might be due to the type and compositional difference of the fats. The quantity and quality of fat shape the host physiology and lead to further downstream alterations in the intestinal microfloral count (Patterson et al., 2014). Fatty acids are a necessary waste product required to balance redox equivalent production in the gut environment (van Hoek and Merks, 2012). The variations in the fatty acid composition among fat sources observed in the present study might have swayed the intestinal and consequently the excreta microfloral population. The temperature, moisture, pH and environment can shape the growth of microorganism; especially, the yeast and mold growth is esteemed by the presence of moisture content of the materials (Maciorowski et al., 2007). In the current study, both the feed material as well as the excreta material could be the source of higher yeast and mold count in the fat groups (FT4 and FT5) due to moisture content.

Excreta noxious gas emissions

The emission of odor from the animal industry as well as from broiler industry is common phenomena which affecting the sustainability of the broiler industry. The emission of gases in and around the poultry production facilities can be a health and performance issue for birds and their caretakers (Patterson, 2005). The emission of the noxious gases are important factors and issues to be considered for broiler industry because higher emission of ammonia and sulfur-containing compounds causes poor performance, susceptibility to disease and mortality in broilers (Kristensen and Wathes, 2000; Wang et al., 2011).Therefore, along with performance parameters, microbial and gaseous concentrations were measured in the present study. Diets usually formulated to meet the nutritional requirements, however, undigested components excretion can govern gastro intestinal microbiology, excreta microbiology, pH and moisture content which all combinedly can have impact on the emission of odorous or noxious components (Sharma et al., 2017). Among nutrients, fat sources are provided in major to ensure energy requirements as well as fatty acid sources, however, utilization and excretion of undigested substances of all nutrients as well fats could be varied due to difference in composition. Increased excreta fat content after feeding animal fat (lard) in case of broiler was reported by Latour et al. (1994). Although we did not analyze the fat content of the broiler excreta in this study, however, it is postulated that, the phenomena of increment of fecal fat content might enhance the chance of elevation of ammonia gas in the animal fat group except the FT2 group in comparison to the FT1 group. Dietary addition of fatty acid can affect the energy loss through excreta and liver weight, where energy substrates can influence the microbial and gaseous emissions; and liver function can affect the utilization of nutrients, secretion of enzymes and hormones and consequently the microbial growth and gaseous emissions (Terpstra et al., 2002). An investigation of dietary oil inclusion reported that it can alter ammonia nitrogen emissions from the manure via the effects of fatty acid on microbial activity in the intestine (Leek et al., 2004). Present result apparently indicated that, mixing of two animal fats (FT4: tallow and lard) resulted elevation of both the NH_3 and H_2S gas which is the negative outcome. Such types of result might be due to inappropriate proportion due to degree of unsaturation of fatty acids into that fat.

The emission of noxious gases from the excreta of animals depends on the utilization of nutrients and the gastrointestinal microbial ecosystem (Ferket et al., 2002). Where the other factors are also associated, such as diet, pH and moisture content, chemical environment, and physical-chemical interactions and compositions (Elliott and Collins, 1982; Carr et al., 1990), presence of different microorganisms and numerous gaseous substances (Patterson, 2005). The utilization of the major and minor nutrients usually happens into the gastrointestinal tract (GIT) of the chicken. Fatty acids can influence the secretion of cholecystokinin hormone secretion and consequently secretion of pancreatic enzymes into the small intestine to utilize the nutrients (McLaughlin et al., 1998), where unsaturated fatty acids are more influential to the stimulation of cholecystokinin than saturated fatty acids (Bradford et al., 2008). Cholecystokinin hormone influence to secrete pancreatic enzymes and bile production, where pancreatic enzymes can affect protein, fat and carbohydrate utilization (Bender, 2004). After exhausting of the carbohydrate sources into the intestine, the protein sources materials are utilized as salvage energy (Macfarlane and Macfarlane, 1995). By the action of putrefactive bacteria, proteins and amino acids formed systemic toxins and carcinogens; where the common toxic end products are phenols, indoles, amines and ammonia generated by deamination, fermentation and decarboxylation into the GIT (Macfarlane and Macfarlane,

1995). The concentration of NH_3-N (associated with NH_3 emission) can be decreased by the vegetable oils (pulm oil, linseed oil and whole soybean) by reducing the deaminating bacteria; where microbial growth depends on the presence of energy substrates and fatty acids in the digestive tract (Russell et al., 1992; Doreau and Ferlay, 1995). Among fatty acids, UFA has the negative impact on the bacterial membrane (Maia et al., 2007). Therefore, in the present study, it could be anticipated that, the variant fatty acid content and available energy in the FT1, FT2, FT3 and FT5 than FT4 might causes reduction of NH_3 and H_2S emission with the association of microbes; because the end product of the metabolism and fermentation by the microbes in the lower intestinal part can consequently influence the pH and other associated factors as well (Rinttilä and Apajalahti, 2013).

Addition of fat to the diet can influence the secretion of enzymes which can swayed the metabolism and excretion of fibre, protein and lipid and finally the gaseous emissions (Canh et al., 1998a, b; Evans et al., 2002; Hiraoka et al., 2003). Among the fatty acids, especially the higher content of linoleic acid in the soybean oil and chicken fat (35 and 20%) than the tallow and lard (2% and 11%) can impact on the pancreatic enzyme which can shaped the utilization, fermentation in the GIT and excretion of nutrients, and finally the emission of gases (Evans et al., 2002; Hiraoka et al., 2003; Beccaccia et al., 2015). Hydrogen sulfide is the most prominent volatile sulfur generated by bacterial sulfate reduction and decomposition of sulfur-containing organic compounds under anaerobic conditions (Arogo et al., 2000). Lower efficiency of nutrients utilization causes excretion of unutilized nutrients through the urine and feces, which then undergoes anaerobic microbial decomposition, resulting in generation of odorous compounds (volatile amines and sulfurs, phenols, volatile fatty acids and indoles (Gilley et al., 2000). Volatile sulfur generation by the action of anaerobic bacteria involves reduction of dissimilatory sulfate and metabolism of sulfur containing amino acids (Kiene and Hines, 1995; Ushida et al., 2003). There was emitted higher SO_2 in FT5 of the current study which might be attributed to the excretion of higher fat and other substances in the excreta of broilers (although it was not measured) relative to other fat groups according to the findings of Latour et al. (1994). In addition, since fatty acids affects the secretion of cholecystokinin hormone and pancreatic enzymes and utilization of protein, fat and carbohydrate (Bender, 2004), which can influence the nutrient excretion and results variation in the emission of H_2S and SO_2 gases. Sulfur-containing gas emissions are influenced by the strong interaction between amino acids and carboxylic acids (e.g. methionine and benzoic acid) (Eriksen et al., 2010). The elevation of the sulfur gases might be attributable to the reflection of the excreta microbial population and substrates availability (Levine et al., 1998); where organic compounds like cysteines, mucin and taurocholate bestowed the readily utilizable substrates for the H_2S and SO_2 emission (Levine et al., 1998). As a whole, the variation of NH_3 and H_2S in FT1 and FT2 with FT3 and FT4; and variation of SO_2 in FT1, FT2, FT3 and FT4 relative to FT5 might be attributed to the fatty acid composition, secretion and function of pancreatic enzymes, differences in digestibility of nutrients, and excretion of indigestible nutrients in the feces and urine.

Conclusion

Effects of corn-soybean meal based basal diet with soybean oil (FT1), chicken fat (FT2), tallow (FT3), tallow plus lard (FT4), and pork fat/lard (FT5) on the performance, immunity, excreta microbiology and noxious gas emissions in broilers were investigated. The result of the present study revealed that overall body weight and feed intake did not differ among the fat treatments, however there was found a tendency of better feed conversion efficiency of broilers after feeding FT1 than the other fat groups. The result of the serum immunoglobulins data indicated that, a significant elevation of serum immunoglobulin M (IgM) was observed after dietary addition of FT1, FT2 and FT5 relative to FT4. In addition, for the excreta microbiology, higher yeast and mold count was displayed in FT4 and FT5 group relative to FT1 and FT2 group. Furthermore, excreta noxious gas emissions (NH_3, H_2S and SO_2) were lower in the FT1 and FT2 groups than that of other fat groups. Overall, the results of the present study suggested that FT1 and FT2 can be prioritized in the diet of broilers with positive influence on body weight gain and feed efficiency, and substantial reduction of noxious gas emissions. Further detail study could be conducted to investigate the single and combination of different dietary fats (with different ratio) on performance and meat quality indices.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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