Journal Homepage: - www.journalijar.com
INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)
Article DOI: 10.21474/IJAR01/2348
DOI URL: http://dx.doi.org/10.21474/IJAR01/2348
ISSN NO. 2320-5407

RESEARCH ARTICLE

EVALUATION OFHALAL TALLOW AND HARAM LARDCOMBINATIONS ON GROWTH PERFORMANCE, IMMUNITY, CECALMICROBIOLOGY AND NOXIOUS GAS EMISSIONSIN BOILERS.

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Manuscript Info

Manuscript History

Received: 30 September 2016
Final Accepted: 30 October 2016
Published: November 2016

Key words:-
Growth performance, Internalorgan weight, Cecalmicrobiology, Excreta gaseous concentrations, Broiler.

Abstract

The study was conducted with an aim to study the animal fat as single (halal tallow) and combined state (halal tallow and haram lard combination) on growth performance, relative organ weight, cecal microbiology and noxious gas concentration in broilers on the aspect of halal ethical issue. Following completely randomized design, a total of 96 day old Ross broiler chicks were allocated into two treatments (TLO: Basal diet with tallow; TPL: Basal diet with tallow+lard), where there was six replications with eight birds per replicate pen. Feed and water was provided *ad libitum* with dividing starter (0 to 3 weeks) and finisher (4 to 5 weeks) period. The result revealed that, growth performancedid not differ between TLO and TPL ($P>0.05$); however, immunity (IgM) was higher in TLO in comparison to TPL ($P<0.05$). Among the internal organs, small intestine, large intestine and cecum weight was higher in TLO relative to the TPL ($P<0.05$).In addition, cecal and excreta pH was lower in TLO than the TPL ($P<0.05$); where, among cecalmicrobiology, *Bacillus* sp. count was higher in TLO compared to TPL ($P<0.05$). Excreta noxious gas concentration data revealed that, ammonia gas was lower in TLO during 0 hour, 3 hour and 12 hour incubation period, while the average value also significantly lower in TLO in relation to the TPL ($P<0.05$). The hydrogen sulfide and sulfur dioxide gas did not differ between TLO and TPL ($P>0.05$). Economic analysis data elucidated that, there was no significant difference for cost per unit of gain between TLO and TPL ($P>0.05$). The result suggested that, tallow and lard combination has no negative impact on the growth performance; however, there was higher excreta noxious gas concentration, which is one of the important issues for the environmental aspects from the broiler industry. Therefore, single use of animal fat (halal tallow) for halal broiler production are preferred instead of combination with another animal fat (haram lard).

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Introduction:-

There are different types of fats and oils which are utilized as the feed ingredients for both human and animals. Fats and oils are composed of different chemical constituents which have impact on health. Some of them are from plants and some are from animals. Use of fats for animal feed has many advantages and the principal function of using the fat source is to ensure the energy content of the diet since it is aconcentrated source of energy. Specially, fat supplementation in diets has been proven a valuable method for fulfilling the high energy requirements of rapidly growing broiler chickens. High energy diets have been shown to improve growth and feed efficiency (Zaman et al., 2008; Hosseini-Vashan et al., 2010). Other benefits ofincluding the fat sources in the diet are improvement of the growth rates and feed efficiency, decrement of feed intake, source oflinoleic acid and increment of palatability of feeds (Firman et al., 2008). Fat has extra caloric effect and influence in the nutrient availability of other ingredients (Jensen *et al*., 1970; Horani and Sell,1977), helps to slow gut transit of other feeds, resulting in increased digestibility (Firman and Remus, 1994; Firman et al., 2008). It has been well documented that the growth performance and feed conversion ratio of the broilers are influenced by dietary supplementation with fat (Sahito et al., 2012).

Fat has been shown to be a practical and economical means by which to increase energy levels in poultry diets and stimulate growth (Hill and Dansky, 1954; Donaldson *et al*., 1957; Griminger, 1986; Latour*et al*., 1994). Utilization of fat increased rate of gain and can decrease the age at market (Firman et al., 2008). In previous research, it has been determined that dietary fat supplementation induces an increase in growth and an alteration in meat quality (Cherry, 1982). Sanz et al. (2000a) reported that fat content from 1 to 5% did not improve performance or meat quality in broilers. While it was reported that, different fat sources while used as portion of complete ration there is no significant differences on the performance parameters (Seidler*et al*., 1955; Young, 1961; Fuller and Rendon, 1979; Pesti*et al*., 2002; Quart *et al*., 1992). Carver *et al.* (1955) found that beef tallow was well utilized when added at a level of 3% to a practical broiler ration. Peebles *et al*., (1997) reported feed conversion ratio was improved between 35 and 42 day but during other period no improvement was observed while broilers were provided 3 or 7% lard. However, the effects of dietary fat content remain controversial due to their composition. Fat sources are composed of saturated and unsaturated fatty acids. The function of fat mainly depends on the degree of saturation, number of carbon in the composition of the fatty acid chain, concentration of fatty acids, position of the glycerol molecules, and interaction between the unsaturated and saturated fatty acids (Rener and Hill, 1961; Ketels and DeGroote, 1989; Dvorin et al., 1998; Leeson and Summer, 2001). Among different saturated fatty acids, stearic acid is not utilized well by the chick (Carver and Johnson, 1953); while polyunsaturated fatty acid is utilized wellby the birds (Crespo and Esteve-Garcia, 2001). Differences in fat deposition as the result of different dietary oil levels was reported by Foglia et al. (1994)which might be due the association with metabolic differences between chicken lines (Foglia et al., 1994). An alternative therapeutic approach exploration was explained by Marion-Letellier et al., (2013) where it was reported that, thereis an impact of fatty acid on innate immunity. Supplementation of combination of vegetable and animal fat sources in case of broiler diet was reported beneficial on the aspects of improvement in the growth performance and carcass characteristics (Poorghasemi et al., 2013). Combination of tallow and soybean oil (25:75) was reported best on the aspect of performance without detrimental impact of carcass yield (Fascina et al., 2009). However, combination of different animal fats are beneficial or harmful, there is no detail research has been conducted yet. On the other hand, according halal concept, there is an ethical issue that, halal species of animals must be provided halal feed ingredients without any contamination of haram feed ingredients to ensure halal meat production; and reared separately from the haram species of animals. Therefore, present study was undertaken to investigate the single state of halal tallow and combination of halal tallow and haram lard on growth performance, immunity, internal organ weight, cecal microbiology and noxious gas concentration in broilers.

Materials and Methods:-

Experimental design, dietary treatments and bird's husbandry:-

Experimental birds were reared at the Sunchon National University experimental farm, Suncheon, Republic of Korea. Based on completely randomized design a total of ninety six day old Ross broiler chicks were randomly allocated into two treatments with six replications (eight birds per replicate) in a completely randomized design. Dietary treatments were: 1) TLO (basal diet with halal tallow); 2) TPL (basal diet with halal tallow + haram lard). The basal diet was formulated to meet the Nutrient Requirements of Poultry (following both the National Research Council, 1994, Washington DC, USA and Korean feeding standard for Poultry, 2012). Feed was provided for a total of 5 weeks in two stages: starter (0 to 3 weeks) and finisher (4 to 5 weeks). All diets were in mashed form. The

ingredients, chemical composition, and vitamin and mineral content of the basal diets are shown in Table 1.

The animal fat added with the basal feed ingredients were purchased from Daejon and Suwon local market of Republic of Korea. Halal tallow and haram lard were collected from the abattoir of Suwon and Daejon respectively. Halal tallow was collected from the slaughterhouse, where cattle were slaughtered in halal way, were inspected properly by the veterinarian to ensure disease free. After collection of raw fat, it was chopped and sliced into smaller chunks of equal size (1 inch), then was placed into 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 minutes, followed by an additional 30 minutes 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 manner as 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) (O'fallon, J. V., Busboom, J. R., Nelson, M. L., & Gaskins, C. T. (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.). The fatty acid composition of the experimental fat and oil sources are presented in Table 2.

Birds 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. 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 at 3°C per week, where it was maintained until the end of the experimental period. The internal relative humidity was maintained at around 50% throughout the experimental period.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. 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 for the present study.

Measurement of growth performance:-

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

Collection and analyses of blood samples:-

At the termination of the feeding trial, 2 birds close to the 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 selected birds into a 10-mL anticoagulant-free vacutainer tube (Greiner Bio-One GmbH, Kremsmunster, Austria). The samples were 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). Then, the serum samples were carefully transferred to plastic vials and stored at −20°C until immunoglobulin analysis was performed. The concentrations of serum IgG, IgA, and IgM were assayed using appropriately diluted samples by a sandwich ELISA with chicken-specific IgG (Cat. No. E30–104), IgA (Cat. No. E30–103), and IgM (Cat. No. E10–101) ELISA quantitation kits (Bethyl Laboratories Inc., Montgomery, TX) according to the manufacturer's instructions. 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 IgG, IgA, and IgM were determined using standard curves constructed from the respective immunoglobulin standards and the results were expressed as mg/ml of serum.

Carcass characteristics and internal organ weight:-

Before selection of bird for slaughter, birds were left 12 hours off feed with *ad libitum* water supply. Birds from the each replicated pen were randomly selected based on similar body weight to slaughter. Three birds per replicate pen were slaughtered in halal way following light electrical stunning. After slaughter, bleeding was done for 5 minutes and then internal organs were separated carefully and weight individually. After evisceration and skinning, the carcass weight was taken individually. The carcass traits, breast and thigh meat weight was recorded, internal organ weight were recorded. Breast and thigh muscles of birds were removed by trained personnel and were weighed

individually. Abdominal fat pad (including fat surrounding gizzard, bursa of Fabricius, cloaca, and adjacent muscles) was removed and weighed individually per replication of treatments. Other internal organs: crop, Proventriculus, gizzard, pancreas, spleen, liver, gall bladder, heart, kidney, spleen, and bursa of fabricius weight was measured. After weighing all the meat and organs, relative weight was calculated based on live weight of birds.

Collection and analyses of cecal microbiology:-
Selected chickens were slaughtered at the end of 5th week of experimental period to measure the microflora concentration of caeca, where cecal contents were collected carefully from each bird. Feed withdrawal period of 12 hour were maintained. The collected cecal contents were serially diluted in sterile saline in the 1:10 dilution and then cultured on agar media (duplicate for each). The culture media for total bacteria,*E. coli*, *Salmonella*, *Lactobacillus*, and yeast and mold were Tryptic soy agar, MacConkey Sorbitol Agar; Salmonella Shigella Agar; *Lactobacilli* MRS (Mann, Rogosa and Sharpe) Agar; and Potato Dextrose Agar, respectively. Incubation in the anaerobic condition at 37°C for 24 hours (*E. coli* and *Salmonella)* and 48 hours (Total bacteria, *Lactobacillus* and yeast and mold) were done followed by the smearing of supernatant of 100 µl onto the agar plate. Following enumeration of microbial colonies in the duplicate incubated agar plates, microbial counts were expressed as $\log_{10}$CFU/ml.

Collection of excreta sample and gas measurements:-
After finishing the growth trial, fecal samples (mixtures of feces and urine) were collected from the each replicate pen of all treatments. The total sampled manure from each pen was thawed and then 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 (model AP-20) gas sampling pump (Gastec Corp., Kitagawa, Japan) and Gastec detector tube (No. 3M and 3LA for NH_3; 4LT and 4L for H_2S; and 5Lb for SO_2). 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 48h. The concentration of NH_3,H_2S and SO_2 was expressed as ppm of excreta.

Economic analysis:-
The economic efficacy of the broilers fed TLO and TPL diet was measured following the feed cost for per unit of gain for the starter (0 to 3 weeks), finisher (4 to 5 weeks) and overall period (0 to 5 weeks).

Cost per unit of gain = FCR× unit price of feed
Where,
FCR: Feed conversion ratio
Price of per kg of feed in (Korean currency, KRW; 1 USD = 1,120KRW)

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), internal organ weight, fecal gaseous emissions; whereas an individual bird served as the experimental unit for serum immunoglobulins and cecal microbiology. A probability level of $P<0.05$ was considered as statistically significant and a level of $P<0.10$ was considered as statistical tendency.

Results:-

Growth performance of broilers:-
Table 3 shows the growth performance of broilers after dietary treatments of TLO and TPL, where it was observed that, there was no significant differences on the body weight gain, feed intake and feed efficiency between TLO and TPL fed birds ($P<0.05$).

Serum immunoglobulins in broilers:-
The serum immunoglobulin data was shown in Figure 1, result revealed that, there was no differences on immunoglobulin G (IgG) and Immunoglobulin A (IgA), however, there was difference in immunoglobulin M (IgG)) between TLO and TPL ($P<0.05$).

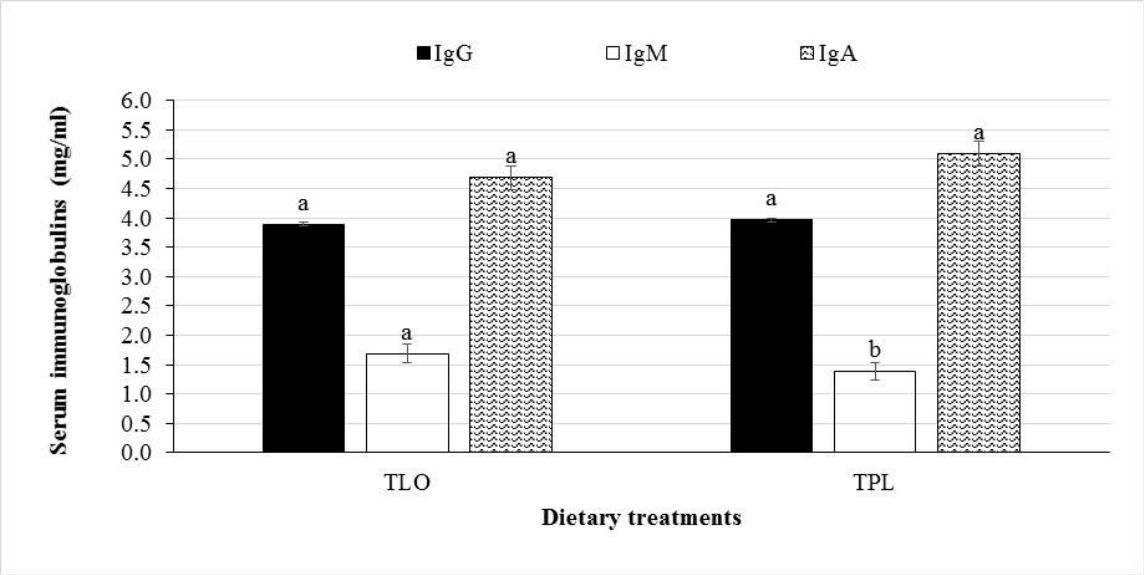

Figure 1:- Effect of tallow and lard combination on serum immunoglobulins in broilers

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

Dietary treatments: TLO: basal feed with halal tallow; TPL: basal feed with halal tallow and haram lard/pork fat

Carcass characteristics and relative organ weight:-
Table 4 shows the carcass characteristics and internal organ weight of TLO and TPL fed broilers. Carcass characteristics data did not differ significantly between TLO and TPL(P>0.05). Among internal organ weight, liver, spleen, pancreas, small intestine and large intestine weight was found higher in TLO relative to TPL fed broilers (P<0.05).

Cecal microbiology, pH and excreta noxious gas emissions:-
Cecal microbiology data indicacted that, the Bacillus count was lower in TLO than TPL (P<0.05) (Table 5). The cecal and excreta pH was lower in TLO in comparison to TPL fed group (P<0.05). Table 6 shows the excreta noxious gas emissions, where it was observed that, ammonia gas was significantly downtrended in the TLO group during 0 hour, 3 hour, and 12 hour of incubation period (P<0.05). Where the average value of ammonia emission also was lower in TLO than the TPL fed group (P<0.05).

Economic analysis:-
The economic analysis data elucidated that, there was no significant difference in the cost per unit of gain between TLO and TPL group (P>0.05) (Table 7).

Discussion:-

Growth performance and Immunity in broilers:-
The result of the present study elucidated that, although there was found a higher numerical value of BWG and FI in the TLO-fed group than the TPL-fed group, there was no significant differences in BWG, FI and FCR between treatments (P<0.05). The result indicated that, there was no remarkable differences between single state (halal tallow) and combined state (halal tallow + haram lard) utilization of animal fat. No significant effect of animal fat was reported by Crespo and Esteve-Garcia, (2001) in broiler study. Other studies also reported no significant differences between various fat sources while fed to young broilers and market broilers (Pesti et al., 2002; Firman et al., 2008). It was reported that, addition of fat usually beneficial after the first week of age (Sibbald, 1978; Lessire et al., 1982); however, impact of combination of animal fats are not well documented. Feed efficiency can be affected by the degree of unsaturation of the dietary fat which influence the digestibility (Alao and Balnave, 1985; Pinchasov and Nir, 1992; Su and Jones, 1993; Zollitsch et al., 1997). 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). Dvorin et al. (1998) reported that, the interaction of saturated and unsaturated fatty acid is balanced while animal fat and vegetable oils are well mixed. Mixture of halal tallow and haram lard might not balance due to improper interaction between saturated and unsaturated fatty acids, which consequently not positively impacted on the performance of the broilers in the current study.Diets supplemented with a combination of tallow and pork fat/lard resultedreduced body weights in female broilers while compared with the birds supplemented with either tallow or lard as single fat sources (Hulan et al., 1984). Where it was reported that, the body weight differences were significant ($P<0.05$) during 28 day of experimental period but not during 48 day of experimental period.Retardation of growth performance in broiler was reported after feeding fatty acids (linoleic acid) in broilers (Belury&Kempa-Steczko, 1997; Szymczyk et al., 2001).

Usually modern commercial broilers are marketed at 5 to 6 weeks of age due to fast growth and there is interacting with the farm environment, which resulting immunosuppression of birds and exposing to different stressors and pathogens (Cheema et al., 2003; Shira et al., 2005). Therefore, dietary intervention of chicken to modulate immunity of birds are important consideration by the animal scientists (Calder, 2001; Klasing, 2007). Immunity of birds in general can be influenced by nutrition, through modulation and regulation of the immune process, cellular proliferation, cellular activation and movement, anatomical development of lymphoid tissues, intracellular killing of pathogens, mucus production and synthesis of immunologically active substances (Butcher and Miles, 2002). Immune response of chicken can be significantly affected by dietary fat sources (Fritsche et al., 1991). Lipids are considered as the fatty acid substrates provider which are necessary for the proper functioning of immune system (Gonzalez et al., 2011). Fat sources affect the proliferation of lymphocyte and consequently the immune system (Fritscheet al., 1991). Fatty acids act as immunomodulatory molecules which is then influence in the mediation of cellular communication, fluidity of membrane and elaboration of second messenger (Klasing, 1997; Watkins, 1991); where the n-3 ad n-6 PUFA are beneficial to the immune system at higher concentration compared to the normal concentration in the diet (Butcher and Miles, 2002).The metabolism of arachidonic acid modulated by the cyclooxygenase and lipoxygenase pathways which resulted prostaglandin and leukotriens synthesis affect the immune system mechanism (Blumberg, 1994).Oleic acid could be reported as an anti-inflammatory fatty acid playing a role in the activation of different pathways of immune competent cells (Carrillo et al., 2012). Lower level of linoleic acid can improve the antibody production in the broilers thereby can influence the immunity of birds (Friedman and Sklan, 1995). Conjugated linoleic acid can stimulate the immune system in chicken and rat (Cook et al., 1993). Impact of conjugated linoleic acid as immunostimulator also reported by Wong et al. (1996).

Carcass characteristics and relative organ weight:-

There was found no significant differences between TLO and TPL in the carcass characteristics like live weight, carcass weight, relative breast and thigh meat weight ($P<0.05$). However, there was observed differences in the internal organ weight of TLO and TPL-fed birds. The increment of the relative small intestine weight in the TLO-fed group relative to TTPL-fed group might reveal the changes in the histology of the gastro-intestinal tract. In general, the increase of the villus height is linked parallally with the increase of digestive and absorptive function of the gastro-intestine due to the increment of absorptive surface area, brush border enzyme expression and transport of nutrient (Pluske et al., 1996). Greater villus height is the indicator of functional intestinal villi being activated (Langout et al., 1999; Shamoto and Yamaguchi, 2000).

Increase of the size of the pancreas can increase the release of lipase lipase enzyme which helps to hydrolyze the fat into monoglycerides and free fatty acids (*Chapus et al., 1988); where free fatty acids stimulate to release the cholecystokinin (CCK) and contracting the gallbladder (*Guimbaudet al., 1997*)*.Pancreatic lipase, also known as pancreatic triacylglycerol lipase, is an enzyme secreted from the pancreas. As the primary lipase enzyme that hydrolyzes (breaks down) dietary fatmolecules into the Gastro-intestinal tract, it is one of the main digestive enzymes, converting triglyceride substrates found in ingested oils to monoglycerides and free fatty acids.Sheppard et *al.* (1980) have shown that relative liver weight in broilers is suppressed with utilization of fat in the diet.The lower liver weight in the TPL-fed group than the TLO-fed group might unable to secret more bile and influence the formation of micelle, thereby unable to enhance the absorption of saturated fatty acids and not affected the performance of the birds in the current study. The presence of fatty acids represent important role in the secretion and function of bile and formation of micelle. Feedstuffs dominated in unsaturated fatty acids are influential in the improvement of the digestion process by increasing bile secretion for micelle formation, enhancement of the absorption of saturated fatty acids, benefiting the bird's performance (Fascina et al., 2009).Bile salts secreted from the liver and stored in gallbladder are released into the duodenum, where they coat and emulsify large fat droplets into smaller droplets, thus increasing the overall surface area of the fat, which allows the lipase to break apart the fat

more effectively. The resulting monomers (2 free fatty acids and one 2-monoacylglycerol) are then moved by way of peristalsis along the small intestine to be absorbed into the lymphatic system by a specialized vessel called a lacteal (belongs to the pancreatic lipase family).

Higher weight of spleen in the TLO-fed group in the current study might indicate more activities in filtering of blood cell which can elevate the antibodies and immunity of birds and consequently improve the livability of birds (Walsh et al., 1994). The important function of spleen is removal of harmful bacteria and viruses into the blood stream. The white pulp of spleen (consist of Malphigian capsules) associated with the immune system through producing antibodies of immunoglobulins, neutralizing the harmful antigens of bacteria and viruses into the blood vessel (The spleen and its role in immune function; retrieve from http://www.laparoscopic.md/digestion/spleen). Higher cecal weight in the current study in the TPL-fed birds than the TLO-fed birds might indicated the presence of higher bacterial community, since ceca is one of the important source of microbial abundance among the gastrointestinal tract of birds (Barnes, 1979; Lee & Newell, 2006; O'hara& Shanahan, 2006).

Cecal microbiology, pH and excreta noxious gas emissions:-

The gastrointestinal tract of bird is considered as the microbial metacommunity which is comprised of several local microbial communities. Each of the gastrointestinal compartment is composed of unique physico-chemical characters and have the special microbial concentration (Dethlefsen et al., 2007). In the upper gastrointestinal tract the majority of the microbes is the lactobacilli, representing 80 to 90% of the total bacterial, while the other bacteria are *enterobacteria* and *enterococcus* (Lu et al., 2003; Apajalahti and Kettunen, 2006). Where in the lower intestine the majority of the microorganisms are under the order *Clostridiales*, representing 50% of the cecal bacteria, and the other bacteria are under the order *Bacteroidales* and *Lactobacillales* (Apajalahti and Kettunen, 2006; Zhu et al., 2002). There was found higher cecaltotal and *Bacilli* count in the TPL-fed broilers group than that of TLO-fed group. The higher cecal weight might be influential in the higher total bacterial population and *Bacilli* population in the current study in case of TPL-fed birds. The lower pH and lower transition time of the intestinal digesta can be influential in the total number of 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, therefore the microbial population was differed between TLO and TPL group in the present study (Peebles et al., 2000; Latshaw, 2008).

After exhausting of the carbohydrate sources into the cecum of chicken, the protein sources materials are utilizied as salvage energy (Macfarlane et al., 1992). By the action of putrefactive bacteria, proteins and amino acids formed systemic toxins and carcinogens; where the common toxic end products are indoles, phenols, ammonia and amines generated by fermentation, deamination and decarboxylation into the gastrointestinal tract; among them ammonia and amines have negative impact on the chicken's physiological process (Macfarlane et al., 1992; Macfarlane and Macfarlane, 1995;Capano, 1994). The end product of protein fermentation by the microbes in the lower intestinal part resulted to elevation of the pH (Rinttilä and Apajalahti, 2013). The general hypothesis is that, the lower pH is beneficial for the gut health of the chicken, because, acid-sensitive pathogenic microbes are suppressed with lower pH of the gut while higher pH causes opposite phenomena (Apajalahti, 2005). In the current study, the pH of the TPL-fed group was higher than the TLO-fed group, which might be attributed to the function of the cecal microbes on the protein and amines and formation of toxic end products, specially the ammonia and amines. The result of the ammonia concentration of the current study also indicated that, there was higher average ammonia concentration in the TPL-fed group relative to TLO-fed group. Ammonia emitted from poultry litter is influenced by a number of factors, including diet, the chemical environment, pH and moisture content of the litter, and physical-chemical interactions occurring in the litter (Taraba *et al*., 1980; Elliott and Collins, 1982; Carr*et al*., 1990), endotoxins, microorganisms, and numerous gases (Patterson, 2005). Poultry excreta as the combination of feces and urine, the ammonia emission is generally happened withportion of ammonia which is originated from decomposition of urea nitrogen in the urine with the presence of urease producing microbes; and some portion of ammonia is originated with the action of non-urease producing bacteria and other microorganisms (Vince *et al*., 1973; Muck and Steenhuis, 1981). 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). Ammonia (NH_3) emissions have the potential to contaminate surface waters and are an environmental concern on both a local and global scale. These emissions in and around poultry production facilities can be a health and performance issue for birds and their caretakers (Patterson, 2005). Therefore, along with performance parameters, microbial and gaseous concentrations were measured in the present study.

Ammonia is produced either by hydrolysis of urea by bacterial urease or by deamination of protein and other nitrogenous substances (Wolpert et al., 1970). The main portion of ammonia which is originated from decomposition of urea nitrogen in the urine with the presence of urease producing microbes like *Bacteriodes, Proteus* spp.,*Bifidobacteria* and other microorganism (Muck and Steenhuis, 1981). Among other bacteria, *E.coli* do not have urease activity, therefore, in that case ammonia is released by the deamination process of organic nitrogen rather than urea (Vince et al., 1973). It has been reported that uric acid produced in the chicken liver is partially excreted into the intestine and hydrolyzed into ammonia by microbial urease (Wrong, 1981; Karasawa et al., 1988). Growth of urease-producing bacteria (Yeo and Kim, 1997) either by influencing the pH and subsequently reducing ammonia levels in chicken ceca (Endo and Nakano, 1999; Endo et al., 1999). The higher cecal weight and higher pH in the TPL group in comparison to TLO group might be attributed to the lower emission of NH_3 from the excreta of broilers. Variation in the diet with fatty acid content may influence the pH of cecal and excreta thereby reduces the gaseous emissions.

Economic analysis:-

Combination of fat sources might be beneficial with the hypothesis that, synergistic action in the absorption of saturated fatty acids with the added amount of unsaturated fatty acids (Ketels et al., 1986; Ketels and DeGroote, 1987); however, if there is no balance between saturated and unsaturated fatty acids, there might be no synergisms. The nonsignificant feed efficiency and cost per unit of gain between TLO-fed group and TPL-fed group might indicated the absence of synergism due to combination of two animal fats (halal tallow and haram lard).

Table 1:- Feed ingredients and chemical composition of the experiment diets

Item	**Starter (d 1 to 21)**			**Finisher (d 22 to 35)**	
Ingredients	**TLO**	**TPL**		**TLO**	**TPL**
Corn	50.00	50.00		56.00	56.00
Soybean meal	37.00	37.00		28.84	28.84
Corn gluten meal	0.50	0.50		1.00	1.00
Wheat-10%	6.00	6.00		7.00	7.00
Limestone-Small	2.03	2.03		1.92	1.92
Salt-Proc	0.25	0.25		0.25	0.25
DCP-18%	0.40	0.40		0.46	0.46
L-lys Sulfate 70%	0.30	0.30		0.18	0.18
Minemix	0.20	0.20		0.20	0.20
Vitamix	0.05	0.05		0.05	0.05
L-threonine-98%	-	-		0.01	0.01
MHA-Liquid	0.26	0.26		0.29	0.29
Sunphase5000FTU	0.01	0.01		0.01	0.01
Tallow	3.00	-		3.85	-
Lard	-	-		-	-
Tallow + Lard	-	3.00		-	3.85
Calculated composition					
ME (kcal / kg)	3,090.20	3,090.20		3,210.90	3,210.90
Crude protein (%)	22.04	22.04		19.00	19.00
Crude fat (%)	5.35	5.35		6.23	6.23
Crude ash (%)	5.71	5.71		5.26	5.26
Crude fiber (%)	2.59	2.59		2.39	2.39
Ca (%)	1.09	1.09		1.04	1.04
Phosphorus (%)	0.45	0.45		0.43	0.43
Lysine (%)	1.34	1.34		1.07	1.07
Methionine (%)	0.57	0.57		0.55	0.55

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).

Table 2:- Fatty acid composition of experimental fats

Fatty acids (% of total fatty acids)	TLO	TPL
Myristic acid (C14:0)	4.23	8.84
Palmitic acid (C16:0)	25.12	25.70
Stearic acid (C18:0)	17.26	15.57
Palmitoleic acid (C16:1n-7)	3.97	6.74
Oleic (C18:1n-9)	37.12	41.74
Eicosenoic acid (C20:1n-9)	1.87	0.78
Linoleic acid (C18:2 n-6)	5.44	7.67
Linolenic acid (C18:3 n-3)	3.64	2.78
Arachidonic acid (C20:4 n-6)	2.12	1.84
SFA	46.61	51.26
MUFA	42.96	44.26
PUFA	11.20	10.29

Dietary treatments: TLO: Corn-soybean meal based basal diet + Halal tallow; TPL: Corn-soybean meal based basal diet + Halal tallow and haram lard.

Table 3:- Effect of tallow and lard combination on growth performance in broilers

Item	**TLO**	**TPL**	**SEM**	**P-value**
IBW(g/bird)	39.672	39.782	0.389	0.848
FBW (g/bird)	1,772.580	1,723.330	43.443	0.450
0 to 3 weeks				
BWG (g/bird)	634.426	587.784	21.035	0.167
FI (g/bird)	914.192	857.326	21.965	0.122
FCR	1.444	1.462	0.019	0.518
4 to 5 weeks				
BWG (g/bird)	1,098.480	1,095.770	33.380	0.956
FI (g/bird)	1,880.880	1,866.760	46.502	0.836
FCR	1.716	1.702	0.023	0.692
0 to 5 weeks				
BWG (g/bird)	1,732.910	1,683.550	43.552	0.450
FI (g/bird)	2,795.070	2,724.080	56.727	0.402
FCR	1.615	1.618	0.016	0.871

[a,b]Means with different superscripts within the same line are significantly different ($P < 0.05$).
SEM = standard error of mean.

Dietary treatments: TLO: basal feed with halal tallow; TPL: basal feed with halal tallow and haram lard/pork fat

Table 4:- Effect of tallow and lard combination on carcass characteristics and internal organ weight in broilers

Item	**TLO**	**TPL**	**SEM**	**P-value**
Live weight at slaughter (g)	1,503.440	1,501.930	46.242	0.982
Carcass weight (g)	1,032.600	1,031.950	32.440	0.989
Breast meat (%)	14.954	14.868	0.362	0.872
Thigh meat (%)	13.686	13.824	0.140	0.548
Internal organ weight (% of live weight)				
Crop	0.248	0.251	0.006	0.741
Proventriculus	0.413	0.408	0.010	0.748
Gizzard	2.021	2.212	0.082	0.207
Heart	0.497	0.530	0.024	0.385
Liver	1.885a	1.854b	0.007	0.022
Gall bladder	0.153	0.154	0.013	0.966
Spleen	0.076a	0.070b	0.002	0.056
Pancreas	0.263a	0.238b	0.002	0.0297
Smallintestine	2.861a	2.045b	0.062	0.001

Large intestine	0.168a	0.127b	0.010	0.037
Cecum	0.440b	0.609a	0.031	0.006
Kidney	0.737	0.771	0.036	0.521
Bursa	0.253	0.316	0.022	0.083
Abdominal fat	1.443	1.610	0.075	0.169

[a,b]Means with different superscripts within the same line are significantly different (P < 0.05).
SEM = standard error of mean.

Dietary treatments: TLO: basal feed with halal tallow; TPL: basal feed with halal tallow and haram lard/pork fat

Table 5:- Effect of tallow and lard combination on cecal microbiology and pH in broilers

Item	**TLO**	**TPL**	**SEM**	**P-value**
Cecal microbiology (log_{10}CFU/g)				
Total bacteria	11.12b	11.921a	0.121	0.032
Lactobacillus sp.	10.017	10.067	0.077	0.661
Yeast and mold	9.888	9.848	0.035	0.450
Bacillus sp.	10.008b	10.275a	0.040	0.001
E. coli	8.018	7.982	0.145	0.865
Salmonella	7.912	8.073	0.062	0.147
Cecal pH	5.494b	6.129a	0.114	0.005
Excreta pH	5.68b	6.234a	0.213	0.021

[a,b]Means with different superscripts within the same line are significantly different (P < 0.05).
SEM = standard error of mean.

Dietary treatments: TLO: basal feed with halal tallow; TPL: basal feed with halal tallow and haram lard/pork fat

Table 6:- Effect of tallow and lard combination on excreta noxious gas emissions in broilers

Item	**TLO**	**TPL**	**SEM**	**P-value**
NH_3				
0 hour	31.800b	46.200a	2.185	0.001
3 hour	22.400b	36.067a	0.971	0.001
6 hour	58.300	59.333	4.662	0.881
12 hour	61.600b	72.333a	2.440	0.012
24 hour	77.400	80.000	2.672	0.509
48 hour	116.600	120.600	1.879	0.167
Average	61.350b	69.087a	1.148	0.001
H_2S				
0 hour	61.500	64.200	4.474	0.679
3 hour	150.000	148.000	11.420	0.918
6 hour	106.000	115.200	11.493	0.590
12 hour	122.500	129.600	9.608	0.613
24 hour	66.400	85.468	12.052	0.290
48 hour	39.600	45.600	4.090	0.359
Average	90.998	98.012	3.876	0.306
SO_2				
0 hour	0.450	0.420	0.014	0.180
3 hour	0.550	0.550	0.018	1.000
6 hour	0.550	0.575	0.050	0.791
12 hour	0.675	0.650	0.041	0.715
24 hour	0.660	0.700	0.032	0.400
48 hour	0.650	0.675	0.059	0.773
Average	0.589	0.595	0.025	0.881

SEM = standard error of mean.
Dietary treatments: TLO: basal feed with halal tallow; TPL: basal feed with halal tallow and haram lard/pork fat

Table 7:- Effect of tallow and lard combination on economic analysis in broilers

Item	TLO	TPL	SEM	P-value
Cost per unit of gain (USD)				
0 to 3 week	0.582	0.589	0.007	0.536
4 to 5 weeks	0.692	0.687	0.009	0.726
0 to 5 weeks	0.651	0.653	0.006	0.872

Significance level considered at $P < 0.05$; SEM = standard error of mean.

Dietary treatments: TLO: basal feed with halal tallow; TPL: basal feed with halal tallow and haram lard/pork fat

Conclusion:-

The animal fat in single state (TLO: halal tallow) and combined state (TPL: halal tallow along with haram lard) was evaluated to investigate the growth performance, immunity, carcass characteristics, internal organ weight, cecal microbiology and excreta noxious gas emissions in broilers. The result of the present study elucidated that, the growth performance and carcass characteristics did not differ between TLO-fed and TPL-fed birds; however, immunity (serum IgM) was better in TLO-fed group relative to TPL-fed group. In addition, among internal organ relative weight, liver, spleen, pancreas, small intestine and large intestine weight was lower in TLO-fed group in comparison to TPL-fed group; whereas, cecal weight was lower in TLO-fed group in relation to TPL-fed group. Furthermore, the excreta pH, total bacteria and *Bacillus* sp. count was lower in TLO-fed group compared to TPL-fed group. The excreta noxious gas concentration (NH_3) was substantially lower in the TLO-fed group relative to TPL-fed group; whereas H_2S and SO_2 did not differ significantly between the treatments. Moreover, economic analysis data depicted that, there was no significant difference on the aspect of cost per unit of gain between the TLO-fed and TPL-fed group. Present study suggested that, combination of animal fat (halal tallow and haram lard) exhibited no further benefit on the aspect of productive performance, rather it increases the risk of higher ammonia gas concentration in broilers. Therefore, according to halal ethical issue, single state of halal tallow utilization could be preferred instead of combined state (with haram lard) for halal broiler meat production.

Acknowledgements:-

This work was conducted with the support of the Cooperative Research Program for Agriculture Science & Technology Development (Project title: Development of livestock production manual for halal certification and substantiation studies (Phase 1), Project No. PJ0122822016), Rural Development Administration, Republic of Korea.

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