EFFECT OF MEDIUM CHAIN TRIGLYCERIDES ON GROWTH PERFORMANCE AND MICROFLORA COUNT ON BROILER CHICKENS

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ABSTRACT

This experiment was conducted at the Poultry Unit of Faculty of Agriculture, University Putra Malaysia for two weeks to determine the effect of giving the Medium Chain Triglyceride (MCT) on the growth performance and the number of microflora count in the starter broiler chicken. Initial body weight, final weight, average weight gain, feed intake ratio and mortality were used to determine growth performance while the concentration of enterobacteriacae and lactic acid concentration were used to determine the number of microflora count. Results showed that the highest MCT concentration had significant influence ($p<0.05$) the final body weight, diet (T5) had the highest final body weight of 488.0 g compared with the control (T1-0%), 466.9 g. In addition, the results showed significant different ($p<0.05$) for average daily gain. T5 diet had the highest average daily gain of 31.42 g comparing with T1, 30.08 g diet control. As for food intake, the feed conversion ratio and mortality rate showed no significant different ($p<0.05$), T1 diet had the highest intake of 534.7 g compared with T2, 513.7 g. While T1 has the highest feed conversion ratio of 1.271 compared with T5, 1.199. For the percentage of deaths among diets is 0%. For the results of the microflora count parameter show significant different ($p<0.05$), T2 has the highest value of 5.96 ± 0.03 Log CFU/g compared to diet T5, 4.82 ± 0.10 Log CFU/g for Enterobacteriacae concentration. T4 has the highest value in lactic acid bacteria concentration of 8.40 ± 0.01 Log CFU/g compared to T1, 7.83 ± 0.05. In conclusion, supplementing 0.8% MCT in starter commercial diet significantly ($p<0.05$) increase final body weight, average daily gain and lactic acid bacteria count and significantly ($p<0.05$) decrease enterobacteriacae bacteria count in starter broiler chicken. However, the MCT (0.2-0.8%) in the diet significantly did not significantly ($p<0.05$) influence the feed intake, feed conversion ratio and mortality rate.

KESAN PEMBERIAN TRIGLISERIDA RANTAI SEDANG (MCT) KEATAS PRESTASI PERTUMBUHAN DAN BILANGAN MIKROFLORA PADA AYAM PEDAGING PEMULA

ABSTRAK

Eksperimen ini telah dijalankan di Unit Poltri, Fakulti Pertanian,Universiti Putra Malaysia selama dua minggu bagi menentukan kesan pemberian Trigliserida Rantai Sedang (MCT) sebanyak 0%, 0.2%, 0.4% 0.6% dan 0.8% keatas prestasi pertumbuhan dan bilangan mikroflora pada ayam pedaging pemula. Parameter berat badan awal, berat badan akhir, purata kenaikan berat badan harian, pengambilan makanan, nisbah pertukaran makanan dan kadar kematian digunakan bagi menentukan prestasi pertumbuhan manakala kepekatan enterobakteria dan kepekatan asid laktik digunakan untuk menentukan bilangan mikroflora. Keputusan menunjukkan ($p<0.05$) diet T5 yang paling tinggi kandungan MCT (0.8%) mempunyai berat badan yang paling tinggi 488.0 g berbanding dengan kawalan (T1-0%), 466.9 g. Selain itu, keputusan menunjukkan ($p<0.05$) diet T5 mempunyai purata kenaikan yang paling tinggi, 31.42 g, berbanding dengan kawalan diet T1, 30.08 g. Bagi keputusan parameter untuk pengambilan makanan, nisbah pertukaran makanan dan kadar kematian menunjukkan tidak ketera dengan ($p<0.05$) iaitu diet T1 mempunyai pengambilan makanan yang paling tinggi, 534.7 g berbanding dengan T2, 513.7 g. Manakala T1 mempunyai bagi nisbah pertukaran makanan paling tertinggi iaitu 1.271 berbanding dengan T5, 1.199. Bagi peratusan kematian di antara diet adalah 0%. Bagi keputusan parameter bilangan mikroflora menunjukkan ($p<0.05$) ,T2 mempunyai nilai paling tinggi iaitu 5.96 ± 0.03 Log CFU/g berbanding T5, 4.82 ± 0.10 Log CFU/g dalan kepekatan enterobakteria . T4 mempunyai nilai paling tinggi dalam kepekatan asid laktik iaitu 8.40 ± 0.01 Log CFU/g berbanding dengan T1, 7.83 ± 0.05 . Kesimpulannya penambahan 0.8% MCT dalam diet komersil peadaging pemula meningkatkan berat badan akhir dengan ketara ($p<0.05$), meningkatkan purata harian dan bilangan mikroflora Bakteria Laktik, dan dengan ketara ($p<0.05$) menurunkan bilangan mikroflora bakteria enterobakteria ayam pedaging pemula. Walau bagaimanapun, MCT (0.2-0.8%) dalam diet ketaranya ($p<0.05$) tidak mempengaruhi pengambilan makanan, nisbah penukaran makanan dan kadar kematian.

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LIST OF SYMBOLS, UNIT AND ABBREVIATION

%	Percentage
<	Less than
ANOVA	Analysis of Variance
AVG	Average Daily Gain
CFC	Cumulative Feed Conversion Ratio
CFI	Cumulative Feed Intake
cm	Centimeter
CWG	Cumulative Weight Gain
FBW	Final Body Weight
g	Gram
GC	Gas Chromatography
GLM	General Linear Model
H_0	Null Hypothesis
H_a	Alternative of Variance
IBW	Initial Body Weight
Kg	Kilogram
L	Liters
LCT	Long Chain Fatty Triglycerides.
MCT	Medium Chain Triglycerides
mL	Milliliters
SSA	School of Sustainable Agriculture
UPM	University Putra Malaysia
USDA	United States Department of Agriculture (USDA)

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Based on the study conducted by Mohd Syauqi *et.al.* (2015) show that Malaysian preferred meat as source of protein such as broiler chicken meat. On the year 2010, broiler production make up to 53.2% of the total livestock production that value about RM10.85 billion (Tapsir *et al.*, 2011). Broiler Industry in Malaysia has reaches its self-sufficient level of 128.1% in 2011.The concern of broiler industry drastically changes due towards presence of common disease within Asian country into such as Newcastle disease, infectious bursal disease, infectious bronchitis, Marek's disease and leukosis, fowlpox virus, infectious coryza, fowl cholera and aspergillosis. The most common zoonotic disease that being reported are *Salmonella enteriditis* and *typhimurium* that concern the health issue of the consumer (MoA, 2011).

Afford to maintain the sufficient usage of antibiotic are highly use as feed additives in promoting growth performance of the broiler chicken by gradually regulate the presence of disease. The rise concern of using antibiotic in developing antibiotic resistance that being reported by Guerra *et al.* (2007). Alternative use of replacement of antibiotic are using Medium chain fatty acids (MCT) that can be describes as organic acids that used as alternatives feed supplement in promoting growing performance that claimed by European Union (EU) in 2006, that beginning to ban the use of antibiotic as feed supplement for the growth of the chicken. In which clearly reported that the function of MCT has also strong antibacterial activity that able to improve the growth of

newly hatch chicks that claim by Hanczakowska (2017) that can be used as feed supplements on chicken diet for improvement of growth performances.

Hence, in order to maintain the 121% self-sufficiency of broiler chicken in Malaysia, there is a requirement to understand the effect of Medium Chain Triglycerides as feed supplement on poultry production to sustain the poultry industry and the value of this enterprise as well as its financial benefits to Malaysian economy.

1.2 Justification

Salmonella infection cause by several strains of salmonella such as *Salmonella gallinarum* (SG) and *Salmonella pullorum (SP)* that cause health deterioration such as fowl typhoid that lead death of a chicken. Basically the infection are severe on young chicks due to readily colonized by the bacteria, and the bacteria can persist in the host for some weeks or during rearing period that claimed by Revolledo *et.al.* (2006).

If this project is successful, the farmer will taking issues of exposing of chicken to high antibiotic usage induces an increase bacteria resistance. Therefore, conducting a research on investigating the effect of Medium Chain Triglycerides will gradually changes the microbial presence within the gut environment and also growth performances will be really meaningful to increase the production of poultry in Sabah and Malaysia.

1.3 Objective

1) To determine the effect of Medium Chain Triglycerides supplementation in the diet on growth performance of starter broiler chicken.

2) To determine the effect of Medium Chain Triglycerides supplementation in the diet on microflora count in starter broiler chicken

1.4 Hypotheses

H_o: There is no significant difference in the growth performance of starter broiler chicken as given Medium Chain Triglycerides as feed supplement

H_A: There is significant difference in the growth performance of starter broiler chicken as given Medium Chain Triglycerides as feed supplement

H_o: There is no significant difference in the microflora count in the gut of starter broiler chicken as given Medium Chain Triglycerides as feed supplement

H_A: There is significant difference in the microflora count in the gut of starter broiler chicken as given Medium Chain Triglycerides as feed supplement

CHAPTER 2

LITERATURE REVIEW

2.1 History of Broiler Production in Malaysia.

Based on the study conducted by Mohd Syauqi *et al.* (2015) showed that Malaysian preferred meat as source of protein such as broiler chicken meat. On the year 2010, broiler production make up to 53.2% of the total livestock production that value about RM10.85 billion (Tapsir *et al.*, 2011). Broiler Industry has reaches its self-sufficient level of 128.1% in 2011 (MoA, 2011).

In review conducted by several authors Panda, (1989); Hu, (1990); Ideris, (1993) indicated the most common disease presence in Asia Country can be divide into several such as Newcastle disease, infectious bursal disease, infectious bronchitis, Marek's disease and leukosis, fowlpox virus, infectious coryza, fowl cholera and aspergillosis. The most common zoonotic disease that being reported are Salmonella enteriditis and typhimurium that concern the health issue of the consumer.

The most common loss in broiler industry such as intensification of poultry industries inevitably increases the prevalence of disease and losses that claimed by Biggs (1982). Besides that, report indicate the loss of broiler industry due to intensification recorded up to 20% of the value of poultry production, and about 3 times the losses from mortality in the USA. Whereas it's also that happen to the country of Asia. Therefore to prevent losses in broiler industry afford of controlling disease is highly require.

2.2 Microbiome Affects Intestinal Morphology and Physiology of Chicken

On the basis of the development of intestinal region of the newly hatch chicks is depend on diet uptake by the young chicken. Importance of early development of intestinal has significant affect the growth and health of the starter broiler chickens due to transition stages in between nutrient source from the yolk to carbohydrate- and protein-based diet that claimed by Gilbert *et al.* (2010). Therefore, rapid transition stages require rapid adaptation by the chicks through changes in both anatomical and physiological of digestive organs of the newly hatch chicks that cause early post-hatch period as a critical stage in the growth of chicks that reported by Uni *et al.* (1999).

Ideal niche for microbial colonization to begin is during rapid transition stages. The presence of microbial colonization within the intestinal changes the rate of development of anatomical and physiological of digestive organs of the starter broiler chickens. In previous study conducted by Furuse and Okumura (1994) using germ-free chickens as a comparison of commercial chicken. The result shows that the germ-free chickens have intestinal morphological of the small intestine and cecum with reduced weight and also thinner Gabriel and Mallet (2006). Besides that, comparison of germ free chicken and commercial chicken shows that intestinal villi are shorter and the crypts are shallower in germ-free chicken that claimed by Gabriel and Mallet (2006). In other study conducted by Chae *et al* (2012) indicate as the chicken is given with feed with probiotic microorganism such as *Lactobacillus acidophilus*, *Bacillus subtilis*, and *Saccharomyces cerevisiae* has improvement of villus height in duodenum and villus height: crypt depth ratio in the ileum of broilers.

The presence of enteric pathogens has influenced the morphological development of the intestine. The example presence of enteric pathogens such as *Eimeria. perfringens*-induced necrotic enteritis causes major changes of the morphological of the digestive organs, the example changes such shorter development of the villus structure and crypt depth. In the study conducted by Fasina *et al.* (2010) *indicate* that chicken with inducing of *Salmonella typhimurium* shows a poor development result of villus height, villus area, crypt depth, and villus height: crypt depth (Fasina *et al.,* 2010*).* The development of digestive organs is highly important in the

process of absorption of nutrient uptake. Therefore, a better development of digestive organs is highly depend upon the concentration presence of the types of microbe's colonization.

2.3 Importance Mucin Fluid on Growth Performance of Broiler Chicken

Mucin can be considered as a protective layer that surrounds the inner surface of the avian gut that stated by Forder *et. al.* (2012). Role of mucin layer as protective layer through the function of both loose part and the compact part of mucin. The loose part of the mucin gradually acts as a site for the colonization of bacteria to take place, while the compacted part of the mucin acts as counterpart on repelling most of the bacteria. The compacted part of the mucin acts a degree of the 1st line of defends in the protection of the chicken from harmful bacteria that can cause disease that might affect growth performance of the chicken (Hansson and Johansson, 2010). The example of the type of mucin is Mucin2 (MUC2) that require to replenish and maintain a suitable thickness of the mucous layer in the intestine, the mucous layer of the intestine will gradually be sloughed off during intestinal movement that claimed by Dharmani *et al.* (2008); Horn *et. al.* (2009).

The basic of development of better immune system for the growth performance of the chicken are highly depend upon the colonization of intestinal microflora. The intestinal microflora plays a major role in depend Mucin via stimulation mucin gene expression (Smirnov *et al.*, 2005). The maintaining of high replenish of mucin as protective layer, give rise to resistance towards the entry of *Salmonella* and also *camplyobacteria* that allow better intestinal function, growth performance of the chicken, feed conversion and weight gain (Dalloul *et al.*, 2003; Vila *et al.*, 2009;). The example taken by a study conducted by Smirnov *et. al.* (2005) indicate the beneficial microorganism by probiotic bacteria has the role of producing more MUC2 gene in the chicken jejunum as the major defense system of the chicken. Therefore knowing the content of mucous layer mucin presence at the avian gut wall is important as protection for the chicken against the entry of harmful bacteria. The content of mucin is regulated by the MUC2 genes that highly depend on presence of the types of microbe's presence within the gut of the avian.

2.4 Importance of Lactic Acid Bacteria on Growth Performance of the chicken

Antibiotic are highly used as feed additives in promoting growth performance of the broiler chicken. The rising concern of using antibiotic in developing antibiotic resistance that being reported by Guerra *et. al.* (2007). Alternative use of replacement of antibiotic by the introduction of a probiotic product as feed additives gives good response to the researcher. Lactic Acid Bacteria has to played a major role in regulates the microbial environment within the gut such as production of beneficial antimicrobial compounds mainly organic acids and bacteriocins that gives rises LAB uses as a probiotic product as compare towards other microorganisms (Marteau *et al.,* 1993; Salminen *et al., 1998*).

The example of two species of LAB such as *Lactococcus lactis* CECT 539 or *Lactobacillus casei* CECT 4043 and their ferment product show great influence on improvement of growth performances such as feed conversion efficiency that stated by Apata. (2009). Lactic acid bacteria is important as shown to be regulating the concentration of the composition of the intestinal microflora that affecting the immunity status of the chicken in an enhancement of improving the growth performance of the chicken. Guerra *et al.* (2007) claimed by using two strains of mention Lactic Acid Bacteria have the beneficial effect on the growth of chicken. Beneficial effect that gradually mentions are nonpathogenic, able to survive during processing and storage, resistant to bile and acid environment and producer of inhibitory compounds (organic acids and antibacterial activity).

Mode of action of Lactic Acid Bacteria in regulating gut microflora environment in reducing the incidence of disease for poultry. The action are divided into 4 mechanisms that claimed by several researchesr such as maintaining normal intestinal microflora by competitive exclusion and antagonism that claimed by Nurmi and Rantala (1973); Jin LZ *et al.* (1998), altering metabolism by increasing digestive enzyme activity and decreasing bacterial enzyme activity and ammonia production claimed by Cole *et. al.* (1987); Yoon *et al.* (2004), improving feed intake and digestion reported by Dierck (1989); Awad *et al.* (2006) and stimulating the immune system suggested by Kabir *et. al.* (2004). Induction of Lactic Acid Bacteria into the gastrointestinal tract promoted numerous attraction of enzymes into substances into the intestines for development of intestinal

milieu. The example such as, in a study conducted by Marteau and Rambaud. (1993) indicate that supplementation that content the LAB such as L. acidophilus or a mixture of Lactobacillus cultures given to the chicken show significant improvement of levels of amylase after 40 d of feeding that supported by Jin *et al.* (2000). LAB bacteria is responsible for secreting the production of enzymes that increase the activity of amylase that claimed by Duke (1977); Sissons (1989).The ability alternation of pH flora of intestine of host chicken increase activity of intestinal enzymes and digestibility of nutrients better (Dierck, 1989).

Therefore, with the presence of the lactic acid bacteria in the microbial population are able to enhance the growth performance of the chicken by increase the immune system of the chicken and also aid digestion process. The lactic acid bacteria had the properties such as antibacterial substances and inhibitory primary metabolites such as acetic acid, lactic acid, propionic acid, ethanol, hydrogen peroxide, bacteriocins and antibiotic-like substances that able to combat with the enterobacteria that might cause the death of the chicken that claimed by Earnshaw (1992).

2.5 Effect of Enterobacteriacae Bacteria on Growth Performance of Broiler Chicken

The Enterobacteriacae can be describes as that live prominently in the intestine example group such as *Salmonella and E.coli O157.*

Salmonella infection cause by several strains of salmonella such as *Salmonella gallinarum* (SG) and *Salmonella pullorum (SP)* that cause health disease such as Fowl Typhoid that lead death of a chicken. Basically the infection are severe on young chicks due to readily colonized by the bacteria, and the bacteria can persist in the host for some weeks or during all of the rearing period that claimed by Revolledo *et al.* (2006). Usually the colonization of the bacteria are located at few parts of the body of the chicks at upper part of the small intestine and in the gizzard and proventriculus (Fanelli *et al.*, 1971). Fowl typhoid causes heavy death losses in chicks and reduces the productivity of adult birds. Infected chicks will usually death within 3 weeks after hatching that show several clinical sign such as depression, loss of appetite, somnolence, droopy wings, huddling, dehydration, thirst, ruffled feathers, and weakness that claimed by Institute

for International Cooperation in Animal Biologic. (2005).The loss appetite cause reduction of feed intake as the result leads towards poor growth performance.

Transmission route of the disease is through Oral route but might consider the transmission route through nasal and cloacal route in newly hatch chicken. The transmission route can be vertical transmission that can be 2 possibility such as cloaca faeces from infected or carrier hens or trough infected ovary of the hens. The disease can be highly infected by adult chickens but might infect very young chickens, mostly 2–3 weeks of age. Whereas the disease might be very severe towards old chicken but can be lethal towards young chicken high morbidity and mortality in young chickens that reported by Hall (1949).

E. coli O157 can be classify in the group of Enterobacteriacae that can cause high mortality, loss of weight and reduction of egg production that claimed by Bandyopadhay and Dhawedkar (1984). The infected chicken show characteristic such as follow depression, loss of appetite, tendency to huddle respiratory distress, reduction of weight gain, dropped wing, closed eyes, cyanosis and labored breathing (Barnes, 1994). Therefore to increase the production level of the chicken gradually decrease the amount population of Enterobacteriacae that can be bring detrimental effect to the chicken. The most severe contact disease via E. coli is Avian colibacillosis disease appear to give high morbidity and mortality on to chicken that increase the lost profit to occur. Its cause severe disesase infection unto the production of chicken such case are yolk sac infection, omphalitis, respiratory tract infection, swollen head syndrome, septicemia, polyserositis, coligranuloma, enteritis, cellulitis and salpingitis. Colibacillosis of poultry is characterized into two forms such as acute form and sub-acute form. The example of acute form is septicemia and sub-acute form is peri-carditis, airsacculitis and peri-hepatitis that cause high morbidity and mortality of the chicken (Calnek *et al.,* 1997). Avian colibacillosis infected the chicken throughout the birds of all ages, the transmission of E.coli through the faecal contamination of egg. The mode of transmission is as follow penetration of *E. coli* through the shell and may spread to the chickens during hatching and is often associated with high mortality rates, or it may give rise to yolk sac infection.

Therefore as the result harmful Enterobacteriacae presence in the gut of chicken might cause severe problem towards the raising of the chicken. The bacteria presence will lead to exposure of disease that might cause morbidity and mortality of the chicken in which affecting the growth performance of the chicken as classify as loss of economic profit value.

2.6 Review of Benefits of Using Organic Acid

Antibiotic in the past have given a sufficient result of an improvement of growth of the chicken and also feed conversion efficiency that being recorded in the pass by Smith, 1968; Levy *et al.*, 1976; Armstrong, 1984; Neu, 1992) but the use of antibiotic rise the concern of resistance antibiotic bacteria that make the clinical disease to be more difficult to be treat. The concern making the European Union ban the use of antibiotic on the farm basis on chicken production (Glynn *et al.*, 1998; Monnet, 1999; Koutsolioutsou *et al.*, 2001). The benefits raise the awareness of using the source of alternative organic acids as the source of feed additives in the aspect of regulating the harmful bacteria has highly influenced research activity on the discovery of non-antibiotic chemical compounds.

Organic acid is generally used as feed preservative by extending the shelf life of the food and also food additives as the effort in the improvement of growth performance of the chicken. The general characteristic of the organic acid that includes the presence group of saturated straight-chain monocarboxylic acids and their respective derivatives (unsaturated, hydroxylic, phenolic, and multi carboxylic versions) and are often generically referred to as fatty acids, volatile fatty acids or carboxylic acids that reported by Cherrington *et. al.* (1991).

Mode of action of an organic acid in regulating the population of bacteria are not fully understood but the assumption has been made such as organic acids exhibiting bacteriostatic and bactericidal properties. The properties highly depend on both physiological status of the animal and the physiochemical characteristics of the external environment. The ability of organic acid to form undissociated acid due to organic acid can be considered as weak acid in nature that claimed by Davidson (2001). The formation of undissociated acid has the function of penetration into the lipid membrane

of bacteria cell and internalized towards the neutral pH of the cytoplasm of the bacteria the undissociated acid gradually dissociate into anions and protons (Eklund, 1983, 1985; Salmond *et al.*, 1984; Cherrington *et al.*, 1990, 1991; Davidson, 2001). Therefore changes the content of anion and protons cause the export of excess protons to take place in order to balance neutral cytoplasm. The exportation of the excess protons require a high amount of energy that comes from cellular adenosine triphosphate (ATP) of the bacteria and depletion may result of depletion of ATP will cause the bacteria to be eliminated. In other study stated by Russell (1992) indicate the toxicity of effect of organic acids by the accumulation of anion as the mode of action for eliminating the bacteria but the theory is not highly significant apply to some bacteria that has ability to decline their internal pH for survival. The other suggestion of mode of action of organic acid as follow interference with nutrient transport, cytoplasmic membrane damage resulting in leakage, disruption of outer membrane permeability, and influencing macromolecular synthesis (Cherrington *et al.*, 1991; Denyer and Stewart, 1998; Alakomi *et al.*, 2000; Davidson, 2001).

2.7 Review of Benefits of Using Essential Oil.

The study conducted to replace antibiotic as the feed supplement for growth by using natural feed supplements derived from plants known as essential oil (EO) that claimed by Simitzis *et. al.* (2011). the use of essential oil as antimicrobial effects has been carry out due to public concern on the uses of synthetic agents for animal growth. The concern is such as potential on carcinogenicity, acute toxicity, teratogenicity and slow degradation periods. The development on the study on essential oil has greatly impacted the world of research on antibacterial effect improve the quality characteristics and shelf life of animal products that claimed by Faleiro (2011).

The mode of action is such as based on the hydrophobic characteristic that enables them to partition lipids in the bacterial cell wall and mitochondria. Therefore increase the lipid layer and lead towards disruption membrane integrity and ion transport processes that cause the disturbance on cell osmatic pressure. Disturbance on the cell osmatic pressure generally cause the growth bacteria to decrease by the trans-membrane electric potential in a bacterial cell is reduced and the proton permeability of the membrane that claimed by Burt (2004).

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