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Evaluation of a synthetic emulsifier product supplementation on broiler chicks

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ABSTRACT

The digestive physiology of young chickens is characterized by inefficient digestion and absorption of fat due to a low level of natural endogenous lipase production. These evidences have increased the interest on the use of emulsifiers to improve utilization of fats in young chickens and growth performance of broiler. The effect of a synthetic emulsifier on growth performance, meat quality, caecum microbial count, plasma metabolites and hepatic apolipoprotein gene expression was investigated in male broiler chicks. A total of 600 one-day-old ROSS 308 broiler chicks were assigned to 2 experimental groups consisting of 15 pens with 20 birds/ per pen each, to compare the different dietary treatments: control diet (CTR) or diet supplemented with AVI-MUL TOP (AMT) at 1 g/kg from day 0 to 12, 0.75 g/kg from day 12 to 22 and 0.5 g/kg from day 22 to 44. Growth performance was determined on days 0, 12, 22, and 44. At the end of the trial (day 44), one chick from each pen was chosen on body weight (BW) basis and sacrificed and samples of blood, liver, caecum content and breast were collected for analysis. AMT supplementation increased BW on days 12 and 22 ($P = 0.02$; $P = 0.02$) and ADG from day 0 to 12 ($P = 0.02$), while reduced FCR from day 22 to 44 ($P = 0.047$) and from day 0 to 44 ($P = 0.02$). AMT supplementation modified carcass and meat characteristics, increasing dressing percentage ($P = 0.01$) and b* (yellowness) ($P = 0.01$) compared to control group. Moreover, AMT dietary supplementation increased total cholesterol ($P = 0.02$) and HDL cholesterol ($P = 0.02$) plasma concentrations. No differences between the two treatments were observed in caecum microbial counts and hepatic apolipoprotein gene expression. In conclusion, our findings show that AMT supplementation to broiler chicks may have a beneficial effect on growth performances (BW, ADG and FCR) and carcass dressing and may affect meat colour (b* yellowness) and lipid metabolism (cholesterol and HDL).

1. Introduction

In poultry nutrition, the use of feed additives is a consolidated routine in order to help the birds express their genetic potentials. The digestive physiology of young chickens is characterised by low levels of natural endogenous lipase production, which may alter

Abbreviations: CTR, basal diet without supplementation; AMT, CTR + Avi-Mul Top; ADG, average daily gain; BW, body weight; ADFI, average daily feed intake; FCR, feed conversion ratio; WHC, water-holding capacity; Apo A-I, apolipoprotein A-I; Apo B, apolipoprotein B; HDL, high-density lipoproteins; LDL, low-density lipoproteins; NEF, Anon-esterified fatty acids

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the digestive metabolism (Noy and Sklan, 1995). Therefore, supplementation of emulsifiers, particularly at early stages of developmental growth, allows the chicks to improve digestion and absorption of the fats usually added to their diet in order to increase energy concentration and growth performance (San Tan et al., 2016).

Emulsifiers can reduce the surface tension of water, increase penetration and improve the distribution of water in press meal (van der Heijden and de Haan, 2010). Roy et al. (2010) reported that supplementation of exogenous emulsifiers in diets containing moderate quantities of added vegetable fats might substantially improve broiler performance. These results were confirmed by Bontempo et al. (2016) who showed that supplementation with a synthetic emulsifier could improve growth performance of broiler chicks.

Emulsifier, such as lecithin, has been reported to reduce free fatty acid absorption, probably by increasing the size of bile salt micelles, which diffuse more slowly through the luminal water interface, hindering the delivery of free fatty acids to the absorptive cell surface (Saunders and Sillery, 1976). Furthermore, Zhang et al. (2011) demonstrated that the supplementation of emulsifier improved the growth performance of broiler chickens in the starter period by increasing fatty acid digestibility. However, the effects of emulsifiers – in association with vegetable oils – on fat utilization have not been thoroughly investigated yet, even though the interest in using exogenous emulsifiers has increased in the last several decades. Thus, this study was conducted to assess the effect of a synthetic emulsifier product AVI-MUL TOP (AMT), consisting of a vegetal bi-distilled oleic acid and glycerol polyethylene glycol ricinoleate. In particular, the effect of AMT addition was evaluated on: growth performance, meat quality and caecum microbial counts, plasma metabolite profile and hepatic gene expression of Apolipoprotein A-I (Apo A-I) and Apolipoprotein B (Apo B) as relevant fat transportation related genes in male broiler chicks.

2. Materials and methods

The experimental protocol was reviewed and approved by the Animal Care and Use Committee of the University of Milan. The experiment was performed at the facility of Animal Production Research and Teaching Centre of the Polo Veterinario, Università degli Studi di Milano (Lodi, Italy). The emulsifier product AVI-MUL TOP (AMT, SEVECOM S.P.A., Milan, Italy), consisting of 50 g/kg vegetal bi-distilled oleic acid emulsified with 50 g/kg ethoxylated castor oil E484, which belongs to the glycerol polyethylene glycol ricinoleate family (Community Register of Feed Additives - EU Reg. No. 1831/2003), was mixed with the other ingredients before the pelleting process to increase the humidity, reduce the pellet press energy consumption and improve pellet quality by modulating the moisture content during the pelleting process.

Animal fat consisting of 50 g/kg poultry fat and 50 g/kg lard was used in the grower and finisher phases, while vegetable oils (soybean oil) was used in the starter phase.

2.1. Animals and housing

A total of 600 male birds ROSS 308 were obtained from a local hatchery at one day of age, weighed and randomly assigned to one of two experimental groups: control diet without emulsifier supplementation (CTR) and control diet containing AMT (1 g/kg inclusion rate of feed from day 0 to 12, 0.75 g/kg of feed from day 12 to 22 and 0.5 g/kg of feed from day 22 to 44 of the trial). Each group consisted of 15 pens (replicate) containing 20 birds per pen (2.5 m × 1.00 m). All chickens were vaccinated against Marek's disease, Newcastle disease and infectious bronchitis *via* coarse spraying at hatching.

The diets (Table 1) were formulated to meet the nutrient requirements defined by the National Research Council (NRC) (1994) for a starter from day 0 to 12, a grower from day 12 to 22 and a finisher from day 22 to 44. Pens, equipped with chain feeder system and automatic nipple cup drinker for water distribution, were bedded with shavings of white wood. Feed and water were provided for *ad libitum* consumption. Light cycle and temperature were the same in the two experimental groups. The photoperiod was 24 h of light from day 0 to day 7 and 23 h of light from day 7 to the end of the trial. Room temperature was 35 °C from day one to day three and was then decreased by 2.5 °C per week to a final temperature of 21 °C at the end of the trial (44 days).

2.2. Data collection and sampling

Body weight and feed intake were recorded at 0, 12, 22, and 44 days of age for each replicate to determine average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR). Mortality was recorded twice daily and dead animals were removed and weighted. At the end of the trial (44 days), one bird from each pen was selected on average pen weight basis and sacrificed by exsanguination from jugular vein. Previously, the birds were stunned in a water bath (125 Hz AC, 80 mA/birds, 5 s). Blood samples were collected into heparinized test tubes and immediately centrifuged at 3 000 × *g* for 10 min at 4 °C. Plasma aliquots were stored at −20 °C until analysis for plasma metabolite profile. Portions (50–100 mg) of liver were sampled, snap-frozen in liquid nitrogen and stored at −80 °C until analysis for lipid content and apolipoprotein gene expression

The dressing was calculated by dividing the eviscerated weight by the live weight. Breast muscle was removed and weighed and the breast muscle yield was calculated as the percentage of eviscerated weight. Breast muscles were stored individually in plastic bags at 4 °C until subsequent analysis of meat quality.

2.3. Meat quality

Breast muscle pH was tested at a depth of 2.5 cm below the surface 24 h after sacrifice, using a combined glass-penetrating

Table 1
Ingredients and chemical analysis of the basal diets for broiler chicks (as-fed basis).

	Starter 1 (0-12 d)	Grower 2 (12-22 d)	Finisher 3 (22-44 d)
Ingredients, g/kg			
Maize	449.1	427.1	350.4
Soybean meal, 490 g CP/kg	330.0	250.0	255.5
Wheat	150.0	200.0	300.0
Sorghum	0	100.0	150.0
Soybean full fat	0	50.0	0
Soybean oil	30.0	0	0
Animal fat	0	38.0	64.0
Sodium chloride	4.0	3.5	2.6
Calcium carbonate	11.5	10.0	9.3
Dicalcium phosphate 180 g/kg	15.5	12.0	9.5
DL-Methionine	3.0	2.8	2.0
L-Threonine	0.8	0.7	0.2
L-Lysine HCl	2.4	2.4	2.2
Premix[a]	2.5	2.5	3.0
Choline HCl 750 g/kg	1.2	1.0	0.9
Xylanase	0	0	0.4
AMT group[b]	-/1.0	-/0.75	-/0.5
Calculated nutrient content			
ME, MJ/kg	12.77	13.19	13.65
Crude protein, g/kg	218.9	201.7	190.8
Crude fat, g/kg	51.8	66.9	82.7
Crude fiber, g/kg	26.8	28.7	25.7
Calcium, g/kg	9.4	7.9	6.9
Phosphorous, g/kg	6.6	5.9	5.4
Lys, g/kg	13.3	12.2	11.2
Met + Cys, g/kg	9.6	9.0	7.9
Thr, g/kg	8.9	8.0	7.1
Oleic acid, g/kg	12.6	22.2	30.6
Linoleic acid, g/kg	27.1	18.7	15.6
Linolenic acid, g/kg	2.4	1.5	1.3

[a] Provided the following per kg of diet: vitamin A, 11,250 IU; vitamin D_3, 5000 IU; vitamin E, 60 mg; $MnSO_4 \cdot 1H_2O$, 308 mg; $ZnSO_4 \cdot 1H_2O$, 246 mg; $FeSO_4 \cdot 1H_2O$, 136 mg; $CuSO_4 \cdot 5H_2O$, 39 mg; KI, 2.4 mg; Na_2SeO_3, 657 µg; 6-Phytase EC 3.1.3.26, 750 FTU; Endo-1, 4-beta-xylanase EC 3.2.1.8, 2250 U.

[b] AMT group: 1 g/kg from day 0 to 12; 0.75 g/kg from day 12 to 22; 0.5 g/kg from day 22 to 44.

electrode (Ingold, Mettler Toledo, Greifensee, Switzerland). Colour measurements were performed on the carcass surface over the breast muscles and on a freshly exposed cut surface of muscle. A Minolta CR-300 chromameter (Minolta, Osaka, Japan) was set to the L* (lightness), a* (redness), and b* (yellowness) according to the CIE scale (CIE, 1986).

Water holding capacity (WHC) was determined on breast muscle using the method of Jauregui et al. (1981), with some modifications. Briefly, 1.5 ± 0.3 *g* of lean muscle was inserted into a pre-weighed (W1) funnel made of four layers of grade 1 filter paper (Whatman International, Maidstone, UK). The funnel with the sample was weighed (W2), put into a centrifuge tube and centrifuged at 15 000 rpm for 15 min at 4 °C. The muscle sample was then removed from the funnel, which was weighed again (W3). WHC was calculated as the percentage of water weight lost from the sample, according to the following formula:

$$(W3\text{-}W1)/(W2\text{-}W1)^* 100$$

where W3-W1 = water weight (absorbed by the paper), and W2-W1 = initial meat weight.

For cooking loss determination, each breast was weighed and sealed in a plastic cooking bag and cooked by immersion in an 85 °C water bath until the internal endpoint temperature reached 80 °C. Internal temperature was measured with cooking thermometers introduced into the thickest part of each breast muscle in each cooking batch. After cooking, the samples were chilled by immersion of the bags in an ice water bath for 30 min. Each piece of breast was then removed from its bag and weighed, and cooking loss was calculated.

Muscles were tempered at 20 °C for 30 min to equilibrate temperatures, and then six probes (1.27 cm in diameter) were obtained from the centre of each muscle in line with the fibres. The probes were cut parallel to the longitudinal orientation of muscle fibres; the peak shear force was measured (Warner-Bratzler blade speed 200 mm/min). The tenderness of the cooked breast samples was determined as shear force by an Instron universal testing machine (Model 5 542, Instron Engineering Corp., Canton, MA, U.S.A.), and the mean values were recorded (expressed in Newtons, N).

2.4. Caecum microbial counts

The caecum content of each sacrificed chick was collected during necropsy. Each sample was placed in a small sterile container

and immediately sent to the laboratory for microbiological analysis in refrigerated conditions. The analyses were performed on the same day. From each sample, the caecum was isolated and after external disinfection, the content was aseptically collected and used for the microbiological analyses period. Pools of three subjects from the same dietary treatment were randomly obtained (5 CTR and 5 AMT pools of caecum). One to three grams of each sample were diluted 1:10 with sterile saline solution (NaCl 0.85 g/L, tryptone 0.1 g/L) and homogenized for 60 s in a Stomacher 400 (Seward Medical, London, UK). Serial 10-fold dilutions were spread by sterile spatula onto De Man, Rogosa and Sharpe agar medium incubated in anaerobic jars (Anaerojar, Oxoid, Basingstoke, UK) with an Anaerogen kit (Oxoid) at 37 °C for 48 h for the enumeration of *Lactobacilli*, and on tryptone bile *X*-glucuronide agar (Oxoid) incubated aerobically at 44 °C for 24 h for the enumeration of *Escherichia coli* (International Organization for Standardization, ISO 16649-2). Moreover, one to three grams of caecal content was diluted 1:10 with buffered peptone water (Oxoid) and incubated at 37 °C for the detection of *Salmonella* spp. according to the International Organization for Standardization (ISO) 6579 method. From the obtained counts, the *Lactobacilli/E. coli* ratio was calculated as the log difference between the two parameters (Abu-Tarboush et al., 1996).

2.5. Plasma metabolite profile

The concentrations of selected plasma parameters were measured with an automated spectrophotometer with commercial assay kits developed and validated for use with ILAB 300 plus (ILAB 300 plus, Instrumentation Laboratory S.p.a., Milan, Italy). Plasma samples were analysed for cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL), glucose, total protein and urea. Low-density lipoprotein cholesterol (LDL) was obtained by difference (cholesterol-HDL). For analysis of non-esterified fatty acids (NEFAs), kits from Randox Laboratories Ltd. (Crumlin, Co. Antrium, UK) were used.

2.6. Determination of lipid content in liver samples

Lipids were extracted by Folch's method (Folch et al., 1957) with slight modifications. Briefly, liver samples (± 1 g) were thawed and homogenized in an excess of chloroform:methanol (2:1) solution for two minutes. The homogenate was filtered, placed in separator funnels and mixed with a saline solution containing KCl 0.88 g/L. After separation in two phases, the lipid chloroform fraction (bottom layer) was evaporated using a rotary evaporator and subsequently weighed.

2.7. Hepatic expression of Apo A-I and Apo B genes

Total RNA was extracted from liver tissue using a commercial kit (SV Total RNA isolation system, Promega, Milano, Italy) according to the manufacturer's protocols. The RNA integrity was assessed by gel electrophoresis to detect 18 s and 28 s rRNA bands. An aliquot of total RNA was reverse transcribed using the iScript cDNA synthesis kit (Biorad, Milan, Italy). The primer sequences used are listed in Table 2. Primer pairs were first tested for their specificity in qualitative PCR, using the pooled cDNA as a template (Jiang et al., 2014). The cycling profile for the assay consisted of 95 °C for 10′, followed by 40 cycles of amplification (95 °C for 15 s, 63° for 60 s). The quantitative analysis of mRNA was carried out by SYBR green methodology as reported by Jiang et al., 2014 using a real time PCR system (Strategene Mx 3000p). The comparative CT method was used (Livak and Schmittgen, 2001), determining fold changes in gene expression, calculated as $2^{-\Delta\Delta CT}$. The relative quantity of Apo A-I and Apo B values were normalized to mRNA levels of 18 s rRNA and GAPDH genes. The 18 s rRNA was chosen to calculate the threshold cycles because it had previously been shown to be constant under all conditions used

2.8. Statistical analysis

Data were analysed by one-way ANOVA using the MIXED procedure of SAS v. 9.4 (SAS, 2018). The pen represented the experimental unit for growth performance parameters, while individual chicks were the experimental units for the carcass characteristics, meat quality, cecum microbial count, plasma metabolite profile and hepatic gene expression. Probability values of ≤ 0.05 were considered significant.

Table 2
Oligonucleotide primer sequences.

Gene	Primer	Sequence	Reference	Product size, bp[a]
Apolipoprotein A-I (Apo A-I)	Forward	5'GTGACCCTCGCTGTGCTCTT3'	Jiang et al. (2014)	217
	Reverse	5'CACTCAGCGTGTCCAGGTTGT3'		
Apolipoprotein B (Apo B)	Forward	5'GACTTGGTTACACGCCTCA3'	Zhang et al. (2007)	196
	Reverse	5'TAACTTGCCTGTTATGCTC3'		
18s rRNA	Forward	5'GCGGCTTTGGTGACTCTA3'	Ocon-Grove et al. (2008)	194
	Reverse	5'CTGCCTTCCTTGGATGTG3'		
GAPDH	Forward	5'TGCTAAGGCTGTGGGGAAAG3'	Huang et al. (2013)	158
	Reverse	5'CAGCAGCCTTCACTACCCTC3'		

[a] Base pair.

Table 3
Effect of AVI-MUL TOP (AMT) on the growth performance of male broiler chicks.

Item	CTR	AMT	SEM	*P*-value
No. Pens	15	15		
BW (g)				
Day 0	40.89	40.97	0.19	0.80
Day 12	378.0	387.6	2.7	0.02
Day 22	1070	1092	6	0.02
Day 44	3411	3475	28	0.12
Day 0 to 12				
ADG (g/d)	28.09	28.88	0.22	0.02
ADFI (g/d)	32.28	32.72	0.28	0.28
FCR	1.149	1.133	0.008	0.17
Day 12 to 22				
ADG (g/d)	69.15	70.49	0.51	0.08
ADFI (g/d)	92.29[b]	94.45[a]	0.66	0.03
FCR	1.335	1.340	0.005	0.47
Day 22 to 44				
ADG (g/d)	106.4	108.3	1.2	0.27
ADFI (g/d)	180.9	180.6	1.7	0.91
FCR	1.700	1.669	0.011	0.047
Day 0 to 44				
ADG (g/d)	76.60	78.06	0.63	0.12
ADFI (g/d)	120.2	120.7	0.9	0.74
FCR	1.570	1.547	0.007	0.02
Mortality (%)	2.00	2.33	0.72	0.64

Different superscript letters indicate significant difference ($P \leq 0.05$) significant.
BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio.

3. Results

3.1. Growth performance and carcass yield

The effects of AMT on body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), feed conversion ratio (FCR) and mortality of male chicks are shown in Table 3. The emulsifier supplementation increased BW on days 12 and 22 ($P = 0.02$; $P = 0.02$) and improved ADG from days 0 to 12 ($P = 0.02$) compared to CTR chicks. Average daily feed intake (ADFI) from days 12 to 22 was higher in supplemented chicks than in control ($P = 0.03$), while FCR was lower in chicks receiving AMT from day 22 to 44 and for the whole period of the trial ($P = 0.047$ and 0.02, respectively). Mortality was not statistically different between the two experimental groups. The effects of AMT on the carcass characteristics are shown in Table 4. AMT supplementation increased the dressing percentage compared with the CTR group ($P = 0.01$).

3.2. Meat quality and caecum microbial counts

The effects of AMT on meat quality of male chicks are shown in Table 5. Dietary AMT increased b* (yellowness) ($P = 0.01$), whereas no effects were observed for a* (redness) and L* (lightness) indexes. None of the analysed samples showed the presence of *Salmonella* spp., No significant differences were observed for *E. coli* and *Lactobacilli* counts between the two groups. Consequently, no changes on the *Lactobacilli/E. coli* ratio were observed. Water holding capacity (WHC), cooking loss and shear force did not show significant differences between the two groups.

3.3. Plasma metabolite parameters

The effects of AMT on selected metabolic parameters are shown in Table 6. AMT supplementation increased cholesterol and HDL

Table 4
Effect of AVI-MUL TOP (AMT) on the carcass characteristics of male broiler chicks.

Item	CTR	AMT	SEM	*P*-value
No. Chicks	15	15		
Dressing (g/kg)	751.4	760.9	0.26	0.01
Breast muscle (g/kg)	319.6	326.7	0.58	0.41

Probability values of $P \leq 0.05$ were considered statistically significant.

Table 5
Effect of AVI-MUL TOP (AMT) on meat quality and caecal microbial count of male broiler chicks.

Item	CTR	AMT	SEM	*P*-value
No. Chicks	15	15		
pH_{24}	6.18	6.12	0.04	0.17
Colour				
L^* (lightness)	54.77	54.11	0.84	0.86
a^* (redness)	−0.12	−0.14	0.36	0.83
b^* (yellowness)	6.64	8.58	0.39	0.01
WHC (g/g)	36.07	35.27	0.99	0.53
Cooking loss (g/g)	26.64	26.45	1.58	0.52
Shear force (N)	17.25	18.89	1.14	0.22
E. coli (log_{10} cfu/g)	7.34	7.60	0.16	0.28
Lactobacilli (log_{10} cfu/g)	8.10	7.98	0.28	0.77

Probability values of P ≤ 0.05 were considered statistically significant.
pH_{24}, pH 24 h after sacrifice; WHC, water-holding capacity N, newton.

Table 6
Effect of AVI-MUL TOP (AMT) on some plasma metabolic parameters of male broiler chicks.

Item	CTR	AMT	SEM	*P*-value
No. Chicks	15	15		
Cholesterol (mg/dl)	123	134	3	0.02
HDL (mg/dl)	74.37	81.91	2.14	0.02
LDL (mg/dl)	48.46	51.81	1.82	0.21
NEFA (mmol/l)	0.82	0.64	0.10	0.24
Glucose (mg/dl)	256	256	4	1.00
Total protein (g/dl)	2.90	2.96	0.06	0.52
Triglyceride (mg/dl)	37.57	36.26	3.32	0.78
Urea (mg/dl)	5.32	4.97	0.41	0.55

Probability values of $P \leq 0.05$ were considered statistically significant.
HDL, high-density lipoproteins; LDL, low-density lipoproteins; NEFA; non-esterified fatty acids.

contents compared with the CTR group ($P = 0.02$; $P = 0.02$). No differences were observed for the other parameters (LDL, NEFA, glucose, total protein, trygliceride and urea).

3.4. Lipid content and gene expression of apolipoprotein in liver

In the present study, total hepatic lipid content of AMT group (7.39 ± 1.89%) did not differ from CTR group (6.54 ± 1.76%). Furthermore, AMT supplementation did not modify the hepatic expression of Apo A-I and Apo B genes compared to the control group.

4. Discussion

The results from the present study show that the emulsifier supplementation could improve growth performances (BW, ADG, ADFI and FCR) of broilers despite the short production system. These data are in agreement with the findings of our previous study (Bontempo et al., 2016). In the current study, the AMT supplement was mixed with feed compounds before the pelleting process, which may increase the humidity, reduce the pellet press energy consumption and improve pellet quality by modulating the moisture content during the pelleting process, consequently improving feed intake and animal performance. In other studies, the addition of an emulsifier improved the digestibility of major nutrients (Dierick and Decuypere, 2004), reduced the viscosity of the digestive contents and increased the transit of the digesta as well as the feed intake (Lázaro et al., 2004). In this study, the incorporation of vegetal bi-distilled oleic acid and glycerol polyethylene glycol ricinoleate may also have improved the growth performance of animals *via* the emulsification of supplemental fatty acids (Xing et al., 2004). In relation to carcass characteristics, Scheele (1997) observed that the growth of the pectoral muscles primarily occurs during the late stages of developmental growth in fast-growing birds. In the present study, an increased dressing was observed in the treated group, suggesting that the relatively rapid growth of the AMT-fed chicks in the finisher phase may contribute to the increase of carcass yield.

In our study, the AMT supplementation influenced the colorimetric indexes of breast. Meat colour is one of the first characteristic of customer interest, especially in boneless products. Birren (1963) underlined that colour has an important impact on processors and consumers and is often used to determine economic value of food. Akit et al. (2014) demonstrated that dietary emulsifier improved redness values and decreased lightness values but had no effect on pork meat pH and drip loss. In contrast, Kim et al. (2008) found no differences in meat colour of pigs fed lecithin or control diets. Ali et al., (2017) reported the effect of emulsifier on chicken breast meat quality. The authors reported a decreased lightness, an increased redness and no significative effect on yellowness of breast muscle. The changes in b* (yellowness) in the breast muscle observed in the group fed with AMT diet may be explained by the role of

emulsifier in increasing lipid-soluble pigments (*e.g.* xanthophyll) accumulation in breast muscle (Laudadio and Tufarelli, 2010).

Many previous *in vitro* studies suggested that dietary emulsifiers might directly and detrimentally impact the microbiota, leading to societal incidence of obesity/metabolic syndrome and inflammatory diseases (Chassaing et al., 2015; Viennois and Chassaing, 2018). However, our study observed that there was no dietary effect on *E. coli* and Lactobacilli counts and consequently on the Lactobacilli/*E. coli* ratio, which may be due to the components of emulsifier and tested parameters in this study. Thus, the effect of AMT supplementation on intestinal inflammation and diversity should be investigated in further researches.

Few authors investigated the effects of emulsifier supplementation on the serum lipid profile in avian species and the results are not univocal. In our study, AMT supplementation increased cholesterol and HDL contents, thus indicating an improved lipid metabolism and transport. A study conducted by Wang et al. (2016) showed increased concentrations of total and LDL cholesterol, but not HDL, in the emulsifier treated chicks compared to the control group. The authors suggested that the response could be related also to the type of fat sources (animal *vs* vegetable) added in the diet and to the inclusion level. Roy et al. (2010) using an exogenous emulsifier suggested a higher removal rate of lipids from the liver. It is clear that multiple potential mechanisms are involved in the regulation of plasma cholesterol concentration, including hepatic uptake of high-density lipoprotein and/or post-absorptive lipid metabolism.

It is well-known that liver is the primary site of lipid biosynthesis in avian species and almost all fat accumulation in broiler adipose tissue derives from the liver or the diet (Jiang et al., 2014). The liver and the intestine are major sites of lipoprotein synthesis and secretion in the adult chicken, and pharmacological, dietary and physiological factors might affect concentration of apolipoprotein A-I (Apo A-I) and consequently plasma HDL (Jiang et al., 2014). Apo A-I and apolipoprotein B (ApoB) are the major protein components of chicken plasma high density and very low-density lipoproteins (HDL and VLDL, respectively). In the present study the expression of Apo A-I and ApoB genes in chicken liver was investigated for their essential role in the assembly and secretion of triglyceride-rich lipoproteins and lipid transport. The lack of significant gene expression modulation of Apo A-I and ApoB genes might be due to an adaptive response to the AMT supplementation (44 days of treatment). The adaptive response was previously observe by Huang et al., 2013 who investigated the role of epigallocatechin gallate as lipid metabolism modulator in ROSS 308.

5. Conclusion

Our results show that AMT supplementation to broiler chicks may have a beneficial effect on growth performances (BW, ADG and FCR) and carcass dressing. and may affect meat colour (b* yellowness) and lipid metabolism (cholesterol and HDL). Overall, our findings emphasize the possible future utilisation of exogenous emulsifier as a tool for enhancing fat utilization in high-yielding chicken through diets.

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References

Abu-Tarboush, H.M., Al-Saiady, M.Y., Keir El-Din, A.H., 1996. Evaluation of diet containing lactobacilli on performance, fecal coliform, and lactobacilli of young dairy calves. Anim. Feed Sci. Technol. 57, 39–49.

Akit, H., Collins, C.L., Fahri, F.T., Hung, A.T., D'Souza, D.N., Leury, B.J., Dunshea, F.R., 2014. Dietary lecithin improves dressing percentage and decreases chewiness in the longissimus muscle in finisher gilts. Meat Sci. 96, 1147–1151.

Ali, S.F., Chao, W., Xiaoli, W., Jintian, H., Mingfa, W., El-Hack, M.E.A., Tian, Z.X.W., 2017. Growth, serum biochemical indices, antioxidant Status and meat quality of broiler chickens fed diet supplemented with sodium Stearoyl-2 Lactylate. Pak. Vet. J. 37, 445–449.

Birren, R.F., 1963. Color and human appetite. Food Technol. 17, 553.

Bontempo, V., Comi, M., Jiang, X.R., 2016. The effects of a synthetic emulsifier supplementation on growth performance of broiler chicks and weaned piglets. Animal 10, 592–596.

Chassaing, B., Koren, O., Goodrich, J.K., Poole, A.C., Srinivasan, S., Ley, R.E., Gewirtz, A.T., 2015. Dietary emulsifiers impact the mouse gut microbiota promoting colitis and metabolic syndrome. Nature 519, 92–96.

CIE, 1986. Commission International d'Eclairage). Colorimetry, 2nd ed. Publication CIE 15.2, Vienna.

Dierick, N., Decuypere, J., 2004. Influence of lipase and/or emulsifier addition on the ileal and faecal nutrient digestibility in growing pigs fed diets containing 4% animal fat. J. Sci. Food Agric. 84, 1443–1450.

Folch, J., Lees, M., Sloane-Stanley, G.H., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497–509.

Huang, J., Zhang, Y., Zhou, Y., Zhang, Z., Xie, Z., Zhang, J., Wan, X., 2013. Green tea polyphenols alleviate obesity in broiler chickens through the regulation of lipid-metabolism-related genes and transcription factor expression. J. Agric. Food Chem. 61, 8565–8572.

International Organization for Standardization, 2001. ISO 16649-2:2001 Microbiology of Food and Animal Feeding Stuffs. Horizontal Method for the Enumeration of Beta-Glucuronidase-Positive *Escherichia Coli*. Part 2: Colony-Count Technique at 44 Degrees C Using 5-bromo-4-chloro-3-indolyl Beta-D-Glucuronide. International Standards Organisation, Geneva, Switzerland.

International Organization for Standardization, 2002. ISO 6579:2002. Microbiology of Food and Animal Feeding Stuffs – Horizontal Method for the Detection of Salmonella Spp. International Standards Organisation, Geneva, Switzerland.

Jauregui, C.A., Regenstein, J.M., Baker, R.C., 1981. A simple centrifugal method for measuring expressible moisture, a water-binding property of muscle foods. J. Food Sci. 46, 1271–1273.

Jiang, R.R., Zhao, G.P., Zhao, J.P., Chen, J.L., Zheng, M.Q., Liu, R.R., Wen, J., 2014. Influence of dietary nicotinic acid supplementation on lipid metabolism and related gene expression in two distinct broiler breeds of female chickens. J. Anim. Physiol. Anim. Nutr. 98, 822–829.

Kim, W.T., Shinde, P., Chae, B.J., 2008. Effect of lecithin with or without chitooligosaccharide on the growth performance, nutrient digestibility, blood metabolites and pork quality of finishing pigs. Can. J. Anim. Sci. 88, 283–292.

Laudadio, V., Tufarelli, V., 2010. Growth performance and carcass and meat quality of broiler chickens fed diets containing micronized-dehulled peas (Pisum sativum cv. Spirale) as a substitute of soybean meal. Poultr. Sci. 89, 1537–1543.
Lázaro, R., Latorre, M.A., Medel, P., Gracia, M., Mateos, G.G., 2004. Feeding regimen and enzyme supplementation to rye-based diets for broilers. Poultr. Sci. 83, 152–160.
Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2-\Delta\Delta CT$ method. Methods 25, 402–408.
National Research Council, 1994. Nutrient Requirements of Poultry, 9th revised edition. National Academy Press, Washington, DC, USA.
Noy, Y., Sklan, D., 1995. Digestion and absorption in the young chick. Poultr. Sci. 74, 366–373.
Ocon-Grove, O.M., Krzysik-Walker, S.M., Maddineni, S.R., Hendricks, G.L., Hendricks 3rd, R., Ramachandran, R., 2008. Adiponectin and its receptors are expressed in the chicken testis: influence of sexual maturation on testicular ADIPOR1 and ADIPOR2 mRNA abundance. Reproduction 136, 627–638.
Roy, A., Haldar, S., Mondal, S., Ghosh, T.K., 2010. Effects of supplemental exogenous emulsifier on performance, nutrient metabolism, and serum lipid profile in broiler chickens. Vet. Med. Int. 2010, 9. http://dx.doi.org/10.4061/2010/262604. 262604.
San Tan, H., Zulkifli, I., Soleimani Farjam, A., Meng Goh, Y., Croes, E., Karmakar Partha, S., Kiat Tee, A., 2016. Effect of exogenous emulsifier on growth performance, fat digestibility, apparent metabolisable energy in broiler chickens. J. Biochem. Microbiol. Biotechnol. 4, 7–10.
SAS Institute, 2009. SAS/STAT® 9.4 User's Guide, 2nd ed. SAS Institute Inc., Cary, NC.
Saunders, D.R., Sillery, J., 1976. Lecithin inhibits fatty acid and bile salt absorption from rat small intestine *in vivo*. Lipids 11, 830–832.
Scheele, C.W., 1997. Pathological changes in metabolism of poultry related to increasing production levels. Vet. Quart. 19, 127–130.
Van der Heijden, M., de Haan, D., 2010. Optimizing moisture while maintaining feed quality. All About Feed 1, 29–31. http://www.allaboutfeed.net/Processing/Cooling–Drying/2010/10/Optimising-moisture-while-maintaining-feed-quality-AAF011514W/.
Viennois, E., Chassaing, B., 2018. First victim, later aggressor: how the intestinal microbiota drives the pro-inflammatory effects of dietary emulsifiers? Gut Microbes 66, 1–4.
Wang, J.P., Zhang, Z.F., Yan, L., Kim, I.H., 2016. Effects of dietary supplementation of emulsifier and carbohydrase on the growth performance, serum cholesterol and breast meat fatty acid profile of broiler chickens. Anim. Sci. J. 87, 250–256.
Xing, J.J., van Heugten, E., Lit, D.F., Touchette, K.J., Coalson, J.A., Odgaard, R.L., Odle, J., 2004. Effects of emulsification, fat encapsulation, and pelleting on weanling pig performance and nutrient digestibility. J. Anim. Sci. 82, 2601–2609.
Zhang, S., Shi, H., Li, H., 2007. Cloning and tissue expression characterization of the chicken APOB gene. Anim. Biotechnol. 18, 243–250.
Zhang, B., Haitao, L., Zhao, D., Guo, Y., Barri, A., 2011. Effect of fat type and lysophosphatidylcholine addition to broiler diets on performance, apparent digestibility of fatty acids, and apparent metabolizable energy content. Anim. Feed Sci. Technol. 163, 177–184.