Evaluation of a synthetic emulsifier product supplementation onbroiler chicks

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Abstract

The digestive physiology of young chickens is characterized by inefficient digestion and ab-sorption of fat due to a low level of natural endogenous lipase production. These evidences haveincreased the interest on the use of emulsifiers to improve utilization of fats in young chickensand growth performance of broiler. The effect of a synthetic emulsifier on growth performance,meat quality, caecum microbial count, plasma metabolites and hepatic apolipoprotein gene ex-pression was investigated in male broiler chicks. A total of 600 one-day-old ROSS 308 broilerchicks 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 day22 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 andsamples of blood, liver, caecum content and breast were collected for analysis. AMT supple-mentation 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 dietarysupplementation increased total cholesterol (P= 0.02) and HDL cholesterol (P= 0.02) plasmaconcentrations. No differences between the two treatments were observed in caecum microbialcounts and hepatic apolipoprotein gene expression. In conclusion, ourfindings show that AMTsupplementation 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)

Keywords: Broiler chicks, Synthetic emulsifier, Growth performance, Meat quality, Plasma metabolite profile, Hepatic gene expression

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 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 (vander Heijden and de Haan, 2010). Roy et al. (2010) reported that supplementation of exogenous emulsifiers in diets containingmoderate 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 broilerchicks. Emulsifier, such as lecithin, has been reported to reduce free fatty acid absorption, probably by increasing the size of bile saltmicelles, which diffuse more slowly through the luminal

water interface, hindering the delivery of free fatty acids to the absorptivecell 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 effectsof emulsifiers–in association with vegetable oils–on fat utilization have not been thoroughly investigated yet, even though theinterest in using exogenous emulsifiers has increased in the last several decades. Thus, this study was conducted to assess the effect ofa synthetic emulsifier product AVI-MUL TOP (AMT), consisting of a vegetal bidistilled oleic acid and glycerol polyethylene glycolricinoleate. In particular, the effect of AMT addition was evaluated on: growth performance, meat quality and caecum microbialcounts, 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, Universitàdegli Studi di Milano (Lodi, Italy). The emulsifier product AVI-MUL TOP (AMT, SEVECOM S.P.A., Milan, Italy), consisting of 50 g/kgvegetal bi-distilled oleic acid emulsified with 50 g/kg ethoxylated castor oil E484, which belongs to the glycerol polyethylene glycolricinoleate family (Community Register of Feed Additives - EU Reg. No. 1831/2003), was mixed with the other ingredients before thepelleting process to increase the humidity, reduce the pellet press energy consumption and improve pellet quality by modulating themoisture content during the pelleting process.Animal fat consisting of 50 g/kg poultry fat and 50 g/kg lard was used in the grower andfinisher 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 oneof two experimental groups: control diet without emulsifier supplementation (CTR) and control diet containing AMT (1 g/kg in-clusion 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'sdisease, Newcastle disease and infectious bronchitisviacoarse spraying at hatching.The diets (Table 1) were formulated to meet the nutrient requirements defined by theNational Research Council (NRC) (1994)fora 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 andautomatic nipple cup drinker for water distribution, were bedded with shavings of white wood. Feed and water were provided foradlibitumconsumption. Light cycle and temperature were the same in the two experimental groups. The photoperiod was 24 h of lightfrom 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 andsacrificed 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 ×gfor 10 min at 4 °C. Plasma aliquotswere 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

105 weighed andthe breast muscle yield was calculated as the percentage of eviscerated weight. Breast
106 muscles were stored individually in plastic bagsat 4 °C until subsequent analysis of meat quality.
107
108 *2.3. Meat quality*
109 Breast muscle pH was tested at a depth of 2.5 cm below the surface 24 h after sacrifice, using a
110 combined glass-penetrating electrode (Ingold, Mettler Toledo, Greifensee, Switzerland). Colour
111 measurements were performed on the carcass surface over the breast muscles and on a freshly
112 exposed cut surface of muscle. A Minolta CR-300 chromameter (Minolta, Osaka, Japan) was set to
113 the L* (lightness), a* (redness), and b* (yellowness) according to the CIE scale (CIE, 1986).
114 Water holding capacity (WHC) was determined on breast muscle using the method of Jauregui et al.
115 (1981), with some modifications. Briefly, 1.5 ± 0.3 g of lean muscle was inserted into a pre-weighed
116 (W1) funnel made of four layers of grade 1 filter paper (Whatman International, Maidstone, UK).
117 The funnel with the sample was weighed (W2), put into a centrifuge tube and centrifuged at 15 000
118 rpm for 15 min at 4 °C. The muscle sample was then removed from the funnel, which was weighed
119 again (W3). WHC was calculated as the percentage of water weight lost from the sample, according
120 to the following formula:
121 (W3-W1)/(W2-W1)* 100
122 where W3-W1=water weight (absorbed by the paper), and W2-W1=initial meat weight.
123 For cooking loss determination, each breast was weighed and sealed in a plastic cooking bag and
124 cooked by immersion in an 85 °C water bath until the internal endpoint temperature reached 80 °C.
125 Internal temperature was measured with cooking thermometers introduced into the thickest part of
126 each breast muscle in each cooking batch. After cooking, the samples were chilled by immersion of
127 the bags in an ice water bath for 30 min. Each piece of breast was then removed from its bag and
128 weighed, and cooking loss was calculated.
129 Muscles were tempered at 20 °C for 30 min to equilibrate temperatures, and then six probes (1.27 cm
130 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

156 The concentrations of selected plasma parameters were measured with an automated
157 spectrophotometer with commercial assay kits developed and validated for use with ILAB 300 plus
158 (ILAB 300 plus, Instrumentation Laboratory S.p.a., Milan, Italy). Plasma samples were analysed for
159 cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL), glucose, total protein and urea.
160 Lowdensity lipoprotein cholesterol (LDL) was obtained by difference (cholesterol-HDL). For
161 analysis of non-esterified fatty acids (NEFAs), kits from Randox Laboratories Ltd. (Crumlin, Co.
162 Antrium, UK) were used.
163
164 *2.6. Determination of lipid content in liver samples*
165 Lipids were extracted by Folch's method (Folch et al., 1957) with slight modifications. Briefly, liver
166 samples (±1 g) were thawed and homogenized in an excess of chloroform:methanol (2:1) solution for
167 two minutes. The homogenate was filtered, placed in separator funnels and mixed with a saline
168 solution containing KCl 0.88 g/L. After separation in two phases, the lipid chloroform fraction
169 (bottom layer) was evaporated using a rotary evaporator and subsequently weighed.
170
171 *2.7. Hepatic expression of Apo A-I and Apo B genes*
172 Total RNA was extracted from liver tissue using a commercial kit (SV Total RNA isolation system,
173 Promega, Milano, Italy) according to the manufacturer's protocols. The RNA integrity was assessed
174 by gel electrophoresis to detect 18 s and 28 s rRNA bands. An aliquot of total RNA was reverse
175 transcribed using the iScript cDNA synthesis kit (Biorad, Milan, Italy). The primer sequences used
176 are listed in Table 2. Primer pairs were first tested for their specificity in qualitative PCR, using the
177 pooled cDNA as a template (Jiang et al., 2014). The cycling profile for the assay consisted of 95 °C
178 for 10′, followed by 40 cycles of amplification (95 °C for 15 s, 63° for 60 s). The quantitative analysis
179 of mRNA was carried out by SYBR green methodology as reported by Jiang et al., 2014 using a real
180 time PCR system (Strategene Mx 3000p). The comparative CT method was used (Livak and
181 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.

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 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

234 incorporation of vegetal bidistilled oleic acid and glycerol polyethylene glycol ricinoleate may also
235 have improved the growth performance of animals via the emulsification of supplemental fatty acids
236 (Xing et al., 2004). In relation to carcass characteristics, Scheele (1997) observed that the growth of
237 the pectoral muscles primarily occurs during the late stages of developmental growth in fast-growing
238 birds. In the present study, an increased dressing was observed in the treated group, suggesting that
239 the relatively rapid growth of the AMT-fed chicks in the finisher phase may contribute to the increase
240 of carcass yield.
241 In our study, the AMT supplementation influenced the colorimetric indexes of breast. Meat colour is
242 one of the first characteristic of customer interest, especially in boneless products. Birren (1963)
243 underlined that colour has an important impact on processors and consumers and is often used to
244 determine economic value of food. Akit et al. (2014) demonstrated that dietary emulsifier improved
245 redness values and decreased lightness values but had no effect on pork meat pH and drip loss. In
246 contrast, Kim et al. (2008) found no differences in meat colour of pigs fed lecithin or control diets.
247 Ali et al., (2017) reported the effect of emulsifier on chicken breast meat quality. The authors reported
248 a decreased lightness, an increased redness and no significative effect on yellowness of breast muscle.
249 The changes in b* (yellowness) in the breast muscle observed in the group fed with AMT diet may
250 be explained by the role of emulsifier in increasing lipid-soluble pigments (e.g. xanthophyll)
251 accumulation in breast muscle (Laudadio and Tufarelli, 2010).
252 Many previous in vitro studies suggested that dietary emulsifiers might directly and detrimentally
253 impact the microbiota, leading to societal incidence of obesity/metabolic syndrome and inflammatory
254 diseases (Chassaing et al., 2015; Viennois and Chassaing, 2018). However, our study observed that
255 there was no dietary effect on E. coli and Lactobacilli counts and consequently on the Lactobacilli/E.
256 coli ratio, which may be due to the components of emulsifier and tested parameters in this study.
257 Thus, the effect of AMT supplementation on intestinal inflammation and diversity should be
258 investigated in further researches.

259 Few authors investigated the effects of emulsifier supplementation on the serum lipid profile in avian
260 species and the results are not univocal. In our study, AMT supplementation increased cholesterol
261 and HDL contents, thus indicating an improved lipid metabolism and transport. A study conducted
262 by Wang et al. (2016) showed increased concentrations of total and LDL cholesterol, but not HDL,
263 in the emulsifier treated chicks compared to the control group. The authors suggested that the
264 response could be related also to the type of fat sources (animal vs vegetable) added in the diet and
265 to the inclusion level. Roy et al. (2010) using an exogenous emulsifier suggested a higher removal
266 rate of lipids from the liver. It is clear that multiple potential mechanisms are involved in the
267 regulation of plasma cholesterol concentration, including hepatic uptake of high-density lipoprotein
268 and/or post-absorptive lipid metabolism.
269 It is well-known that liver is the primary site of lipid biosynthesis in avian species and almost all fat
270 accumulation in broiler adipose tissue derives from the liver or the diet (Jiang et al., 2014). The liver
271 and the intestine are major sites of lipoprotein synthesis and secretion in the adult chicken, and
272 pharmacological, dietary and physiological factors might affect concentration of apolipoprotein A-I
273 (Apo A-I) and consequently plasma HDL (Jiang et al., 2014). Apo A-I and apolipoprotein B (ApoB)
274 are the major protein components of chicken plasma high density and very low-density lipoproteins
275 (HDL and VLDL, respectively). In the present study the expression of Apo A-I and ApoB genes in
276 chicken liver was investigated for their essential role in the assembly and secretion of triglyceride-
277 rich lipoproteins and lipid transport. The lack of significant gene expression modulation of Apo A-I
278 and ApoB genes might be due to an adaptive response to the AMT supplementation (44 days of
279 treatment). The adaptive response was previously observe by Huang et al., 2013 who investigated the
280 role of epigallocatechin gallate as lipid metabolism modulator in ROSS 308.
281

282 **5. Conclusion**

283 Our results show that AMT supplementation to broiler chicks may have a beneficial effect on growth
284 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.

Acknowledgements

This work was funded by SEVECOM S.P.A., Milan, Italy. The authors gratefully acknowledge Dr Roberto Bombardieri and Dr Roberto Zaupa and Paolo Chignola (Veronesi Group) for their support and technical assistance. The authors wish to thank Dr. Cinzia Margherita Bertea (Unito) for technical support.

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Table 1

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

386 **Table 2**

387 Oligonucleotide primer sequences.

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

388 [a] Base pair.

389

390

391 **Table 3**

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

393

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

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395 Different superscript letters indicate significant difference ($P \leq 0.05$) significant.

396 BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion

397 ratio.

398

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		
pH24	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 (log10 cfu/g)	7.34	7.60	0.16	0.28
Lactobacilli (log10 cfu/g)	8.10	7.98	0.28	0.77

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

pH24, pH 24 h after sacrifice; WHC, water-holding capacity N, newton.

411 **Table 6**

412 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

413

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

415 HDL, high-density lipoproteins; LDL, low-density lipoproteins; NEFA; non-esterified fatty acids.