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To cite this article: Sihem Dabbou, Achille Schiavone, Francesco Gai, Silvia Martinez, Josefa Madrid, Fuensanta Hernandez, Andrés L. Martínez Marín, Dominga Soglia, Stefano Sartore, Isabelle D. Kalmar, Laura Gasco & Joana Nery (2019) Effect of dietary globin, a natural emulsifier, on the growth performance and digestive efficiency of broiler chickens, Italian Journal of Animal Science, 18:1, 530-537, DOI: [10.1080/1828051X.2018.1547127](https://doi.org/10.1080/1828051X.2018.1547127)

To link to this article: <https://doi.org/10.1080/1828051X.2018.1547127>



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Published online: 10 Jan 2019.



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Effect of dietary globin, a natural emulsifier, on the growth performance and digestive efficiency of broiler chickens

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ABSTRACT

The feed utilisation of young chicks is characterised by a suboptimal fat digestibility, which can be improved by means of dietary emulsifiers. The objective of this study was to evaluate the effect of dietary Globin on the energy efficiency and digestibility of starter feeds and on the production performance of broilers throughout the whole rearing cycle.

A total of 224-day-old ROSS 708 chickens (14 birds/pen, 8 replicates/treatment) were fed *ad libitum* with either a basal diet (C) or a basal diet with the addition of 0.05% Globin during the starter (d1–10), growing (d10–25) and finisher (d25–35) periods. Nutrient digestibility (aD), protein metabolisability (aM_{CP}), energy efficiency (EE) and net energy for production (NE_p) were assessed during the starter period. The average daily feed intake (ADFI), average daily gain (ADG), feed conversion ratio (FCR) and protein efficiency ratio (PER) were measured of each growth period.

Globin significantly decreased FCR ($p = .020$) and increased aD_{fat} ($p = .021$), EE ($p = .028$) and NE_p ($p = .011$) during the starter period. aM_{CP} ($p = .049$) and PER ($p = .039$) were higher in the Globin group than in the Control group. The increased availability of energy from dietary fat, as a result of Globin supplementation, possibly shifted the use of the absorbed amino acids towards an anabolic metabolism, and this could explain the increased aM_{CP} and PER but similar aD_{CP}. The overall performance was similar between groups, although Globin tended to increase PER ($p = .064$) overall.

HIGHLIGHTS

- Globin dietary supplementation was studied in broiler chickens.
- Globin improved digestibility and nutrient efficiency utilization in the first period.
- The overall performance was similar between groups.

ARTICLE HISTORY

Received 21 June 2018
Revised 17 October 2018
Accepted 25 October 2018

KEYWORDS



Broiler; digestibility; energy efficiency; globin; performance

Introduction

Actipro[®] Globin (Veos, 8750 Zwevezele – Belgium) is a protein-based emulsifier that is used in meat products and animal feeds. The active compound is a hydrophilic protein which is manufactured from the red cell fraction of food-grade porcine blood according to a proprietary process by which the final product acquires emulsifying properties. The pH of the proximal gastrointestinal tract (GIT) of chickens ranges between approximately 3 and 5 (crop, proventriculus and gizzard), and reaches pH 6 in the proximal small intestine (Klasing 1998). With an isoelectric point of around pH 4.5, Globin likely precipitates in the acid gastric environment and exerts its

emulsifying properties in the duodenum and jejunum, in a similar way to endogenous and other exogenous emulsifiers (e.g. glyceryl polyethyleneglycol ricinoleate (Bredol[®]), polyethylene glycol ricinoleate, soy lecithin or milk derived casein; Udomprasert and Rukkamsuk 2006; San Tan et al. 2016; Siyal et al. 2017b; Guerreiro Neto et al. 2011). In general, the dietary supplementation of emulsifiers in broiler chickens positively influences digestibility (Prola et al. 2013) and growth performance (Kalmar et al. 2014).

Lipid digestion and fat retention is low in newly hatched chicks, compared to older chickens, due to an insufficient lipase production and bile salt synthesis

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(Al-Marzooqi and Leeson 1999; Prola et al. 2013). Noy and Sklan (1995) observed increased bile acid and lipase secretion in chicks of up to 21 days of age. Moreover, an increase in the intestinal passage rate in chicks up to 14 days of age, leads to a similar fatty acid (FA) digestibility during the first 21 days after hatching. Van der Klein et al. (2017) suggested that fat metabolism could differ between male and female broilers, which may contribute to the differential growth of lean and fat tissues between male and female broilers. In fact larger abdominal fat pad has been observed in females (van der Klein et al. 2017). Lower gastrointestinal weights, but higher liver weights were found in females than in male broilers after a 35 days rearing period (van der Klein et al. 2017). A previous study on the use of 0.05% Globin in poultry feeds with palm oil as the main added fat source, did not show any significant improvement in the feed conversion ratio (FCR) of the starter feed (d1–10). Interestingly, a slight decreasing overall trend (3%) was observed in the FCR during the grower and finisher periods (d11–42) (Arnouts and Lippens 2006). Therefore, this emulsifier could be used in animal nutrition to increase the emulsification, and hence the absorption of FAs and to improve fat digestibility in broiler chickens.

The objective of the present study has been to evaluate the effect of dietary globin on the energy efficiency and digestibility of starter feeds and on the production performance of broilers over during the complete rearing cycle.

Materials and methods

Birds and husbandry

A total of 224-day-old broiler chicks (ROSS 708) were used for this experiment. The chicks were individually identified using a wing tag at the onset of the trial. Two treatments were studied, and each treatment comprised 8 replicates. Each replicate involved 14 chickens (7 males and 7 females) per pen. Sixteen pens were installed in a commercial poultry house in Airasca (TO) (Italy). Hence, the trial was performed under commercial rearing conditions. The pens were 1.20 m wide × 1.20 m long, with rice hulls as litter. The feeds and drinking water were provided *ad libitum* in hanging feeders (1/pen) and automatic water dispensers (4/pen), respectively. The poultry house was equipped with automatic ventilation and infra-red lamps. The daily lighting schedule was 18 h light:6 h dark, and the temperature was gradually lowered during the rearing period from 32 to 22 °C. The broilers

Table 1. Ingredients and nutrient composition of basal diets.

| | Starter (d1–10) | Grower (d10–25) | Finisher (d25–35) |
|--|--------------------|--------------------|----------------------|
| Ingredient composition, % | | | |
| Corn meal | 35.41 | 26.51 | 40.17 |
| Soybean meal 48 | 32.76 | 31.26 | 27.29 |
| Wheat | 24.43 | 34.94 | 24.93 |
| Soybean oil | 2.91 | 3.66 | 4.43 |
| Dicalcium phosphate | 1.53 | 1.17 | 1.02 |
| Calcium carbonate | 0.60 | 0.77 | 0.71 |
| Vitamin and mineral premixa | 0.50 | 0.40 | 0.30 |
| L-Lysine | 0.33 | 0.27 | 0.25 |
| DL-methionine | 0.30 | 0.30 | 0.22 |
| Sodium chloride | 0.24 | 0.16 | 0.20 |
| Threonine | 0.15 | 0.11 | 0.08 |
| Sodium bicarbonate | 0.12 | 0.23 | 0.18 |
| 3-Phytase | 0.10 | 0.10 | 0.10 |
| Titanium dioxide | 0.50 | – | – |
| Xylanase | 0.10 | 0.10 | 0.10 |
| Choline chloride | 0.02 | 0.02 | 0.02 |
| Nutrient content | | | |
| Dry matter, % | 90.81 | 89.94 | 90.67 |
| Crude ash, % | 8.20 | 6.27 | 5.72 |
| Crude protein, % | 23.67 | 21.52 | 19.52 |
| Ether extract, % | 6.46 | 5.39 | 6.66 |
| Calcium, % | 0.75 | 0.73 | 0.65 |
| Phosphorus, % | 0.64 | 0.59 | 0.54 |
| Sodium, % | 0.13 | 0.13 | 0.13 |
| Gross energy, MJ/kg | 18.50 | 19.30 | 19.20 |
| Metabolisable energy, ^b MJ/kg | 11.50 | 11.50 | 12.20 |

^aVitamin and mineral premix composition: vitamin A (retinyl acetate), 12,500 U; vitamin D₃ (cholecalciferol), 3000 U; vitamin E (DL- α -tocopheryl acetate), 60 U; vitamin K (menadione sodium bisulfite), 1.02 mg; riboflavin, 2.0 mg; pantothenate, 8.0 mg; niacin, 6 mg; piridossin, 4 mg; folic acid, 0.5 mg; biotin, 0.10 mg; tiamin, 1.0 mg; vitamin B₁₂, 20 mg; Mn, 120 mg; Zn, 80 mg; Fe, 52 mg; Cu, 15 mg; I, 1.5 mg; Se, 0.4 mg; ^bMetabolisable energy (ME), Calcium, Phosphorus and Sodium were calculated from ingredient composition according to McDonald et al. (2011) and Sauvart et al. (2004).

were vaccinated against Newcastle Disease and Infectious Bronchitis on day 1. The experimental protocol was approved by the Ethic Committee of the University of Turin (Italy) (Protocol n. 420078).

Diets

Basal diets of the three periods, that is, a starter (d1–10 of age), a grower (d10–25 of age) and a finisher diet (d25–35 of age), were fed to the control group. The Globin group was fed the same basal diets with the addition of 0.05% porcine Globin (Actipro[®] 95 PGS, Veos, Belgium; 93% dry matter (DM), 92% crude protein (CP), 0.3% fat and 14.4 MJ/kg metabolisable energy (ME) in poultry), which was placed on top during the raw material mixing process. A Globin supplementation dose of 0.05% was used, according to the manufacturer's recommendation. The control and globin supplemented diets were isoenergetic and iso-nitrogenous. All the diets were formulated according to Aviagen (2014) broiler nutrition specifications. The

ingredients and chemical composition of the experimental diet are reported in Table 1.

Growth performances

The clinical signs and mortality were monitored daily during the whole experimental period. Body weight (BW) was recorded individually at the end of each feeding period. The average daily feed intake (ADFI), average daily gain (ADG) and FCR were determined per feeding period and for the overall rearing period. In addition, the protein efficiency ratio (PER) was calculated as the ratio between ADG and CP intake during each rearing period for each treatment.

Nutrient digestibility

For estimation of nutrient digestibility collection trays were installed in floor pens according to Kaczmarek et al. (2015) with slight modifications. For this purpose, all birds were removed from pens and housed in wire-mesh cages (100 cm × 50 cm width × length) for 60 min/day for four consecutive days (d7–10) to collect fresh excreta samples. The excreta in each pen was pooled and stored at – 20 °C, until lyophilisation and analyses. The external marker method, with 0.5% titanium dioxide as the marker, was used to determine the apparent nutrient digestibility. Apparent nutrient digestibility (aD_X) was calculated according to Eq. (1) (McDonald et al. 2011; Kaczmarek et al. 2015; Schiavone et al. 2017). Crude protein digestibility (aD_{CP}) and protein metabolisability (aM_{CP}) were calculated using the excreta CP, corrected for uric acid ($CP_{corrected}$, Eq. (2)), and the excreta total CP, respectively (Schiavone et al. 2017).

$$aD_X (\%) = \left(1 - \frac{[TiO_2]_{diet} * [nutrient X]_{excreta}}{[TiO_2]_{excreta} * [nutrient X]_{diet}} \right) \times 100 \quad (1)$$

$$CP_{corrected} = ([N]_{Kjedahl} - [N]_{uric\ acid}) \times 6.25 \quad (2)$$

where $[nutrient]_{diet}$ and $[nutrient]_{excreta}$ are the analysed concentrations of the nutrients in the diet and excreta samples (g/kg) respectively, and where $[TiO_2]_{diet}$ and $[TiO_2]_{excreta}$ are the analysed concentrations of titanium dioxide in the diet and excreta samples (g/kg).

Nutrient analysis

Excreta and diet samples were analysed to establish the DM content according to AOAC International (2005; procedure number 930.15). Dried samples were ground to 0.5-mm particles and stored pending analysis of the

crude ash, CP (AOAC 2005; procedure numbers 924.05 and 984.13, respectively), ether extract (Folch et al. 1957), gross energy (GE, IKA C7000, Staufen, Germany) and titanium dioxide (Short et al. 1996). The excreta samples were analysed to establish the uric acid (UA) using the spectrophotometric method proposed by Terpstra and De Hart (1974).

Whole body energy content

The whole body energy (GE_{bird} , MJ/bird) was determined on day-old chicks and at 10 days of age (1 male and 1 female per replicate pen), according to Fatufe et al. (2004). The birds were sacrificed by means of an intravenous injection of pentobarbital sodium in the wing vein; they were then homogenised, freeze dried and the GE_{bird} was determined by means of bomb calorimetry. Energy accretion (EA) was calculated as the difference between GE_{bird} at 1 and at 10 days of age. The EA was used to calculate the net energy for production (NEp).

Energy efficiency of the starter feed

The net energy for production (NEp, MJ/kg feed), defined as the energy accretion over the 10-d starter period per unit of feed intake (FI; kg/bird) was calculated as the ratio between EA and FI during the starter period (Eq. (3)). Dietary energy efficiency (EE, %), defined as the energy gain over the 10-d starter period per unit of energy intake (Fatufe et al. 2004), was calculated as the ratio between NEp and GE of the starter feed (MJ/kg feed; Eq. (4)):

$$NEp \text{ (MJ/kg feed)} = \frac{GE_{bird} (d10 - d1)}{FI(d10 - d1)} \quad (3)$$

$$EE (\%) = \frac{NEp}{GE_{feed}} \quad (4)$$

Statistical analysis

Statistics were performed using the SPSS software package (version 25.0 for Windows, SPSS Inc., Chicago, IL, USA). All the data were pooled per pen and statistically analysed with the pen as the experimental unit. Normality and homoscedasticity were tested with the Kolmogorov-Smirnov test and a modified Levine test, respectively. The performance and digestibility traits were analysed using one-way ANOVA. In addition, a univariate General Linear Model analysis was performed for the BW of the birds with diet (D), sex (S) and their interaction ($D \times S$) as the fixed factors. Null hypotheses were considered for the effect of the fixed factors and their interaction on the BW of the birds.

Table 2. Effect of dietary globin at a dose of 0.05% on technical performance of broiler chickens ($n = 8$).

| | Control | Globin | SEM | <i>p</i> -Value |
|---------------------------------|-------------------|-------------------|-------|-----------------|
| BW, g | | | | |
| - BW _{d1} | 43 | 42 | 0.24 | .143 |
| - BW _{d10} | 193 | 191 | 3.90 | .756 |
| - BW _{d25} | 937 | 925 | 13.62 | .663 |
| - BW _{d35} | 1721 | 1741 | 14.13 | .505 |
| ADFI, g/d | | | | |
| - Starter period (d1–10) | 18.30 | 17.20 | 0.43 | .196 |
| - Grower period (d10–25) | 71.40 | 69.30 | 0.96 | .298 |
| - Finisher period (d25–35) | 132.10 | 130.50 | 2.02 | .699 |
| ADG, g/d | | | | |
| - Starter period (d1–10) | 15.00 | 14.80 | 0.38 | .817 |
| - Grower period (d10–25) | 49.30 | 48.60 | 0.73 | .675 |
| - Finisher period (d25–35) | 72.80 | 74.60 | 0.82 | .292 |
| FCR, g/g | | | | |
| - Starter period (d1–10) | 1.22 ^a | 1.16 ^b | 0.01 | .020 |
| - Grower period (d10–25) | 1.45 | 1.43 | 0.01 | .235 |
| - Finisher period (d25–35) | 1.81 | 1.75 | 0.03 | .326 |
| PER, g/g | | | | |
| - Starter period (d1–10) | 3.46 ^a | 3.62 ^b | 0.04 | .039 |
| - Grower period (d10–25) | 3.21 | 3.26 | 0.02 | .212 |
| - Finisher period (d25–35) | 2.84 | 2.94 | 0.04 | .262 |
| Overall performance, g/d | | | | |
| - ADFI, g/d | 73.60 | 71.90 | 0.80 | .312 |
| - ADG, g/d | 46.20 | 46.40 | 0.54 | .871 |
| - FCR, g/g | 1.59 | 1.55 | 0.02 | .212 |
| - PER, g/g | 3.18 | 3.27 | 0.03 | .064 |

^{a,b}Different superscripts indicate significant differences at $p < .050$.

BW: body weight; ADFI: average daily feed intake; ADG: average daily gain; FCR: feed conversion ratio; PER: protein efficiency ratio.

Significance was set at $p \leq .05$ and statistical tendency was considered for $p \leq .10$.

Results

Growth performances

Mortality was considered negligible throughout the whole experimental period because only one chick from the control group was found dead during the growing period. Table 2 summarises the growth performance of the broiler chickens during the three rearing periods. The initial BW of the chicks did not differ between the dietary treatments. Overall, the BW was similar between the tested groups for all the time points. The BW of the males tended to be higher at the end of the starter period ($p = .068$) and was significantly higher at 25 and 35 d of age ($p = .009$ and $p < .001$; Figure 1). A statistical tendency ($p = .088$) of an interaction between sex and diet was observed at 35 d of age, at which time the females of the Globin group showed a higher BW than those of the control group (1674 and 1582 g, respectively), while the BW of the males was similar for the Globin and control group (1826 and 1799 g, respectively; Figure 1).

Globin supplementation led to a lower FCR during the starter period (1.22 and 1.16 in the control and Globin groups, respectively; $p = .020$) (Table 2). The FCR of the growing and finisher periods was similar

between the tested groups (1.45 and 1.43 for the control and Globin groups during the growing period, and 1.81 and 1.75 for the control and Globin groups during the finisher period, respectively). The PER of the starter period was significantly higher in the Globin group than the control group (3.46 and 3.62 in the control and Globin groups, respectively; $p = .039$). Overall, PER tended to be higher in the Globin group (3.18 and 3.27 in the control and Globin groups, respectively; $p = .064$).

Nutrient digestibility, energy efficiency and protein metabolisability during starter period

The effect of Globin supplementation on nutrient digestibility, aM_{CP} , NE_p and the EE of the starter feed is reported in Table 3. Protein digestibility was similar between the tested groups but protein metabolisability was higher in the Globin group than the control group ($p = .049$). The fat digestibility of the starter feed was also higher in the Globin group than in the control group ($p = .021$). The energy efficiency and net energy for production were also increased by the dietary Globin during starter period.

Discussion

The active compound in Globin is a hydrophilic protein that lowers the surface tension of oil-in-water

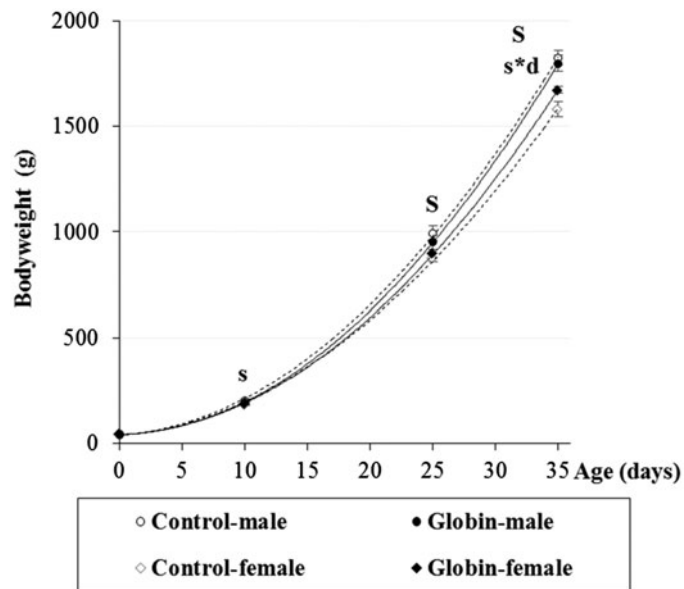


Figure 1. Effect of dietary globin supplementation on body weight (g) at 1, 10, 25 and 35 days of age (MJ/kg) in male and female broiler chickens (mean \pm SEM). **Note:** continuous line represents Globin group and dotted line represents control group; capital letters mean significant effect ($p < .050$) of sex (S) and/or diet (D), lowercase letters mean statistical tendency ($p < .100$) of sex (s) and/or diet (d) between control and Globin groups.

Table 3. Effect of dietary Globin at a dose of 0.05% on nutrient digestibility, protein and energy efficiency and net energy for production of broiler starter feed ($n = 8$).

| | Control | Globin | SEM | <i>p</i> -Value |
|-----------------------|-------------------|-------------------|------|-----------------|
| aM _{CP} , % | 63.9 ^a | 67.8 ^b | 1.04 | .049 |
| aD _{CP} , % | 87.4 | 88.3 | 0.44 | .325 |
| aD _{fat} , % | 74.0 ^a | 78.6 ^b | 1.04 | .021 |
| EE, % | 31.7 ^a | 34.0 ^b | 0.54 | .028 |
| NEp, MJ/kg | 5.9 ^a | 6.4 ^b | 0.11 | .011 |

^{a,b}Different superscripts indicate significant differences at $p < .050$.

aM: apparent metabolisability; aD: apparent digestibility; CP: crude protein; EE: energy efficiency; NEp: net energy for production.

systems and stabilises the emulsion process (Yang and Lin 1998; Tybor et al. 1973). Because of its emulsifying capacity, Globin may be used to enhance fat digestibility of poultry, especially of newly hatched chicks (Arnouts and Lippens 2006). Several studies that used different types of exogenous natural and synthetic emulsifiers in poultry diets have recently been reviewed (Siyal et al. 2017a and b; Abbas et al. 2016; Kaczmarek et al. 2015). Therefore, our study investigated the effect of globin (Actipro[®] Globin) as an emulsifying agent on the growth, performance, digestibility and energy efficiency of broiler chickens. The addition of Globin reduced the FCR and increased the PER, aD_{fat}, aM_{CP}, EE and NEp of the starter feed. The observed increase in feed efficiency could be due to the increased availability of dietary fat as an energy substrate. Consequently, the metabolic fate of the absorbed amino acids may be shifted towards an anabolic metabolism (McDonald et al. 2011). This would explain the observed increase in protein

metabolisability and PER for the dietary Globin, although the protein digestibility was similar to the control.

Dietary Globin resulted in higher aD_{fat} and reduced FCR during the starter period. Our results are in agreement with those reported by Arnouts and Lippens (2006) who observed a tendency of decreased FCR in the grower-finisher periods but no significant effect in the starter period, when 0.05% Globin was added to the feed. Other studies have found a lower FCR and a higher fat digestibility during grower and finisher periods when other emulsifiers were used in broiler diets (Kaczmarek et al. 2015; Upadhaya et al. 2018). In contrast to the present study, Arnouts and Lippens (2006), Kaczmarek et al. (2015) and Upadhaya et al. (2018) did not find any effects on performance or digestibility during the starter period. The differences in effects on performance and fat digestibility as a result of dietary emulsifiers could be related to the dietary content of the saturated and unsaturated FAs, the sex of the birds and/or the type and dosage of the emulsifier. The dietary fat in the study by Arnouts and Lippens (2006) included palm fat (between 73 and 64% of the total fat ingredients during the starter and finisher periods, respectively), Kaczmarek et al. (2015) used rapeseed oil and lard, Upadhaya et al. (2018) used tallow fat as the major fat source, while only soybean oil was used as the fat ingredient in the present study. Zaefarian et al. (2015), who used either soy oil or tallow as dietary fat sources, reported higher feed intakes and weight gains between 1 and 20 days of age, and for the whole rearing period during the early

rearing period in broilers fed diets containing a lysolecithin emulsifier. Nevertheless, Zaefarian et al. (2015) found no effect of emulsifier addition on FCR during the initial rearing period, as has also been observed in the present study. The performance differences could also be related to the amount and type of emulsifier, because Zaefarian et al. (2015) added less emulsifier to the diets that clearly had higher fat contents than the ones presented in this study.

Although ADG and growth were not influenced by the diet throughout the rearing period, a sex \times diet influence on growth was observed at 35 days of age. The higher BW of females fed the Globin diet could be related to the fat deposition process. Van der Klein et al. (2017) observed a significantly higher weight of the abdominal fat pad in females than in male broilers at 35 days of age, together with a higher BW of males than females of the same age. The authors suggested that the higher liver weight in females could also be related to an increased hepatic activity, in particular concerning the fat metabolism. The emulsion properties of Globin could therefore also have led to an increased digestibility of fat, which was translated into a higher BW at the end of the rearing period in the females fed the Globin than those fed the control diet.

Globin dietary supplementation did not affect aD_{CP} , but a higher aD_{fat} and aM_{CP} were observed in the Globin group during the starter period. These results are in line with those observed by Upadhaya et al. (2018), who found a similar protein digestibility, a higher DM and a higher fat digestibility in 35-day old broilers fed a diet supplemented with a commercial blend of emulsifiers (0.05 up to 0.10% supplementation). In a study that included a basal broiler diet supplemented with glycerol polyethylene glycol ricinoleate at concentrations of 1% and 2% of added fat, Roy et al. (2010) found positive effects of emulsifiers on the digestion of fat as well as other nutrients. Zhao et al. (2015) and Upadhaya et al. (2017) reported improvements in fat digestibility when a basal diet was supplemented with graded levels of emulsifier (1,3-diacylglycerol and lysophospholipid) in broilers and weaning pigs. On the other hand, Zaefarian et al. (2015) found a higher protein digestibility in broilers older than 12 days of age fed diets containing soy oil as major fat source with the addition of 0.035% lysolecithin emulsifier, when phytase was supplemented at the maximal level.

The higher aM_{CP} observed in the globin group during the starter period led to a significant increase in PER, compared to the control group. Roy et al. (2010) also found an increased PER as a result of the addition of an exogenous emulsifier in broiler diets during growing,

finisher and overall rearing periods. These authors reported a higher metabolisability of fat and protein throughout the overall rearing period. In addition, they associated the fat digestibility with protein metabolisability, and this might have resulted in an improved growth performance, in particular for FCR.

The higher EE and NE_p in the Globin group than in the control group corroborates the hypothesis that an increased fat digestibility due to dietary Globin, results in an increased availability of energy from fat, which reduces the need for a less-efficient protein catabolism for energetic purposes. These results are in agreement with those of Kaczmarek et al. (2015), who also found an increased apparent metabolisable energy in broilers fed diets supplemented with an exogenous emulsifier during the first 2 weeks of life.

Conclusions

The findings of this study suggest that the addition of 0.05% Globin as an emulsifier to broiler chicken diets decreased FCR and increased fat digestibility, protein metabolisability, PER and the net energy for production and energy efficiency during the starter period. These findings suggest that increasing fat digestibility, through the addition of dietary Globin, could have increases availability of energy from fat, which reduces the need for a less-efficient protein catabolism for energetic purposes. Further research efforts are necessary to investigate the impact of increasing levels of Globin in broiler chicken diets containing different sources and amounts of dietary fat in order to understand the dose-response effect of globin supplementation on the growth performance and nutrient digestibility of broiler chickens.

Acknowledgments

The authors are grateful to Dr. Oreste Massimino, Mr. Roberto Borgogno and Mrs. Marilena Falcetti, "O.R.A. Agricola Srl" (Cherasco, CN, Italy), Dr. Piero Gaidano (Mangimificio Fratelli Borello, Bra, CN, Italy), Dr. Paolo Montersino, Mr. Dario Sola and Mr. Mario Colombano for technical support.

Disclosure statement

The authors declare that there is no conflict of interest associated with the paper. The authors alone are responsible for the content and writing of this article.

Funding

This work was supported by the VEOS, Belgium under Grant [no. SCH_CONTR_FIN_16_01].

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