Lycopene affects the immune responses of finishing pigs

Marcelise Regina Fachinello⁵, Nelson Luis Mello Fernandes⁶, Eliezer Rodrigues de Souto⁷, Tatiana Carlesso dos Santos⁷, Alcides Emanuel Rodrigues da Costa⁴ and Paulo Cesar Pozza⁴

⁴Departamento de Zootecnia, State University of Maringá, Maringá, Brazil; ⁵Departamento de Ciências Veterinárias, Federal University of Parana, Palotina, Brazil; ⁶Departamento de Agronomia, State University of Maringá, Maringá, Brazil

ABSTRACT
The objective of this study was to evaluate dietary lycopene levels on the immune responses of finishing pigs. Forty barrows and 40 gilts, averaging 75.04 ± 1.6 kg of initial weight, were allotted in a randomised blocks design arranged in a 2 × 5 factorial scheme, consisting of two sexes (males and females) and five lycopene levels (0, 12.5, 25.0, 37.5, 50.0 mg/kg of diet). The studied parameters were submitted to the statistical analysis adopting 5% significance. Increasing lycopene in pig diets increased the plasma albumin ($p = .023$). There was no interaction ($p > .05$) between sex and lycopene on the leukocyte profile or haematocrit concentration. As lycopene levels increased in the diet, the lymphocyte concentration increased linearly ($p = .045$). The neutrophil concentration and the neutrophil:lymphocyte ratio were affected ($p < .05$) by dietary lycopene levels, showing a lower neutrophils concentration at 17.49 mg lycopene/kg of diet, and the lowest neutrophil:lymphocyte ratio was observed at 16.46 mg/kg. Eosinophils were also affected ($p = .050$) by lycopene supplementation, estimating the greater response by adding 22.69 mg lycopene/kg of diet. There was an interaction ($p = .011$) between the blood collection period and lycopene levels for anti-BSA IgG, resulting in higher production of anti-BSA IgG with the supplementation of up to 20.06 mg of lycopene/kg of diet. Dietary lycopene supplementation for finishing pigs affected the cellular and humoral immune response, and the highest anti-BSA IgG production was achieved by supplementing 20.06 mg lycopene/kg of diet.

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Introduction
The high animals’ density and the environmental conditions in many pig farms exposes it to an expressive number of pathogenic bacteria, viruses and also some parasites. As a result, dietary nutrients that could be targeted for growth performance are redirected to the immune system, in order to provide protection against pathogenic microorganisms, directly affecting the pig performance. The addition of antioxidants and natural immunomodulatory compounds in the diet is considered a good way to improve animal health and performance (Zhao et al. 2016). In the absence of an infectious process, the need to elaborate an immune response, by itself, is able of affecting the productive capacity. However, activating immune system affects nutrient and energy utilisation, and the immune system activation depends on a very complex mechanism, which is subjected to the effects of internal and external factors (Pechinskii and Kuregyan 2014).

Carotenoids are antioxidants and one of the external factors that may affect the immune system (Pechinskii and Kuregyan 2014), showing a broad spectrum of biological effects and acts as immunomodulator, also affects the function of T and B cells, induces differentiation and inhibit the proliferation of some cells (Darroch 2001) and are able to affect humoral and cellular immunity (Zhao et al. 2016). Lycopene is a carotenoid that shows an expressive sequestering ability of singlet oxygen, possibly due to its two unconjugated double bonds, providing a higher reactivity than other carotenoids (Shami and Moreira 2004).

Lycopene stimulates the immune system acting against the oxidative damage of the lymphocytes’ DNA (Palabiyik et al. 2013). It stimulates lymphocytes by increasing the production of IL-2 and interferon-gamma (INF-γ), a potent activator of T lymphocytes (Yuksek et al. 2013). Lycopene also affects the immunoglobulins production, increasing blood levels of IgA, IgG and IgM, and enhances the immunity (Lumeij
2008), stimulating the communication between cells and raising the immune response (Olson et al. 2008).

In addition, lycopene is a potent antioxidant, as well as an inhibitor of proinflammatory and prothrombotic factors (Türk et al. 2010). Lycopene suppresses inflammation in several tissues, inhibiting the formation of proinflammatory cytokines and chemokines in macrophages (Lee et al. 2012; Marcotorchino et al. 2012), in addition to controlling chronic immune and inflammatory processes and also delaying the maturation of dendritic cells (Kim et al. 2004).

The immunomodulatory role of lycopene has been studied, but there is a lack of information about the dietary supplementation of lycopene on the pigs’ immune system. Thus, this study aimed to evaluate dietary lycopene levels on the immune responses of 75 to 100 Kg pigs.

Materials and methods

The experiment was carried out in the Experimental Farm of Iguatemi – EFI, belonging to Universidade Estadual de Maringá, Paraná State, Brazil (23° 21’S, 52° 04’W and Altitude of 564m), from January to May 2016. All experimental procedures were previously approved by the Animal Care and Use Committee (protocol number 6570200815).

Animals and facilities

The pigs (Piétrain × Landrace × Large White) were housed in an open-sided finishing barn, divided into two wings, each consisting of 20 pens (3 m²). Each pen was provided by a self-drinker and a semiautomatic feeder, providing free access to feed and water throughout the experimental period.

The temperature was registered with the aid of a digital thermometer, installed in the centre of the experimental building, recording the minimum and maximum temperature of 19.62 ± 2.54°C and 32.11 ± 3.2°C, respectively.

Experimental design and diet

Forty barrows and 40 gilts, averaging 75.04 ± 1.6 kg of initial weight, were distributed in a randomised blocks design in a 2 × 5 factorial scheme, consisting of two sexes (barrows and gilts) and five lycopene levels (0, 12.5, 25.0, 37.5 and 50.0 mg/kg of diet), with eight replicates and one animal per experimental unit. The period was the criteria adopted to establish the blocks, since the building was provided with 40 pens.

| Table 1. Ingredients, chemical and energetic composition of basal diet. |
|---------------------------------------------------------------|
| **Ingredients %** | **Barrows** | **Gilts** |
| Corn | 87.14 | 83.89 |
| Soybean meal 45% | 9.61 | 12.47 |
| Soybean oil 0.74 | 0.74 | 0.95 |
| Dicalcium phosphate | 0.79 | 0.97 |
| Limestone | 0.55 | 0.52 |
| Salt | 0.20 | 0.20 |
| L-lysine HCl 78.4% | 0.37 | 0.39 |
| L-threonine 98.5% | 0.07 | 0.07 |
| DL-methionine 99.0% | 0.04 | 0.05 |
| L-tryptophan 98.0% | 0.02 | 0.02 |
| Growth promoter^a | 0.02 | 0.02 |
| Vitamin and mineral supplement^b | 0.40 | 0.40 |
| Lycopene extract^c | 0.00 | 0.00 |
| Inert^d | 0.05 | 0.05 |
| Total | 100.00 | 100.00 |

| Composition |
|----------------|
| Metabolisable energy, MJ/kg | 13.81 | 13.81 |
| Nitrogen, % | 1.86 | 2.03 |
| Calcium, % | 0.45 | 0.49 |
| Available phosphorus, % | 0.23 | 0.26 |
| Potassium, % | 0.42 | 0.47 |
| Sodium, % | 0.10 | 0.10 |
| Chlorine, % | 0.18 | 0.18 |
| SID lysine, % | 0.69 | 0.77 |
| SID met + cis, % | 0.40 | 0.44 |
| SID threonine, % | 0.44 | 0.48 |
| SID tryptophan, % | 0.12 | 0.13 |

^aEnramycin 0.015%.
^bContent/kg diet; vit. A: 30,000 UI, vit. D3: 5000 UI, vit. E: 120 UI, vit. K: 5 mg, vit. B12: 120 mcg, Niacin: 150 mg, Calcium pantothenate: 75 mg, folic acid: 8 mg, Choline chloride: 0.48 g, iron: 350 mg, copper 15 mg, manganese: 250 mg, Zinc 0.75 g, iodine: 10 mg, selenium: 3 mg.
^cLycopene extract at a concentration of 10% included in the basal diet replacing the inert.
^dFine clean sand.

The experimental diets (Table 1) were based on corn, soybean meal, minerals, vitamins and additives, to meet the nutritional recommendations proposed by the National Research Council – NRC (2012). The lycopene source used in the experimental diets consisted of a commercial product containing 10% of lycopene (lycopene extract) that was added in the diets at the expense of 0, 125, 250, 375 and 500 mg of inert/kg of diet, corresponding to 0, 12.5, 25.0, 37.5 and 50.0 mg of lycopene/kg of diet, respectively.

Pig immunisation before blood collection

Pigs were immunised with 1 mg of a bovine serum albumin solution (BSA) (A3912, Sigma-Aldrich, St. Louis, MO, USA) diluted with 0.5 mL of a phosphate-buffered saline (PBS) solution and 0.5 mL of an adjuvant, by subcutaneous immunisation (Li et al. 1999). This procedure was carried out on days 0 and 12 of the experimental period. The Complete Freund’s adjuvant (F5881, Sigma-Aldrich, St. Louis, MO, USA) was used at the first (day 0) inoculation and the incomplete Freund’s adjuvant (F5506 Sigma-Aldrich, St. Louis, MO, USA) was used in the second (day 12) inoculation (Figure 1).
Blood collection

Blood samples were taken at the end of the experiment in order to determine the total protein content and its fractions, haematocrit and leukocyte profile. Pigs were fasted during six hours before blood collection that was performed by a puncture of the jugular vein (Oliveira et al. 2004). Blood samples were also collected on days 0, 12 and 24 of the experimental period to determine serum IgG production.

Blood samples were harvested in glass tubes containing EDTA, to determine total proteins and its fractions, and heparin was used in glass tubes to determine haematocrit and leukocyte profile, as the blood serum was used for IgG determination. After collection, the blood samples were immediately centrifuged at 3000 rpm during 15 min, at the Swine Laboratory of EFI, in order to obtain the plasma and serum, which was extracted from the glass tubes by an automatic pipette and stored in Eppendorf type microtubes at −20 °C.

Determination of haematocrit and leukocyte profile

Glass tubes containing blood samples were placed into an automatic homogeniser during 5 minutes before haematocrit determination. After that, blood samples were transferred into capillary microtubes and centrifuged at 13,416 × g during five minutes. The percentage of haematocrit was determined using a percentage scale.

A blood smear was prepared on glass slides and then stained using the May-Grunwald-Giemsa method. The differential count was performed by using an optical microscope using the immersion objective and the cells were classified into lymphocytes, eosinophils, monocytes, neutrophils and basophils, calculating the proportion of each 100 cells.

Total proteins and fractions

Total proteins (TP) and albumins (AB) were determined using specific kits (Bioclin®), according to the standard operating procedures described for each parameter. The absorbance reading was performed on a Biochemical Analyzer (Bioplus® 2000, Brazil). The globulins (GL) quantification was performed by means of the difference between AB and TP, and the AB:GL ratio was also calculated.

Antibovine serum albumin (anti-BSA) IgG production

The anti-BSA IgG production was determined using the indirect ELISA technique. The 96-well ELISA plates were coated with a solution (100 μL/well) containing 0.01 mg BSA/mL of carbonate buffer (pH 9.6). The plates were then incubated at 4 °C during 16 h. A blocking solution was used at 100 μL/well and the plate was incubated for 1 h at 37 °C. Serum samples were tested in triplicate (100 μL/well, 1:10,000) and incubated in a greenhouse for 2 h at 37 °C. The IgG antipig conjugated to rabbit-produced peroxidase (100 μL/well, 1:30,000, SAB3700420, Sigma-Aldrich, St. Louis, MO, USA) was added and incubated for 1 h at 37 °C. Then, 00 μL/well of the 3,3′, 5,5′ substrate was added, – tetramethylbenzidine (TMB) (T0440, Sigma-Aldrich, St. Louis, MO, USA) and incubated during 30 min at 27 °C. The reaction was stopped by using HCl (2M, 100 μL/well).

The anti-BSA IgG production was quantified by means of absorbance reading, with measurements being performed on an ELISA plate reader (SMP500 – 13334-RCPP coupled to SoftMaxR Pro 5 serial number) at 450 nm and the results were expressed as an optical density. The plates were washed three times with a NaCl and Tween-20 wash solution between each aforementioned step. To determine the optimal dilution of serum and antipig IgG, to take the absorbance readings, a series of dilutions were tested. Serum was tested at the dilutions 1:1000, 1:5000, 1:10,000, 1:15,000, 1:20,000 and the IgG antipig was tested at the dilutions 1:5000, 1:10,000, 1:15,000, 1:30,000, 1:50,000. The best readings were obtained at 1:10,000 and 1:30,000, for serum and IgG antipig, respectively.

Statistical analysis

The UNIVARIATE procedure of SAS (Statistical Analysis System, version 9.0, Cary, NC, USA) was applied to assess the presence of outliers. Subsequently, data were submitted to analysis of variance (ANOVA) and the effects of blocks, sex, lycopene and sex × lycopene interaction were included in the model. Additionally, for the anti-BSA IgG production, the effects of days, sex × days, lycopene × days and days × sex × lycopene interactions were included in the model.
The degrees of freedom regarding lycopene levels and/or collection days were deployed in orthogonal polynomials to fit the regression equations by means of the quadratic and/or linear models, as the Linear Response Plateau (LRP) model was also fitted. Data were submitted to statistical analysis using the SAS (Statistical Analysis System, version 9.0, Cary, NC, USA). The significance level of 5% was adopted for all the statistical procedures.

**Results**

**Total proteins and fractions**

There was no interaction between sex and lycopene (Table 2) on TP ($p = .971$), AB ($p = .430$), GL ($p = .648$) and AB:GL ratio ($p = .527$). The inclusion of lycopene in pig (barrows and gilts) diets affected the plasma protein fractions, with a linear increase ($p = .023$) in plasma albumin concentration as lycopene increased in the diet ($\hat{Y} = 0.0071x + 3.23; R^2 = 0.89$).

The TP, GL and AB:GL ratio were not affected ($p > .05$) by lycopene levels. However, sex affected the plasma protein fractions, with gilts having higher TP ($p = .001$) and GL ($p = .001$) concentrations and lower AB ($p = .049$) and AB:GL ($p = .001$) concentrations than barrows.

**Leukocyte profile and haematocrit**

There was no interaction ($p > .05$) between sex and lycopene levels on leukocyte profile and haematocrit (Table 3). The lymphocyte profile was affected by dietary lycopene levels, with a linear increase ($p = .045$) in lymphocyte concentration as lycopene levels increased ($\hat{Y} = 0.1382x + 68.81; R^2 = 0.58$) in pig diets (barrows and gilts).

Neutrophils were linearly ($p = .024$) and quadratically ($p = .003$) affected by dietary lycopene levels, according to the equations $\hat{Y} = -0.1225x + 19.87$ ($R^2 = 0.35$) and $\hat{Y} = 0.0110x^2 - 0.6710x + 23.26$ ($R^2 = 0.97$), respectively. The LRP model was also fitted ($\hat{Y} = -0.66x + 23.75; R^2 = 1.00$) and its association with the quadratic model showed a lower neutrophil cells at 17.49 mg of lycopene/kg of diet (Figure 2(A)). The neutrophil:lymphocyte ratio was linearly ($\hat{Y} = -0.002592x + 0.328; R^2 = 0.33$) and quadratically ($\hat{Y} = 0.000242x^2 - 0.146x + 0.4024; R^2 = 0.97$) affected by dietary lycopene levels ($p = .005$), and also the LRP model ($\hat{Y} = -0.01x + 0.4114; R^2 = 1.00$). The association of the quadratic and LRP models showed the lowest neutrophil:lymphocyte ratio at the level of 16.46 mg lycopene/kg of diet (Figure 2(B)).
Table 3. Blood leukocyte profile and haematocrit of, barrows and gilts, from 75 to 100 kg, fed diets containing different levels of lycopene.

| Item, cells/total 100 cells | Lymphocyte (mg/kg of diet) | Monocyte | Neutrophil | Basophil | Eosinophil | Neutrophil, % | Haematocrit, % |
|-----------------------------|-----------------------------|----------|------------|----------|------------|--------------|----------------|
|                             | 0  | 12.5 | 25.0 | 37.5 | 50.0 | Medium | 0  | 12.5 | 25.0 | 37.5 | 50.0 | Medium | SEM | Sex × lycopene | Sex | Lin² | Qua² |
| Barrows                     | 63.96 | 72.63 | 78.40 | 73.92 | 75.31 | 72.84 | 68.87 | 71.17 | 72.19 | 73.42 | 72.60 | 71.65 | 1.719 | .747 | .045 | .191 |
| Gilts                       | 23.62 | 13.89 | 10.25 | 14.18 | 17.26 | 15.84 | 23.89 | 16.71 | 16.04 | 15.74 | 17.24 | 17.92 | 1.868 | .946 | .233 | .024 |

* Different letters in the same row differ by F test at 5% probability. SEM: standard error of the mean.

** Linear effect of lycopene.

** Quadratic effect of lycopene.

** Shows a lower concentration than barrows.

** Shows a better fit for the quadratic.

** Anti-BSA IgG production

There was an interaction (p = 0.011) between collection periods (day) and lycopene levels (mg/kg of diet) for the quadratic model (Table 4). The result of this interaction showed a better fit for the quadratic model than for the linear model (p = 0.09) found for the LRP model, showing the best fit among all tested models.

The immune system has a large number of distinct plasma proteins that react with each other to opsonise pathogens and induce inflammatory processes, which help fighting infection. In conditions of inflammatory processes there may be a reduction of plasma proteins and a change in the AB:GL ratio. The eosinophils presented a quadratic response on TP and GL, but AB increased according to lycopene levels of 0, 12.5, 25.0, 37.5 and 50.0 mg/kg of diet. The highest eosinophil levels of 0, 12.5, 25.0, 37.5 and 50.0 mg/kg of diet were observed in this study. In addition, there was no effect on TP and GL, but AB increased according to lycopene levels of 0, 12.5, 25.0, 37.5 and 50.0 mg/kg of diet. The highest coefficient of determination (R² = 0.95) was obtained for the linear model, showing the best fit among all tested models.

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Table 4. Dietary Lycopene for barrows and gilts, from 75 to 100 kg, on IgG production in different collection periods.

| Periods (Days) | Barrows Lycopene (mg/kg of diet) | Gilts Lycopene (mg/kg of diet) | SEM | p value | Linear | Quadratic |
|----------------|----------------------------------|--------------------------------|-----|---------|--------|-----------|
| 0              | Medium 0.012 12.5 25.0 37.5 50.0 | Medium 0.017 0.020 0.016 0.018 | 0.014 | 0.300 0.290 0.270 0.350 | 0.015 | 0.019 0.020 0.016 0.018 | 0.020 | 0.002 |
| 12             | 0.321 0.318 0.318 0.335 0.309 | 0.093 0.275 0.272 0.357 0.307 | 0.261 | 0.036 |
| 24             | 0.589 0.798 1.289 0.966 0.930 | 0.471 0.832 0.872 1.017 0.983 | 0.835 | 0.102 |
| Medium         | 0.308 0.348 0.563 0.420 0.426 | 0.194 0.379 0.388 0.463 0.436 |

Day × Sex × Lycopene .557
Day × Lycopene .110
Day × Sex .218
Sex × Lycopene .625
Lycopene** .000 .001 .010
Day** .001 .001 .001
Sex .257

*Equations fitted for the collection periods: day 0 – NS; day 12 – NS; day 24 – Y = 0.00823x + 0.668914 (R² = 0.57) and Y = −0.0005x² + 0.03098x + 0.526780 (R² = 0.95).

**Equations fitted for lycopene (mg/kg of diet): 0 mg – Y = 0.0215x − 0.0071 (R² = 0.98); 12.5 mg – Y = 0.0330x − 0.0333 (R² = 0.94); 25 mg – Y = 0.0442x − 0.0553 (R² = 0.94); 37.5 mg – Y = 0.0406x − 0.0462 (R² = 0.95); 50 mg – Y = 0.0391x − 0.0391 (R² = 0.96).

Figure 2. (A) Neutrophil concentration/total 100 cells and (B) neutrophil:lymphocytes ratio in the blood of pigs (barrows and gilts) receiving diets containing different lycopene levels.

Figure 3. Absorbance of anti-BSA IgG by using ELISA, at 24 days after starting the dietary lycopene supplementation for pigs (barrows and gilts), from 75 to 100 kg.

Figure 4. Absorbance of anti-BSA IgG by using ELISA, along the 24 days after starting the dietary lycopene supplementation for pigs (barrows and gilts), from 75 to 100 g.
production, or yet, a selective AB loss from blood stream and a high AB:GL ratio suggests a deficiency in immunoglobulin production (Miyada et al. 1997). The AB concentration involves the transport of several exogenous chemical compounds and endogenous metabolites and regulates osmotic pressure, whereas GL are an important part of the immune system (Yuksek et al. 2013). A decrease of AB levels may lead to the use of readily accessible AB storages or a reduced synthesis by hepatocytes and the reduction of GL levels may indicate a reduced immunity, since the liver has become unable to synthesise GL for the purpose of immunological activities (Narra 2016). As aforementioned, the increase in AB levels (Table 2) may indicate that lycopene reduced the oxidative stress in pigs and thereby minimised the degradation.

Plasma proteins are also easily affected by physiological, nutritional, sexual, environmental and genetic factors (Colditz 2002; Yuksek et al. 2013). In this study, gilts showed a higher TP and GL concentrations than barrows, as a lower AB and AB:GL concentrations. The higher TP levels observed for gilts may be due to a higher nutritional requirement for proteins compared to barrows (National Research Council 2012) which could directly affect its blood concentrations.

Leukocytes are the first defence line of the immune system, and when challenged by pathogens the leukocytes directly act on it, firstly by the subgroup of polymorphonuclear leukocytes, including neutrophils, eosinophils and basophils, which supress the invader by phagocytosis. In this study, the lycopene supplementation distinctively affected the leukocyte proliferation. Neutrophils are often associated with subclinical and clinical infections (Mortara et al. 2015) caused by bacteria and the lowest neutrophils concentration was obtained by supplementing 17.49 mg lycopene/kg of diet (Figure 2(A)), according to the association of the quadratic and LRP models. Likewise, eosinophils were affected by lycopene levels and the highest concentration was estimated by the quadratic model when supplementing 22.69 mg of lycopene/kg of diet.

Neutrophils, together with macrophages, are sources of active oxygen species (AOS) and plays an important role in cellular immunity. Neutrophils uses myeloperoxidase to transform the hydrogen peroxide, resulting from the conversion of superoxide anions by the superoxide dismutase, into the hypochlorite ion, a highly active bactericide, whereas macrophages generate a hydroxyl radical (Pechinskii and Kuregyan 2014). The AOS balance is maintained using endogenous and exogenous antioxidants, including carotenoids (Pechinskii and Kuregyan 2014), with the lycopene being the predominant carotenoid in the blood plasma.

The immune system is extremely sensitive to oxidative damage, due to the high concentration of polyunsaturated fatty acids in its plasmatic membrane and due to the production of reactive oxygen species, so it may impair the ability of the immune system to produce an immune response (Meydani et al. 1995). Therefore, an adequate amount of antioxidants are important, as well as positively interfering with the immune response, promoting the neutralisation of free radicals, which have been associated with aging, damage and cell death in animals and humans (Darroch 2001).

The second subgroup are mononuclear leukocytes, which include monocytes (macrophages) and lymphocytes (B cells, T cells and natural killer cells). Monocytes ingest dead or damaged cells (through phagocytosis) and provides immune defences against many infectious organisms, already the lymphocytes identifies foreign and invading substances in the body and produces antibodies and cells that specifically target them. In the present study the lymphocyte cells increased at all levels of lycopene supplementation in pigs diets. These results are in agreement with that observed by Watzl et al. (2003) in which the daily intake of tomato juice (37.0 mg/day lycopene) by healthy men increased the lymphocyte proliferation and the cytotoxicity of natural killer cells. Furthermore, Hasegawa et al. (2010) reported that lycopene may modulate the potential of cytokine production in T cells or indirectly activate T cells.

The neutrophil/lymphocyte ratio are inflammatory biomarkers used as prognostic factors in many inflammatory diseases and infections (Forget et al. 2017). As neutrophil and lymphocyte were affected by dietary lycopene (Table 3), its ratio was also affected, resulting in a better response by adding 16.46 mg lycopene/kg of diet, providing the lowest neutrophil:lymphocyte ratio (Figure 2(B)), also obtained by the association of the quadratic and LRP models.

Despite the obtained results about the effects of lycopene on leukocyte cells, mainly on lymphocytes, little is known about lycopene affecting the activation, proliferation and differentiation of immunoglobulin secreting plasmocytes (antibodies). It is known that lycopene enriched diets and other carotenoids, increases B lymphocytes and serum IgG, after 7 days treatment, suggesting that carotenoid-based dietary supplementation may increase B lymphocyte production and the concentration of circulating immunoglobulins (Garcia et al. 2003).
The specific antibody response to ovalbumin is a characteristic of humoral immunity (Zhao et al. 2016), because immunoglobulins are synthesised and excreted by plasma cells derived from B lymphocytes. IgM is the first antibody formed when white blood cells are initially exposed to an antigen, and when exposed to an antigen for a second time, the pig builds very high levels of antibodies, mostly IgG class, and the white blood cells switch from synthesising IgM to IgG, after a continued antigen exposure (Chu and Song 2013).

Serum IgG is considered the main constituent of the blood immunoglobulin and is the most important antibody of the secondary immune response, besides having high affinity for antigen-specific binding (Chu and Song 2013), is the target of this study. The production of Anti-BSA IgG was evaluated and it was observed that Anti-BSA IgG increased throughout the evaluation days at all levels (0, 12.5, 25.0, 37.5 and 50.0 mg) of lycopene supplementation (Figure 4). This response shows that lycopene affects immunity building, as the affect was observed over time.

However, the maximum stimulus provided by lycopene was not achieved, probably due to a short evaluation period (24th day), suggesting a longer period for the maximum lycopene effect on Anti-BSA IgG production. This stimulation of lycopene on IgG production has been observed in other studies, like that carried out by Luo and Wu (2011), showing that lycopene can increase blood levels of IgA, IgG and IgM, improving the immunity of rats with cancer. Similarly, Neyestani et al. (2007) observed that dietary supplementation of lycopene stimulates the formation of specific antibodies, mainly IgG isotype, reporting that lycopene is not only a common immune enhancer, because it provides an specific immune stimulation.

Lycopene supplementation showed a beneficial effect on the long-term production of anti-BSA IgG, increasing until the 24th day of the experimental period, and the stimulation of humoral immunity was observed supplementing of up to 20.06 mg lycopene/kg of diet (Figure 3). As it becomes an immune mediator not always the highest concentration of nutraceutical supplementation promotes the best immune response (Moraes et al. 2012). Evaluating three dietary levels of vitamin E (30, 65 and 100 mg/kg) for broilers, Silva et al. (2009) observed that the intermediate concentration promoted the highest production of antibodies against Newcastle disease. Likewise, this study also showed that an intermediate lycopene level (20.06 mg of lycopene/kg of diet) provided the best response to the Anti-BSA IgG production. In addition, lycopene affected both cellular immunity, by stimulating leukocyte production, and humoral immunity, by the specific immune response due to the increased IgG production.

Conclusions
Dietary lycopene supplementation for finishing pigs affected the cellular and humoral immune response, and the highest Anti-BSA IgG production was achieved by supplementing 20.06 mg lycopene/kg of diet.

Disclosure statement
No potential conflict of interest was reported by the authors.

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