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# PLANT FEED ADDITIVES AS NATURAL ALTERNATIVES TO SYNTHETIC VITAMIN E IN ORGANIC BROILER STOCK FARMING

*ADDITTIVI DI ORIGINE VEGETALE COME ALTERNATIVA NATURALE ALLA VITAMINA E SINTETICA NELL'ALLEVAMENTO DEL BROILER DA CARNE*

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**ABSTRACT**

The aim of this trial was to evaluate the potential of two plant feed additives commercial products as substitutes to synthetic vitamin E in organic broiler diet.

Two hundred fifty-two 1 day-old Ross 308 male broilers were randomly assigned to 3 dietary groups each divided into 3 replicates of 28 birds, including different antioxidant additives: 1) control group (C), fed a commercial organic feedstuff containing synthetic vitamin E (alfa-tocopherol acetate) as main antioxidant additive; 2) T1 group, receiving the same feedstuff where vitamin E was replaced by a combination of grape seed extract (Resinox 80Q®) and green tea extract; and 3) T2 group, receiving the commercial organic feedstuff including a mix of hydrolysed polyphenols (Oxistim®) as main antioxidant additive. A starter feedstuff was administered until 21 days of age while a grower feedstuff was fed until the end of the trial (42 days of age). The amounts of plant antioxidant additives in T1 and T2 group diets were adjusted to obtain the same dietary antioxidant capacity induced by the vitamin E in the control diet. The antioxidant capacities were equalized among the groups based on an *in vitro* test (ABTS test) performed on the herbal products. Environmental temperature and humidity were recorded daily. During the first growing phase the temperature was on average 40°C and then decreased to an average of 25°C until the end of the trial. During the whole experiment, 16 h of light were provided daily.

Feed and water consumption (FC and WC respectively) were measured daily on a pen basis. Feed conversion rate and feed efficiency were calculated using the available data on feed consumption and body weight.

Health status and body weight (BW) were evaluated weekly. Faeces and litter samples were taken at the same intervals.

At 42 days of age, 108 broilers were sent to the slaughterhouse. During the slaughtering, after jugulation, individual plasma samples were collected for the metabolic profile determination, while an additional blood sample was obtained from a subsample of 3 birds per pen (9 animals per dietary treatment) for the evaluation of the haematological profile. The whole coelomic content, namely the gizzard, heart, liver, pancreas, spleen, bursa of Fabricious, kidneys, small intestine (duodenum, jejunum, and ileum), caecum, colon and rectum were weighted and sampled for histological evaluation. After removal of head, neck and feet, eviscerated carcasses were weighted and then dissected to separate thighs, wings, and breast. Each part was evaluated for pH, meat cooking loss and colour (L*, a*, b*) as meat quality parameters.

The data were analysed using IBM SPSS v6. The statistical significance was set at $P<0.05$.

Weekly individual cumulative feed consumption (g), weekly group feed consumption (g), weekly group cumulative feed consumption (g) and individual cumulative weekly water consumption (ml) were not affected by the treatments. No differences were found for the BW, with exception for the BW at 21 days of age in which the T1 showed a lower average BW compared to T2 (844.1 vs 889.4 g respectively; $P=0.022$) even if they were not different in comparison with the C group. Individual weekly BW, compared with those reported in the genotype standard (Ross 308 management handbook), maintained a continuous lower trend at 7 days of age (177 g of average weight vs 208 g expected), 14 days of age (454 g of average weight vs 519 g expected), 21 days of age (869 g of average weight vs 985 g expected), 28 days of age (1385 g average weight vs 1573 g expected), 35 days of age (1989.93 g average weight vs 2235 g expected) and at 42 days of age (2472.3 g average weight vs 2918 g expected).

Weekly feed:gain ratio and feed efficiency were not affected by the treatments. However, cumulative feed:gain ratio resulted lower in T2 at 42 days of age compared to C and T1 (0.78 vs 0.81 and 0.79 respectively; P=0.036). Furthermore, cumulative feed efficiency showed a statistically relevant difference at the same interval with T2 showing the higher value (1.28 vs 1.26 and 1.24 of C and T1 respectively; P=0.036). The treatments did not affect mortality cases (C=6, T1=8, T2=11; P=0.459). Concerning the metabolic profiles, the treatments with both natural antioxidants, did not affect most of the considered parameters. Exceptions were found for plasmatic vitamin E, which was higher in the C group, as expected, compared to both the natural antioxidants (7.29 vs 5.01 and 5.17 mg/l respectively; P≤0.001) and the creatinine, which was reduced by the T1 treatment (63.0 vs 91.98 and 88.62 µmol/l of C and T2 respectively; P≤0.001). Finally, the ALT value was lower in the T2 in comparison to the C group, the latter being equal to T1 (2.92 vs 3.78 and 3.17 IU/l of C and T1; P 0.012). The natural antioxidants did not affect neither the organs development nor the haematological profile and carcass yield/quality.

Overall, these data suggest that natural plant feed additives can replace synthetic vitamin E in organic broiler farming, since any major variation in performances and health status was observed in the present trial. However, specific *in vivo* and *post-mortem* tests on the antioxidant status of the tissues should be addressed, and the use these natural antioxidants must be preceded by an accurate evaluation of their antioxidant capacity/activity, especially in the case of not standardised natural extracts.

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## Introduction

The broiler farming system is one of the most reliable and productive meat realities nowadays due to the short productive cycle and to the ongoing emerging market.

The fast-growing industry has led to the development of efficient intensive livestock farming models that had brought to a quanti-qualitative standardization of the animals. This has been attained through the exploitation of high-yielding genotypes and the use of nutritionally balanced diets (Haque et al., 2020).

Historically, the broiler system, has been subjected to the use of large scale antibiotics prophylaxis in order to prevent and antagonize diseases and to promote growth: nowadays is a trend that is counteracted both in order to reduce the physiological phenomenon of antibiotic resistance but also to satisfy the consumer perception that antibiotic-free broiler meat is superior to conventional broiler meat (Cervantes, 2015). In addition, consumers are concerned about antibiotic residue and antimicrobial resistance, as well as pesticide residues, additives, nutritional content, flavour, traceability and genetically modified organisms with regards to broiler meat production (Haque et al., 2020). Therefore, alternative approaches to replace antibiotics treatments and the growth-promoters use are needed to avoid antimicrobial resistance and the related public health issues (Haque et al., 2020). In fact, it is often thought by consumers that broiler’s meat contains antibiotics residues; this particular concept was analyses by Sattar et al. (2014) who reported to be found, in his 35 broiler trial during July-December 2012, antibiotic residues mostly in the liver, kidney, thigh meat, and breast meat of broilers.

In this framework the organic stock farming seems a good alternative to classic intensive broiler farming, since it is a means of animals farming with a complex system of contentious inputs allowed by EU disciplinary, directed toward higher conditions of animal welfare, care for the environment, restricted or none use of medical drugs and the preparation of healthy products without residues (Kijlstra and Eijck., 2006).

Organic stock farming presented a huge number of challenges faced by companies that attempt to produce antibiotics-free animals, and this includes production, management, health and animal welfare challenges (Cervantes., 2015).

Several plant feed additives (PFA) have been analysed with the goal to be used as additives into the diets: it has been proven that this wide group of biologically active compounds has a potential positive effect on animal health and productivity (Hashemi and Davoodi, 2011). Furthermore, PFA are reported to positively affect growth by improving the feed conversion ratio, boosting the immune system, and reducing stress. In fact, several PFA has demonstrated to have antioxidant and anti-inflammatory activities (Manuelian et al., 2021; Pitino et al., 2021; Righi et al., 2021; Tsiplakou et al., 2021). Moreover, several recent studies showed that PFA promoted broiler chicken growth and could be used as an alternative to antibiotics (Haque et al., 2020; Windisch et al., 2008a). Furthermore, in the organic farming synthetic vitamins are considered contentious inputs, allowed by the EU organic disciplinary; consequently, to fulfill the vitamin animal requirements, the supplementation with molecules able to acts similarly to the vitamins is of utter importance (Righi et al., 2021).

The PFA include several polyphenols, which operate mainly as antioxidants and may be used as alternatives to the synthetic vitamins: we can divide PFA in phenolic acids, stilbenoids and flavonoids.

To better explain the use of the PFA, the following part will expose the characteristic of a classic rearing system, an organic stock farming module and the use of these molecules so far.

## Chapter 1: Overview on broiler rearing system

The broiler strains genetic selection has brought to ongoing improvements in robustness and overall health while providing the birds an optimal quality care and welfare standards: this survey on the classic broiler rearing system will come from the Ross Broiler Management Handbook (Aviagen, 2014), Ross Nutrition Specification (Aviagen, 2019) and Ross 308 Performance objective (Aviagen, 2019).

The overall broiler performance has been implemented through more than a decade work with the aim of meet a balance range of characteristics: from a sanitary point of view bird welfare, leg health, cardiovascular fitness, and robustness meanwhile from a productive perspective growth rate, feed conversion ratio, liveability, and meat yield are to be seek.

This can be done thoroughly aiming to maintain the following parameters in the supposed ranges: stock density, ventilation, lighting, feed supply, chick quality, health, bird welfare, nutrition, temperature, water supply and vaccination status.

Rearing broilers is the midway phase in the poultry industry, placed in between hatchery and the processing plant: the aim of the bird manager, from a pragmatic point of view, will be to achieve the developing of a good feeding behaviour, to develop immune function, to allow optimum development of skeleton and cardiovascular system, to optimize carcass quality and weight and to maximize bird welfare.

### 1.1 Environmental conditions

When the chicks are placed in the growing site, they must be received in the proper conditions: optimum biosecurity level, air temperature of 30 C°, litter temperature of 28-30 C°, a relative humidity index around 60-70

% onto a 10 cm litter depth in order to isolate and maintain these environmental conditions, fresh free water and a lightning program suitable for their age (23 h of light with 20-30 lumen and 1 h of dark for 7 days). At 27 days the temperature will reach and maintain a minimal level of 20 C°.

### 1.1.1 Ventilation

From the 27$^{th}$ day and along the whole duration of the cycle, ventilation is the main mean of controlling the bird environment. Ventilation provides adequate fresh air, remove the moisture in excess and limits the stocking of potentially harmful gasses: particular attention must be paid to dust, ammonia, carbon dioxide, carbon monoxide and excess water vapour. Humidity needs to be related with temperature, and with the increase of temperature humidity needs to be reduced: with a temperature > 29 C° and Hr > 70 %, health can be affected negatively. Therefore, controlled environment housing with strong ventilation is a common way to minimize the chances that air contaminants will reach toxic levels (Saleh et al., 2005). Additionally, is useful to apply a negative pressure to the structure, in order to create a partial vacuum inside the house of – 20 Pa in relation to the outside pressure, increasing the biosecurity, since the air will only flow from the inside of the housing to the outside, prevent potential pathogens. The methodologies to reach the optimal ventilation are various and will change depending on the width of the house, the angle of the internal ceiling, the shape of the internal ceiling, the type of inlet and the amount of inlet opened: transitional ventilation or tunnel ventilation are the main possibilities. Evaporative cooling systems are to be an additional tool to enhance tunnel ventilation and the maintain of a proper temperature: pad cooling is the main way to achieve that.

**Table 1**: Effects of common broiler house air contaminants as reported by Ross Broiler Management Handbook (Aviagen, 2014).

| | |
|---|---|
| Ammonia | Optimal < 10 ppm |
| | Damage to lung surface > 10 ppm |
| | Increase susceptibility to respiratory diseases > 20 ppm |
| | Reduce growth rate > 25 ppm |
| Carbon Dioxide | Optimal < 3,000 ppm |
| | Cause ascites > 3,500 ppm |
| | Fatal at high levels |
| Carbon Monoxide | Optimal < 10 ppm |
| | Affects bird health > 50 ppm |
| | Fatal at high levels |
| Humidity | Housing: 60-70% |
| | After brooding: 50-60% |

### 1.1.2 Lighting programs and intensity

Lighting will need to comprehend four important parameters: the number of hours of light and dark given in a 24 hour period, how these are distributed during the day, the colour and intensity of the light. To decide whether apply one lighting program or another, some main guidelines need to be followed precisely: at 7 days of age darkness need to be considered optimum between 4-6 hours, all changes in light intensity, duration and colour needs to be gradual, different protocols can variate their functionality related to environmental factors, therefore are not to be considered standardized and at 7 days of age a minimum of 30-40 lux will need to be used.

### 1.1.3 Litter choice and management

Litter management is another central aspect to be considered, but independently from the type of bedding material, a good litter should provide good moisture absorption, biodegradability, bird comfort, low dust level, low number of contaminants, consistent availability from a biosecurity source. The floor needs to be cleaned after the litter material removal, and therefore earth floors or wood floors are not recommended. Material can be selected from a huge variety of alternatives, with singular prerogatives of usage: pine shaving and sawdust, hardwood shaving and sawdust, pine or hardwood chips, pine or hardwood bark, rice hulls, peanut hulls, coconut husks, sand, crushed corn cobs, chopped straw or hay, straw pellets, processed paper, chemically treated straw pellet, peat moss, flax straw, recycled litter of every upon cited type. Poor quality litter can be secondary to poor environmental management, high salt or protein diet, wrong drinker management, hight stock density, poor water quality, poor fat quality of the diet, enteritis or poor quality of litter material and depth (Bolan et al., 2010). A poor litter quality will increase the food pad dermatitis cases and the susceptibility of broilers to potential bacterial pathogens that will inhabit the litter itself (Shepherd and Fairchild, 2010).

### 1.1.4 Stocking density

Stocking density is ultimately a decision based on economic and local welfare legislations, and influence bird welfare, performance, uniformity and product quality. Strict regulation imposed by the EU Broiler Welfare

Directive (2007) direct maximum stocking densities related with the welfare standard of the pen, using as parameter the sum of the broiler's weights of the same pen: 33 kg/$m^2$ as general limitation, 39 kg/$m^2$ if stricter standards are met or 42 kg/$m^2$ if exceptionally hight welfare standards are met over a prolonged period of time. The different stocking choices will be decided taking into consideration the target live weight and age at processing, climate and season, type and system of housing and equipment (particularly ventilation and evaporating cooling systems), local legislations and quality assurance certification requirements.

## 1.2 Feeding and nutritional strategies

The feeding program is usually divided in 3 separate steps which aim to apport the proper nutritional value to the different stages of the broiler growth. A started feedstuff, in the form of crumble pellets, is to be given from the first day of life up to approximately 10 days of age, but a longer period of 14 days can be covered if the appropriate weight is not achieved: the goal of this period is not to enhance performance but to promote the physiological development of the chick. During the second step, a grower feed should be administered to the broilers, also changing the texture into a proper pellet size, for another 14-16 days: this period will aim to provide the adequate aminoacidic quantity and quality with the purpose of supporting the rapid increase of the animal feed consumption. During the last period of life, the animals are fed with a finisher feedstuff, from the 25$^{th}$ up to desired age related to the granted weight. A secondary finisher can be added in some cases to achieve the aspire weight: this will change based on the genetic strain of the animal, which is considered to be for

Ross 308 and average weight of 323g at 10 days, 1366g at 25 days, 3136 ad 42 days, 4962g at 60 days.

**Table 2**: guideline values for broiler feed contaminants as reported by Ross Broiler Management Handbook (Aviagen, 2014).

| | Energy (MJ/kg) | Crude Protein (%) | Total Lysine (%) | Total Methionine and Cysteine (%) |
|---|---|---|---|---|
| Starter | 12.65 | 22-25 | 1.43 | 1.07 |
| Grower | 13.20 | 21-23 | 1.24 | 0.95 |
| Finisher | 13.40 | 19-23 | 1.09 | 0.86 |

### 1.2.1 Protein and essentials amino acids

Since broilers are fed a plant protein-based diets, it is of major importance to formulate the diet to achieve minimum digestible amino acids (AA) constrains to maximise AA utilization, reduce protein intake and promote good enteric health and litter quality. High ratios of digestible Arginine and Lysine promote good biological performances.

**Table 3**: Aminoacidic nutritional specifications for As-Hatched Broiler (target weight 3.5kg) contaminants as reported by Ross Broiler Management Handbook (Aviagen, 2014).

| | Starter Total | Starter Digest | Grower 1 Total | Grower 1 Digest | Grower 2 Total | Grower 2 Digest |
|---|---|---|---|---|---|---|
| Lysine (%) | 1.40 | 1.25 | 1.26 | 1.12 | 1.17 | 1.04 |
| Methionine + Cysteine (%) | 1.05 | 0.93 | 0.97 | 0.85 | 0.91 | 0.80 |
| Methionine (%) | 0.54 | 0.50 | 0.50 | 0.46 | 0.47 | 0.44 |
| Threonine (%) | 0.95 | 0.84 | 0.85 | 0.75 | 0.79 | 0.70 |
| Valine (%) | 1.08 | 0.94 | 0.98 | 0.85 | 0.92 | 0.80 |
| Isoleucine (%) | 0.95 | 0.84 | 0.87 | 0.76 | 0.82 | 0.72 |
| Arginine (%) | 1.43 | 1.29 | 1.29 | 1.16 | 1.21 | 1.09 |
| Tryptophan (%) | 0.22 | 0.20 | 0.20 | 0.18 | 0.19 | 0.17 |
| Leucine (%) | 1.54 | 1.38 | 1.38 | 1.23 | 1.29 | 1.14 |

| | Finisher 1 Total | Finisher 1 Digest | Finisher 2 Total | Finisher 2 Digest |
|---|---|---|---|---|
| Lysine (%) | 1.10 | 0.98 | 1.06 | 0.94 |
| Methionine + Cysteine (%) | 0.87 | 0.76 | 0.83 | 0.73 |
| Methionine (%) | 0.45 | 0.41 | 0.43 | 0.39 |
| Threonine (%) | 0.75 | 0.66 | 0.72 | 0.63 |
| Valine (%) | 0.88 | 0.76 | 0.84 | 0.73 |
| Isoleucine (%) | 0.77 | 0.68 | 0.74 | 0.65 |
| Arginine (%) | 1.14 | 1.03 | 1.10 | 0.99 |
| Tryptophan (%) | 0.18 | 0.16 | 0.17 | 0.15 |
| Leucine (%) | 1.21 | 1.08 | 1.16 | 1.03 |

### 1.2.2 Minerals

A key role is also played by macro- and micromineral elements, such as calcium, phosphorus, magnesium, sodium, potassium, and chloride. An appropriate amount and form of calcium influences positively growth performances, feed efficiency, bone development, leg health, nerve function, and the immune system development and maintenance. Despite that, studies have shown that calcium supplied in excess of requirement may affect negatively the process of digestion owing to formation of insoluble salts with dietary fatty acids in the intestinal lumen, which may lead to a reduction of nutrient availability, decreasing the dietary energy utilisation and reduction in growth and feed efficiency (Ceylan et al., 2020). On the other hand, phosphorus is necessary in the correct form and quantity to fortify the skeletal structure and optimize growth: a ratio of 2:1 Ca:P is appropriate for almost all broiler diets. However, in starter diets, a slightly higher ratio (2.1:1) is beneficial to performance and especially helpful in promoting excellent leg strength. It has to be specified that, in contrast with Aviagen guidelines, a recent study has defined that reducing dietary Ca and P level during starter (0–10 days) and grower-finisher (11–41 days) periods maintained the broiler performance at any of studied levels and even gave better results (Ceylan et al., 2020). The latter author studied 112000 Ross 308, conclude that reducing the addition of almost 20% Ca and P intake of broilers, especially after starter period, will not impair growth and bone development. This can be achieved by keeping the dietary levels as 0.90:0,.45, 0.75;0.38 and 0.60;0.30% Ca and P for starter, grower and finisher phase respectively.

Magnesium requirements are normally met without supplementations: in fact, an excessive magnesium supplementation (> 0.5%) can cause scouring. Sodium, potassium, and chloride are needed to maintain the metabolic homeostasis: high levels of these minerals result in increased water intake and subsequent poorer litter quality, but on the contrary low supplementation can affect negatively feed intake, growth performances, and blood pH.

**Table 4**: Macromineral nutritional specifications for As-Hatched Broiler (target weight 3.5kg) as reported by Ross Broiler Management Handbook (Aviagen, 2014).

| | Starter | Grower 1 | Grower 2 | Finisher 1 | Finisher 2 |
|---|---|---|---|---|---|
| Calcium (%) | 0.96 | 0.84 | 0.78 | 0.72 | 0.68 |
| Phosphorus (%) | 0.48 | 0.42 | 0.39 | 0.36 | 0.34 |
| Magnesium (%) | 0.05 – 0.30 | 0.05 – 0.30 | 0.05 – 0.30 | 0.05 – 0.30 | 0.05 – 0.30 |
| Sodium (%) | 0.16 – 0.23 | 0.16 – 0.23 | 0.16 – 0.23 | 0.16 – 0.23 | 0.16 – 0.23 |
| Potassium (%) | 0.4 - 1 | 0.4 – 0.95 | 0.4 – 0.90 | 0.4 – 0.85 | 0.4 – 0.80 |

### 1.2.3 Vitamins

The vitamins supplementation can be more complex since it needed to be strictly related with the main components of the diets: depending on the main raw materials different quantities of vitamins can be added. This basal quantity can be implemented due to adverse farming conditions that can cause a distress in the animals: in Table 5 are shown the requirements specified by Aviagen for Ross 308. Vitamin deficiencies or excesses can be expressed with classical deficiency signs and nonspecific parameters as lower production and reproduction rates. Therefore, vitamin nutrition should no longer be considered important only for preventing deficiency signs but also for optimising animal health, productivity and product quality (McDowell and Ward, 2009).

**Table 5**: Vitamin nutritional specifications, per kilos, for As-Hatched Broiler (target weight 3.5kg) as reported by Ross Broiler Management Handbook (Aviagen, 2014).

| | Starter Wheat based feed | Starter Maize based feed | Grower 1 Wheat based feed | Grower 1 Maize based feed | Grower 2 Wheat based feed | Grower 2 Maize Based feed |
|---|---|---|---|---|---|---|
| Vitamin A (IU) | 13.000 | 12.000 | 11.000 | 10.000 | 10.000 | 9.000 |
| Vitamin D3 (IU) | 5.000 | 5.000 | 4.500 | 4.500 | 4.000 | 4.000 |
| Vitamin E (IU) | 80 | 80 | 65 | 65 | 55 | 55 |
| Vitamin K (mg) | 3.2 | 3.2 | 3 | 3 | 2.2 | 2.2 |
| Thiamin B1 (mg) | 3.2 | 3.2 | 2.5 | 2.5 | 2.2 | 2.2 |
| Riboflavin B2 (mg) | 8.6 | 8.6 | 6.5 | 6.5 | 5.4 | 5.4 |
| Niacin (mg) | 60 | 65 | 55 | 60 | 40 | 45 |
| Pantothenic Acid (mg) | 17 | 20 | 15 | 18 | 13 | 15 |
| Pyridoxine B6 (mg) | 5.4 | 4.3 | 4.3 | 3.2 | 3.2 | 2.2 |
| Biotin (mg) | 0.30 | 0.22 | 0.25 | 0.18 | 0.20 | 0.15 |
| Folic Acid (mg) | 2.20 | 2.20 | 1.90 | 1.90 | 1.60 | 1.60 |
| Vitamin B12 | 0.017 | 0.017 | 0.017 | 0.017 | 0.011 | 0.011 |

| | Finisher 1 Wheat based feed | Finisher 1 Maize based feed | Finisher 2 Wheat based feed | Finisher 2 Maize based feed |
|---|---|---|---|---|
| Vitamin A (IU) | 10.000 | 9.000 | 10.000 | 9.000 |
| Vitamin D3 (IU) | 4.000 | 4.000 | 4.000 | 4.000 |
| Vitamin E (IU) | 55 | 55 | 55 | 55 |
| Vitamin K (mg) | 2.2 | 2.2 | 2.2 | 2.2 |
| Thiamin B1 (mg) | 2.2 | 2.2 | 2.2 | 2.2 |
| Riboflavin B2 (mg) | 5.4 | 5.4 | 5.4 | 5.4 |
| Niacin (mg) | 40 | 45 | 40 | 45 |
| Pantothenic Acid (mg) | 13 | 15 | 13 | 15 |
| Pyridoxine B6 (mg) | 3.2 | 2.2 | 3.2 | 2.2 |
| Biotin (mg) | 0.20 | 0.15 | 0.20 | 0.15 |
| Folic Acid (mg) | 1.60 | 1.60 | 1.60 | 1.60 |
| Vitamin B12 (mg) | 0.011 | 0.011 | 0.011 | 0.011 |

Vitamin requirements established decades ago have changed little and do not reflect greatly the changes in management procedures of modern poultry operations. Vitamin supplementation allowances need to be set at levels that reflect specific management systems that are high enough to take care of fluctuations in environmental temperatures, energy content of feed and influencing factors (for example, infectious diseases, stress, parasites, biological variations, diet composition, bioavailability and nutrient interrelationships) that might influence feed composition or vitamin requirements (McDowell and Ward, 2009).

**Table 6.** OVN levels as described by McDowell and Ward, (2009) in International Poultry Production - Volume 16 Number 4.

| | OVN |
|---|---|
| Vitamin A (IU/kg) | 12.500 |
| Vitamin D3 (IU/kg) | 4.000 |
| Vitamin E (mg/kg) | 225.000 |
| Vitamin K (mg/kg) | 4.00 |
| Thiamin B1 (mg/kg) | 3.00 |
| Riboflavin B2 (mg/kg) | 9.00 |
| Niacin (mg/kg)) | 60.00 |
| Pantothenic Acid (mg/kg) | 15.00 |
| Pyridoxine B6 (mg/kg) | 6.00 |
| Biotin (mg/kg) | 0.25 |
| Folic Acid (mg/kg) | 2.00 |
| Vitamin B12 (mg/kg) | 40.00 |

A new concept of poultry nutrition is nowadays considered as coherent with the need for optimum intravitam nutrition (OVN). Performance benefits from OVN diets for meat production include increased growth, feed efficiency, oxidative stability of meat, resistance to high density stress and prevention of bone problems. Of particular interest for this introduction is the concept of supra-nutrition supplementation of vitamin E with 400 mg/kg, which is reported to be highly effective decreasing the lipid peroxidation in meat products (McDowell and Ward, 2009).

## Chapter 2: Organic stock farming regulations for broilers

Organic animal farming has experienced an exponential development that also resulted onto changes in the production process (Åkerfeldt et al., 2021).

The organic production is based on a precise set of European regulations (IFOAM, 2005) which sees its pillars into the principles of health, ecology, fairness and care: consumers expect high animal health and welfare, but there are doubts whether the methodology achieves it better than conventional animal farming systems do (Sutherland et al., 2013).

The following points are the "pillars" of the organic broiler farming system, following the baselines thoroughly described in (CE) 834/2007 and 889/2008:

- The use of natural light can be integrated with artificial to a maximum of 16 h/day, with a continuous rest time of 8h with no light.
- Broilers cycle should be a minimum of 81 days of life.
- Cages are prohibited and the animals must have access to an open paddock for at least 1/3 of their life.
- Multilayers systems cannot have more than 3 layers comprehending the ground floor.
- Perches need to be suitable in numbers and shapes for the animals farmed.
- Maximum density should be 21 kg/$m^2$.
- Feeds must be produced by a certified organic line of a feed industry.
- Maximum 3 veterinary allopathic drug treatments may be used only if required by the critical situation, whether other

homeopathic or phytotherapic do not solve the pathology: in case of exceeding the three administration the treated animals are to be considered out of the biological production system.

- Artificial vitamins and amino acids are to be considered contentious inputs: therefore, the use of PFA is strongly encouraged.

## Chapter 3: PFA applied to broiler farming

The present chapter will divide the action of plants extracts based on the parameters evaluated, as described by Righi et al., (2021) and Pitino et al., (2021).

### 3.1 Feed Intake and Average Daily Feed Intake

The feed supplementation with grape pomace (GP; 0.5% to 6% of the diet) in comparison with vitamin e (VitE) (200 mg/kg of the diet) did not affect broilers FI (Brenes et al., 2008; Goni et al., 2007).

Likewise, the dietary inclusion of grape skin (6% of the diet) up to 21 days left unaffected broilers average daily feed intake (ADFI) (Nardoia et al., 2020).

Comparing VitE (200 mg/kg of the diet) with antioxidant compositions (2.5% of rosehip, chokeberry pomace or nettle separately (Loetscher et al., 2013) or rosemary extract (RO; at 0.010, 0.015 and 0.020% of the diet) for 42 days (Yeşilbag et al., 2011) along with VitE with rosemary leaves (RL; at 2.5% of the diet) for 28 days (Loetscher et al., 2013) brought to similar results.

Similar results have been reviewed by Righi et al. (2021) also with milkweed extract (or coneflower CE, 0.056% of the diet), thyme extract (TE, 0.056% of the diet), sage extract (SE, 0.056% of the diet), marigold xanthophyll (MX, 0.02% of the diet), synthetic antioxidants (0.00486% of the diet) or β-apo-8-carotenoic acid ethyl ester (0.004% of the diet) were singularly commensurate with VitE (150 mg/kg of the diet) during a 20 days trial (Koreleski and Šwiatkiewicz, 2007).

The dietary addition of oregano oil (OO) at 0.01% and 0.02% of the diet (Papageorgiou et al., 2003) and 0.01% (Avila-Ramos et al., 2012)] compared with VitE (included at 200 mg/kg of the diet and 10 or 100 mg/kg, respectively) for 42 days did not affect broilers' FI.

Furthermore, Righi et al. (2021) reported no significant variations in FI were noted when broilers were summed with OO, or RO, or fennel volatile oil (FVO; each at 0.01% of the diet - Cetin et al., 2016).

Moreover, olive leaf essence dietary addition (OLE; at 0.2% and 0.4% of the diet) did not affect FI in broilers reared under heat stress during a 14 days trial compared with VitE treatments (Agah et al., 2019).

On the contrary, broilers fed thyme oil (TO; at 0.01 and 0.02% of the diet), showed a higher FI (by 3.7% on average) commensurate with those receiving only a controlled diet (VitE 40 mg/kg).

A similar result (FI +2.0% on average) was observed in comparison with the VitE (200 mg/kg) control group (Bölükbaşi et al., 2006).

Nevertheless, in broilers, the dietary inclusion of rosemary powder (RPO; at 1%) compared with VitE (200 mg/kg) diminished the FI - 4.3% in a 42 days feeding test (Rostami et al., 2015).

In broilers, average daily feed intake (ADFI) remain invariate when GP (from 0.5 to 10% of the diet) was used for 21 (Brenes et al., 2008; Goni et al., 2007) or 42 days (Ebrahimzadeh et al., 2018) compared with VitE (at 200 mg/kg of the diet) group.

Variations were found neither a commercial preparation of essential oils (0.05 and 0.1%) neither VitE (200 mg/kg of the diet) compared with the negative control treatment (only vitamin premix), during a 42 days trial (Botsoglou et al., 2004).

Feeding broilers under HS conditions (34°C) with polyphenols (PP), alone (0.02%) or in association with VitE (PPE, 0.01% PP and 100 mg/kg of VitE) in alternative to two dosages of VitE (100 and 200 mg/kg) did not affect FI (Mazur-Kuśnirek et al., 2019).

Nevertheless, after 28 days of trial, the same variable was higher by the dietary addition of PP (of about 19%) compared with VitE (at 10.60 mg/kg). In other two analogous studies, PP supplementation did not modify FI in broilers disputed with Ochratoxin A (Mazur-Kuśnirek et al., 2019). Generally speaking, all the reported papers on broilers seems to define that the dietary supplementation with different PFA do not pursue a negative impact on FI or ADFI when compared with synthetic vitamins or control treatments (Righi et al., 2021).

### 3.2 Body weight and body weight gain

Following in the work of Righi et al. (2021), an increase (by 10%) in body weight (BW) onto heath stressed (HS, 34°C) broilers, was noticed when their diet was added with grape seed extract (GSE, at 0.03%) compared with VitC (0 or 300 mg/kg of diet) for 42 days (Hajati et al., 2015).

This improvement was progressively in the first 28 days of trial, when HS was not present. However, GSE supplementation (at 2.6 or 5.2% of the diet) alone or in combination with methionine (at 0.15% of the diet), during the 3 weeks trial, let the author (Righi et al., 2021) suggest a dose-dependent deleterious effect on average terminal weight (ATW) and weight gain (WG) compared with the control and VitE (500 IU/g of the diet, equivalent to 335 mg/kg of alpha-tocopherol) diets (Lau and King, 2003).

The negative effect on WG has been confirmed by other authors on the use of grape secondary products at similar doses (Brenes et al., 2008; Ebrahimzadeh et al., 2018; Goni et al., 2007). When broilers were fed a diet including FGS and UGS at 6% for 21 days, Righi et al. (2021) noticed that the average daily gain (ADG) was negatively affected compared with the control group (by 10.9% on average) and the group augmented with VitE (at 200 mg/kg; by 14.43% on average) (Nardoia et al., 2020).

The FSE supplementation (0.01% of the diet) during a 42 days' trial, lessened the negative effect of HS (32°C) on ADG commensurate with the basal and VitC addition (by 6.5% on average) (Wang et al., 2008).

Broilers fed a diet including RO (at 0.01, 0.015 and 0.02%) manifested averagely higher live WG than animals' grantee VitE (by 4.9%) or RL (by 5.2%) after 21 days of trial (Yeşilbag et al., 2011).

Likewise, broilers fed for 21 days with oregano aqueous essence (OAE, at 0.002% of the diet) in comparison with the control and VitE ones (150 mg/kg) enhances (by 8.1 and 11.5% respectively) their BW (Scocco et al., 2017). As stated by Righi et al. (2021) the dietary inclusion of OAE (at 0.02%), compared with VitE (at 150 mg/kg of diet) improved (by an average of 8.1%) broilers BW at 21 days of age, in opposition with the control (by 8.9%) at 42 days of age. In addition, ADG was positively impacted (by 14.7%) exclusively in the 21-42 days interval of treatment, and only in analogy with the negative control group (Forte et al., 2018).

The dietary addition of oregano plant (OP, at 0.05%) alone or in consociation with VitE (170 mg/kg) compared with basal diet, containing only basal levels of VitE (30 mg/kg), increased (by 15.2% on average) broilers WG. Whereas no significant differences were reported by Righi et al. (2021) between OP and VitE group (Giannenas et al., 2005). When broilers were supplemented with a higher quantity of OP (0.5% of the diet) for 42 days the overall WG was increased (by 15.9%) compared with the control group, where we could find only VitE and VitC at low additional quantities (30 and 10 mg/kg respectively) (Florou-Paneri et al., 2006).

On several occasions, the admixture of different PFA unveiled the same effect as the addition of vitamins. In a 38 day diet trial, OO supplemented broilers (0.01% and 0.02%) did not modify their BW, compared with those given a basal (30 mg/kg of VitE) or a VitE rich (200 mg/kg feed) diet (Botsoglou et al., 2002).

In like manner, in broilers fed 2 different diets (crude soybean oil diet or acidulated soybean oil limestone), the supplementation with OO (at 0.01%) did not considerably affect WG in equivalence with 2 different addition levels of VitE (10 and 100 mg/kg) in the diet (Avila-Ramos et al., 2012).

However, when different doses of OO and RO were included in broilers diet, BW was negatively affected (Basmacıoğlu H. and M, 2004), as precisely reported by (Righi et al., 2021) in his review.

The diminished inclusion levels of RO in broilers diet, up to 21 days, reduced (by 5.1%) their BW compared with those consuming VitE at a basal (only 50 mg/kg of VitE) or at the maximum level (200 mg/kg).

The dietary inclusion of OO at the lowest dose alone or in combination with RO (at 0.075% each), reduced broilers BW (by 5.0% average). A reduction (by 5.6% on average) of BW was found in broilers consuming simultaneously OO and RO in two different combinations (0.075 and 0.15%) at the end of the experiment (42 days). Similarly, as specified by Righi et al. (2021), the highest dietary inclusion levels of RO and OO compared with the basal diets reduced (by 4.1% on average) the broilers WG up to 21 days of age. The highest inclusion levels of both RO and OO, resulted in a reduction (by 3% on average) of their WG. Lower values of WG were also noticed with the highest inclusion level of OO, as well as with the intermediate and higher doses of its combination with the RO (by 6.0% on average) (Basmacıoğlu H. and M, 2004). In another trial, also comprehended in the Righi et al. (2021)'s review, feeding broilers with a mixture of RO, OO, and FVO (all at the concentration of 0.04%) for 42 days, improved FBW and WG (by 7.65% on average), commensurate with basal additions (receiving only 50 mg/kg of diet of VitE), but not compared with VitE (200 mg/kg) enriched diet (Cetin et al., 2016).

The dietary supplementation of TO (0.01%) compared with a control diet (VitE deficient) for 42 days led to negative effects on broilers BW and WG (by 0.58 and 0.6%, respectively), as noticed by Righi et al. (2021).

In addition, the BW was higher (by 2.8% on average) in TO (at 0.01 or 0.02% of the diet) matched with VitE (100 mg/kg) fed broilers but diminished (by 0.92%) than those supplemented the highest dose of VitE (200 mg/kg).

The data on WG were coherent with those on BW (Bölükbaşi et al., 2006). Equivalent findings were obtained for WG and final body weight (FBW) in broilers supplemented with different PFA (at 0.2% of the diet). Specifically, tomato skin, orange peel, and green tea leaves were tested in broilers in comparison with VitE (200 mg/kg of the diet) (Marzoni et al., 2014). The dietary addition of RL (at 2.5% of the diet) did not change average WG, daily water consumption, and mortality, neither compared with VitE supplementation (200 mg/kg) nor to the addition of another natural antioxidant (rosehip, chokeberry pomace, and nettle) (Loetscher et al., 2013).

The dietary inclusion with a commercial blend of essential oils (at 0.05% and 0.1% of the diet) compared with VitE (200 mg/kg) for 42 days, did not substantially impact FBW of broilers (Botsoglou et al., 2004).

Similar results were noticed by Righi et al. (2021) for BW in CE (0.056%), TE (0.056%), SE (0.056%), MX (0.02%), synthetic antioxidants (0.00486%) and β-apo-8-carotenoic acid ethylester (0.004%) compared with VitE (150 mg/kg) fed broilers (Koreleski and Świątkiewicz, 2007).

The 14 days's supplement with OLE was not sufficient to decrease the negative effect of heat stress on WG (Agah et al., 2019). The inclusion of the commercial product Proviox® (PP; Provimi, France; at the dose of 0.02%) alone or concomitantly with VitE (PPE; 100 mg/kg) for 38 days, increased (by 18.1%) final BW in HS broilers. Feeding PP also led to an augmented (by 13.8% on average) WG compared with both, the thermoneutral and HS control groups, whereas PPE fed animals showed higher (by 16.8%) WG only compared with the HS one (Mazur-Kuśnirek et al., 2019).

In a similar study on broilers collected by Righi et al. (2021) feeding PP did not mitigate the detrimental effects of Ochratoxin A contaminated grain (Mazur-Kuśnirek et al., 2019). In fact, at day 21 of trial, BW was lower (by 26.3% on average) in PPE and PP groups compared with those consuming a not contaminated diet. Similar results were found also after 35 days, when the BW values were reduced (by an average of 11.3%). The WG was also reduced (by an average of 16.7%) in PPE and PP groups. In another similar case study, the same dietary treatments did not affect BW and WG in broilers fed with low quality oil and no substantial differences were observed between PP and VitE fed animals (Mazur-Kuśnirek et al., 2019).

### 3.3 Feed gain ratio or feed conversion ratio

Prosecuting on the Righi et al. (2021) review, GP (from 0.05 to 10% doses) compared with VitE (200 mg/kg) inclusion in broilers' diet did not undermine feed:gain ratio (FG) after 21 (Goni et al., 2007) or 42 days of age (Ebrahimzadeh et al., 2018). The F:G ratio of broilers was not affected by the dietary addition of CE, TE, SE, MX (Koreleski and Światkiewicz, 2007), RL (Loetscher et al., 2013), OAE (Forte et al., 2018; Scocco et al., 2017), OO (Avila-Ramos et al., 2012; Botsoglou et al., 2002), OO and RO alone or in various combinations (Basmacıoğlu H. and M, 2004), as well as of TP (King and Zeidler, 2004) and RP (Rostami et al., 2015) in comparison with VitE (from 150 to 200 mg/kg of the diet).

Moreover, the dietary addition of different natural antioxidants (Marzoni et al., 2014), OLE under HS (Agah et al., 2019), hesperidin (Simitzis et al., 2011), and polyphenols (Mazur-Kuśnirek et al., 2019c, 2019a, 2019b) compared with VitE (from 150 to 200 mg/kg) did not affect the FG ratio.

Similar results were reported by Righi et al. (2021) when RL and RO were added in broilers' diets for 42 days, albeit a reduction (by 9%) on the F:G ratio of the RO compared with VitE fed animals (at 50 and 20 mg/kg) was observed after 21 days (Yeşilbag et al., 2011). Other studies have reported significant effects of PFA on FG ratio, but only in comparison with VitE lacking diets or with diets without VitE. A reduction (by 8.1% on average) of FG ratio was found when OP alone or in combination with VitE (OP + VitE) or VitC (Oregano plant + Vitamin C) was added in broilers diet for 14 days (Florou-Paneri et al., 2006).

Valuable effects were reported in FSE fed broilers, decreasing (by 34.47%) the FG ratio (Wang et al., 2008). A significant decrease (by an average of 3.8%) of FG ratio in RL fed broilers compared with those consuming VitE at basal or supplemental levels, was found to occur in the initial 2 weeks of the trial (Loetscher et al., 2013).

Feeding broilers with TO (at 0.01% and 0.02% of the diet) increased F:G ratio compared with those consuming either no VitE (by 1.4%), VitE at 100 mg/kg (by 4.1%) or VitE at 200 mg/kg (by 2.6%) (Bölükbaşi et al., 2006). Conversely, the administration of a mixture of OO, RO, and FVO (at 0.04% each), compared with a basal and VitE rich diet reduced (by 8.1% on average) broilers' FG ratio (Cetin et al., 2016).

In addition, Righi et al. (2021) enclosed a decrease (by 5.9%) of FG ratio in broilers fed with GP (at 0.6% of the diet) compared with VitE (200 mg/kg) (Brenes et al., 2008). Broilers under HS supplemented with FSE also reduced (by 4.4%) their FG ratio in comparison with those consuming only the basal but not the VitC enriched diet (Wang et al., 2008).

Conversely, the dietary supplementation of broilers with FGS and UGS (at 3 and 6% each) negatively affected the FG ratio (Nardoia et al., 2020). Indeed, higher FG ratio in UGS compared with basal and VitE fed broilers was found (by about 12 and 10%); moreover, the FG ratio was also higher in relation to unsupplemented animals (by 13.5%) and to VitE ones (by 11.9%) in comparison with those consuming the diet with the highest inclusion level of FGS. Righi et al. (2021) noticed that Gianneas and colleagues (Giannenas et al., 2005) reported beneficial effects of feeding OP (at 0.5% of the diet) alone or mixed with VitE (170 mg/kg) for 42 days on F:G ratio. In fact, the supplemented compared with the unsupplemented broilers reduced (by 5.6%) their FG ratio, without significant differ with VitE fed one.

### 3.4 Nutrients digestibility

PFA have a different biological activity depending on the intestinal tract which they operate into (Florou-Paneri et al., 2019).

The GP (at 1.5% of the diet) compared with VitE integration in broilers diet did not modify apparent ileal fat digestibility (AIDF) (Brenes et al., 2008). Nevertheless, the dietary supplementation with GP (at 3 and 6%), compared with VitE reduced (by 3.9% on average) the AIDF, whereas the apparent ileal protein digestibility (AIDP) was not affected (Brenes et al., 2008). As noticed by Righi et al. (2021), broilers fed with the highest inclusion level of GP than with the lowest one had obviously a higher HP (by 18.35%) and CT (by 289.4%) total intake. Moreover, the GP addition at high levels (3 and 6%) increased HP total intake (by 8.7% and 23.3%, respectively) compared with the basal and VitE rich diets (Brenes et al., 2008).

Ileal digestibility and fecal digestibility of HP (IDHP and FDHP, respectively) had a negative correlation when increasing GP inclusion in broilers diet. The IDHP was lower (by 16.0% on average) compared with both control and VitE groups, whereas FDHP was lower (by 16.8%) compared with control and VitE treated (Brenes et al., 2008). The same findings have been observed for the AIDP (by a mean value of 6.62%) in the UGS (at 6% of the diet) fed animals. Moreover, UGS dietary addition (at 3 and 6%) reduced ileal total extractable polyphenols (TEP) compared with the basal (by a mean proportion of 18.4%), and the VitE (by an average amount of 22.3%) fed diet (Nardoia et al., 2020).

On the other hand, Righi et al. (2021) noticed that FGS did not significantly affect the above parameters. Broilers supplemented with FSE (at 0.1% of the diet) for 42 days, showed greater apparent digestibility of crude protein (on average by 3.3%) and calcium (on average by 5.5%) compared with those consuming the basal and VitC diets (Wang et al., 2008).

The RL (at 2.5% of the diet) compared with rosehip administration for 5 weeks reduced (by 9.1%) the nitrogen metabolization capacity of broilers diet and the metabolizable energy (by 6.4% on average) compared with the control, VitE and the nettle groups (Loetscher et al., 2013).

A higher apparent digestibility of energy (by 3.9%) and phosphorus (by 12.2%) in FSE fed animals was found (Wang et al., 2008). The dietary supplementation with OLE (at 0.4%) in HS broilers for 14 days lowered apparent digestibility of energy (by 8.5%) compared with VitE fed animals, but not in comparison with those consuming the basal diet where this parameter was increased (by 2.7%). The OLE fed animals (at 0.4% of the diet) showed a reduction in apparent digestibility of crude protein (by 14.5%), ash ( by 22.7%) and phosphorous (by 15.7%) (Agah et al., 2019).

**3.5 Serum biochemical parameters**

Following Righi et al. (2021) review, the lower dose of GP (5, 7.5 10%) inclusion in broilers, in a not specified acid profile diet, was not effective in decreasing total CHOL. The same results were observed using VitE (200 mg/kg).

On the other hand, GP (at 10.0% of the diet) compared with VitE or a basal diet increased HDL-C (by 50.1% on average). However, all the GP compared with control groups showed a substantial decrease (by 31.0% on average) for LDL-C, whereas only the highest inclusion level of GP showed lower (by 28.6%) LDL-C compared with VitE (Ebrahimzadeh et al., 2018).

In opposition to these findings, the dietary integration of another vinery by-product in broilers, called grape seed extract (GSE; from 0.015 to 0.045%), did not show a substantial effect on CHOL, HDL-C, LDL-C parameters in comparison with the control and VitC (300 mg/kg) diets under thermoneutral conditions (Hajati et al., 2015). By contrast, within HS conditions, VitC and GSE (at 0.015 and 0.030%) fed animals, compared with the un-supplemented ones showed inferior CHOL (by an average of 8.4%) level.

The lower level of GSE was not efficacious in decreasing CHOL concentration whereas LDL proteins were lowered (by 27%) only with the highest inclusion in comparison with the basal diet. Similarly, the highest inclusion level of GSE compared with the basal diet resulted in lowering (by 10.7%) the very-low density lipoproteins (VLDL).

Based on the information that Righi et al. (2021) collected, it seems that HS plays a central task in the lipid metabolism of broilers, conditioning also GSE impact: this can be since stressors affects CHOL level in plasma of poultry, by the stimulation of the adrenal grand which induces lipolysis.

Feeding GP to broilers brought to a decrease of TG levels (by an average of 39.35%) in comparison with the control group, without significant differences between GP and VitE groups (Ebrahimzadeh et al., 2018).

In contrast to these observations, broilers farmed under thermoneutral conditions and fed with GSE in comparison with VitC did not manifest any significant effect on TG levels (Hajati et al., 2015).

Nonetheless, in the same paper the highest GSE addition level led to a considerable decrease of TG concentration (by 10.8%) compared with the basal diet. However, no significant differences between VitC and GSE groups, and between VitC and control groups were found in the analysis of Righi et al. (2021). In the same research, glucose was at a lower level in GSE compared with the basal (by an average of 12.0%) and VitC (by about 8.6%) fed animals after 28 days of trial, whereas no differences between VitC and control groups was observed. At day 42 Hajati et al. (2015) reported lower values in GSE groups than control (on average 15.6%), and VitC group (mean of 11.7%).

Broilers reared under HS, and fed a diet supplemented with olive leaves (at 0.2% -OL1- and 0.4% -OL2-) or complemented with VitE (250 mg/kg) had an inferior level of CHOL than control, reaching the lowest point in OL1 than control group (by 15.5%) (Agah et al., 2019). The authors also reported lower and comparable TG values in OL and VitE groups in comparison with the control group (on average TG was reduced by 34%). Uric acid was also lesser in OL2 than in OL1 and VitE groups (by 28.85% on average) as well as in the control one (by 0.48%).

Substantial changes of lipid metabolism parameters were noticed by the author Righi et al. (2021) when TO was supplemented to broilers (Bölükbaşi et al., 2006). Precisely, animal complemented with TO (at 0.01% and 0.02%) increased total CHOL (by 7.1% and 10.32%) in comparison with groups supplemented with VitE (100 and 200 mg/kg respectively).

Furthermore, HDL-C was higher (by 8.9%) when adding TO at the lowest level, and lower (by an average of 4.2%) when TO was supplemented at the highest level in comparison with the VitE groups.

Likewise, LDL-C was higher (by an average of 15,7%) in TO treated animals than in VitE groups (Bölükbaşi et al., 2006). Finally, the TO supplementation increased all the mentioned lipid metabolism markers when compared to the control group: this is a clear example of what Is summed in Righi et al. (2021)'s work, the complete lack of the expected hypocholosterolemic effect of thyme oil.

### 3.6 Serum enzyme activities

Other parameters that have been investigated in the Righi et al. (2021)'s review are serum enzyme activities, such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH): supplementing animal diets with PFA will exert an hepatoprotective effect reducing liver damage markers.

While this is supported in many publications (Ahmadvand et al., 2012) on the other hand we need to notice that broilers, exposed to HS conditions (34°C) for 41 days and fed with GP (at 10% of the diet) increased (by 63.5%) their AST activity in plasma compared to those fed the control and VitE (200 mg/kg) diets (Ebrahimzadeh et al., 2018). As suggested by the author Righi et al. (2021) the higher AST might have been related to the hepatocellular necrosis potentially inducted from polyphenols.

The potential hepatoprotective effect of PFA was also confirmed in another study (Agah et al., 2019), where the dietary supplementation with OLE (at 0.4% of the diet) or VitE compared with a basal diet reduced ALT activity (by 18.0 and 25.8% respectively). The OLE compared with VitE and control diet was also able to reduce (by an average of 18.1%) ALP activity (Agah et al., 2019).

Always following the line of the authors, these interesting finding could be partially explained due to the preventive action of olive leaves towards free radicals' generation, hence, to preserve hepatocytes integrity.

### 3.7 Plasma oxidative status

Righi et al. (2021) analyzed several studies that have investigated the effects of PFA on plasma oxidative status markers, defining as of particular interest the superoxide dismutase (SOD) which, along with glutathione peroxidase (GPx), act as antioxidant against reactive oxygen species (ROS) balance.

Broilers fed with RL (at 0.57%, 0.86% and 1.15%) and RO (at 0.01%, 0.015% and 0.02% of the diet) compared with VitE (administered at the doses of 50 and 200 mg/kg) for 42 days did not express significant differences on SOD activity, whereas the high inclusion levels of RL and RO reduced the cited above (Yeşilbag et al., 2011).

In like manner, the review author reported no significant effects on broilers' SOD activity in a trial comparing the dietary inclusion of OL with VitE (Agah et al., 2019). The dietary supplementation with GP (at 7.5% of the diet) in broilers reared under thermoneutral conditions, increased plasma SOD activity compared with control and VitE (by an average proportion of 50.8%) groups. The dietary addition with GP (at 5% of the diet) compared with a basal and VitE diets led to a similar increase of SOD activity, but reduced in time.

On the contrary the dietary inclusion of GP at higher level (10%) did not improve SOD activity compared with both the basal and VitE (200 mg/kg) diets (Ebrahimzadeh et al., 2018).

Broilers exhibit higher GPx activity when fed with GP (at 5% of the diet) in comparison with those consuming a basal (by 46.3%) diet. Higher doses of GP (7.5% of the diet) compared with a basal and VitE diet, increased GPx activity (by a mean value of 46.4%), whereas VitE compared with the basal diet did not change this parameter (Ebrahimzadeh et al., 2018). The dietary supplementation with OLE compared with a basal diet increased GPx (by 24.0%), but not with VitE (Agah et al., 2019).

Another valuable parameter to establish antioxidant capacity of PFA is the malonildialdehyde (MDA), an endogenous marker of lipid oxidation, whose increase is expression of a diminished antioxidant capacity of the organism.

Several papers collected by Righi et al. (2021) in his review have reported favorable effects of supplementing poultry diet with PFA on different markers related with the antioxidant status. For instance, the dietary inclusion of OL (at 0.4% of the diet) compared with VitE and a basal diet reduced MDA values (by 22.5%) in plasma. A reduction of the ferric reducing activity power (FRAP by 19.1%) in broilers fed with OL (0.4% of the diet) compared with VitE was also noticed (Agah et al., 2019).

The GP fed animals (at 7.5% of the diet), compared with those consuming the basal and VitE diets, reduced their MDA plasma content (by 25.2%) (Ebrahimzadeh et al., 2018).

Adding sweet chestnut wood (SCW) extract to broilers in comparison with high PUFA (polyunsaturated fatty acids; from linseed oil) content diets and two levels of VitE (85 mg/kg and 200 mg/kg), showed no significant effects on plasma and liver MDA content, total antioxidant status (TAS), lipid soluble antioxidant capacity (ACL) and both SOD and GPx activities (Voljč et al., 2013). The SCW inclusion reduced (by 42.4%) the DNA damage in comparison only to linseed oil fed group (Voljč et al., 2013).

In broilers fed with natural tocopherols, rosemary, grape seed, green tea and tomato alone, or in various combinations (at 0.01 and 0.02% of the diet) among them, for 42 days, no significant effects on plasma FRAP, and thiobarbituric acid reactive substances (TBARS) and SOD activity as well, were reported.

On the other hand, some effects were observed on GPx activities (Vossen et al., 2011).

The TAS was not affected by polyphenols (PP) addition in broilers diet in three similar studies (Mazur-Kuśnirek et al., 2019). Nevertheless, SOD activity was lower in PP (by 18.3%) and PPE (PP with VitE; by 21.8%) fed animals, compared with those in thermoneutral conditions (TN). The GPx activity was higher (by 49.1%) in PPE group than in those challenged with heat stress (HS), (Mazur-Kuśnirek et al., 2019) as well as in PPE (20.5%) and in PP (11.9%) groups in comparison with those consuming a low-quality oil diet (Mazur-Kuśnirek et al., 2019b). In this last paper, GPx activity was higher in PPE (15.0%) and in PP (14.3%) fed animals. In another similar study highlighted by Righi et al. (2021), in which all diets contained grain contaminated with ochratoxin A, TAS was higher in PPE (by 23.5%) and PP (by 21.6%) groups than in positive control group (only with contaminated grain) (Mazur-Kuśnirek et al., 2019a).

However, SOD activity was lower (by 18.4%) in PP compared with control group which consumed the not tainted grain, but higher (by 10.6%) in PPE than VitE (100 mg/kg) group, and lower in PP (by 12.8%) than in high dose VitE group (200 mg/kg). In the same paper, GPx activity was higher in PPE (by 17.6%) and in PP (by 34.6%) respect the contaminated grain fed broilers, without showing any significant difference with VitE ones. Amongst the different parameters investigated (VitE, total tocopherols, Vitamin C, retinol) in blood and liver, hepatic TBARS values were significantly influenced: PPE and in PP were higher (by 32.0% on average) in comparison with TN group (Mazur-Kuśnirek et al., 2019c).

On the other hand, the supplementation with PPE decreased hepatic TBARS values compared with broilers fed with contaminated grain, demonstrating a synergic effect of PP and VitE in attenuating the detrimental effects of nutritional stress (Mazur-Kuśnirek et al., 2019a).

Dietary supplementation of chickens with GP (3% of the diet) compared with the basal and VitE diet resulted in a higher total intake of extractable polyphenols (by 50.6%), (Goni et al., 2007). Digestibility of extractable polyphenols was also higher (on average 117.2%) in GP than in basal and VitE fed animals (Goni et al., 2007). The Total antioxidant capacity (TAC, measured by ABTS assay) of broilers excreta increased (by 19.9%) when fed with GP compared with basal diet. A similar reduction (by 18.8%) was included in Righi et al. (2021)'s review when the TAC was determined by FRAP assay. However, no difference was observed between VitE and GP treatments in ameliorating this marker (Goni et al., 2007).

In male broiler chicks, the TAC was also affected when GP was included in animals' diets at different levels (1.5, 3, 6%) (Brenes et al., 2008). In fact, the TAC comprehended in diet, ileal content and excreta resulted higher (by averages proportions of 345.43, 102.7 and 41.6% respectively) in GP than the basal diet fed broilers. Meanwhile, the same parameter was determined to be higher (by a mean of 125.6%) in the diet and ileum (by an average of 68.2%) in GP than VitE (200 mg/kg) fed animals. Furthermore, GP supplementation did not affect the serum TAC (sTAC, measured by ABTS assay). On the other hand, the above-mentioned parameter (measured by the FRAP assay) differed between GP and basal diet fed animals (Brenes et al., 2008).

Continuing into the review, in broilers fed a linseed oil rich diet, with a high PUFA content, the addition of SCW (at 0.3% of the diet) reduced the lymphocyte DNA damage (by 42.0%) (Voljč et al., 2013). The HSP70 gene (which expression is expected to increase in heat stress), was found to lower levels (594,3%) in the heart tissue of GSE fed broilers when animals were reared under heat stress. A reduction (by 199.9%) in the expression of HSP70 gene in the liver of GSE fed broilers exposed to heat stress was also noticed (Hajati et al., 2015).

The effects of PFA on selected immunological parameters, in comparison with synthetic vitamins, have also been investigated: different grape polyphenols (GPP) to VitE ratios (C, 0:100; LGPP, 25:75; MGPP, 50:50; HGPP, 75:25) in broiler diets were tested. As interestingly stated by Righi et al. (2021), broilers supplemented with the highest GPP:VitE ratio (HGPP) compared with those receiving only VitE (100 mg/kg) increased their antibody titers (by a mean value of 59.5%) against Newcastle disease and Infectious Bursal Disease (IBD) (Iqbal et al., 2015). However, antibody titers against IBD were also higher in HGPP compared with LGPP and MGPP groups (by an average proportion of 389.0%) (Iqbal et al., 2015). In contrast to these results, dietary supplementation with rosemary powder (RPO; 0, 0.5, 1% of the diet) or VitE for 42 days, did not affect positively broilers antibody titers against random viruses and sheep red blood cells (SRBC) (Rostami et al., 2018).

### 3.8 Effects on macro - and microscopic anatomical features and on intestinal microbiome profile

Several studies collected in Righi et al. (2021)'s review have suggested an immunostimulant activity played by polyphenols on several signal transduction pathways which are responsible for a wide range of cell functions, related, inter alia, to immune system organ function and growth (Zhu et al., 1997).

The broiler spleen and Bursa of Fabricius were not affected at all both when the diet was supplemented with different levels of GP (5, 7.5 and, 10%) either with VitE (200 mg/kg) (Ebrahimzadeh et al., 2018). In a similar way, lower GP addition levels (1.5 to 6% of the diet) did not manifest to influence the relative spleen weight in broilers; only those that were supplemented with low GP levels (1.5% of the diet) compared with VitE, improved the above-mentioned morphological parameter by 20.0%

(Brenes et al., 2008). Comparable findings concerning the weight of thymus, spleen and bursa of Fabricius in RPO (0.5 and 1% of the diet) and VitE (100, 200 mg/kg of the diet) fed broilers were noticed (Rostami et al., 2018) and collected in the author's review.

Moreover, in some papers we see how PFA have effects on small intestine and gut gross morphometry, which can influence absorption and digestion capability. Broilers fed with inclusion of GP (1.5%) compared with VitE, improved relative length of duodenum and ceca (by about 9.0%) (Brenes et al., 2008). On the contrary, RPO addition (from 0.5% to 1%) in broilers' diet afflicted their jejunum length and weight, colon length and weight and right cecum weight, but no such differences were reported concerning the above-mentioned anatomical traits compared with those consuming 2 different levels of VitE (100, 200 mg/kg) (Rostami et al., 2015).

The dietary inclusion with GP (at 5, 7.5 and 10% of the diet) for 42 days affected broilers' mucosa status and its microscopic structure (Ebrahimzadeh et al., 2018): the GP supplemented compared with the control group showed a considerable reduction (by 35.2%) of villus height (VH) in jejunum, while those consuming the highest levels of GP had also shorter villus height (by 35.4%) compared with the VitE fed ones. Furthermore, broilers fed win an addition of GP (at 7.5 and 10% of the diet) in comparison with the control group, had significantly lower VH/crypt ratios especially in jejunum (with a mean reduction of 35.2%), whereas only those consuming the highest GP level compared with those receiving VitE showed a lower value (by 35.4%) (Ebrahimzadeh et al., 2018). As the author stated, this evidence can indicate our belief that polyphenols have a potential effect on the intestinal digestive and absorption functions.

Continuing onto this trend, grape polyphenols added at 0.0025, 0.0050 and 0.0075 % of the diet in broilers diet did not cause any significant effects on histological features of kidneys and liver (Iqbal et al., 2015).
Among the other potentially functions of PFA on poultry metabolic status, some papers collected in Righi et al. (2021)'s review have investigated their effectiveness in affecting intestinal microflora and mucosa integrity (Lee et al., 2004). Dietary addition with oregano aqueous extract (OAE; at 0.02% of the diet), compared with VitE and an control diet enhanced the gut secretion of glyconjugates (Scocco et al., 2017) by the goblet cells, which plays an important role in protecting the intestinal mucosa (Scocco and Pedini, 2010).
The PFA efficiency in regulating pathogens is of great interest in livestock species and especially poultry, with a particular attention to some critical phases in animal life cycle (Windisch et al., 2008b). As collected by Righi et al. (2021), Coliform's bacteria count was lesser in OAE than in VitE and control groups (by an average proportion of 20.8%) (Scocco et al., 2017). Including OAE, compared with VitE, reduced the *Escherichia coli* count in broilers caecum at 21 (by 35.0%) and 42 days (by 9.0%). The dietary a of addition of OAE, in comparison with VitE and a control diet for 3 weeks, diminished (by 32.5%) ileal count of total anaerobia bacteria. Furthermore, in broilers' ileum tract, the treatments groups did not influence the *Staphylococcu*s spp. population, while lactic acid bacteria (LAB) kept a positive trend in OAE group throughout the all-time of the interval. Nevertheless, the different treatment did not modify the count of these beneficial bacteria: enterococci, lactobacilli and staphylococci populations in caecum, at both sampling times, did not manifest a significant change (Scocco et al., 2017). In line with the upon mentioned study, UGS and FGS supplementation in broilers did not significantly

affect LAB, *E. coli* and *Clostridium* spp. population in the ileal contents of 21-d broilers (Nardoia et al., 2020).
Righi et al. (2021) collected a great number of studies that indicate how the dietary inclusion of PFA have some effects on macro- and microscopic anatomical features and on the microbiome profile.

### 3.9 Carcass weight and quality, and internal organ weights

PFAs effects on slaughter traits have been investigated in some articles collected in Pitino et al., (2021)'s review, as the consequence of in vivo growth performances. It should be specified that the possible effects on these traits, are most likely to be attributable to the modulation that PFA can exert on the intestinal microbiota equilibrium (Yang et al., 2015)
Starting with Hesperidin, included in broilers' diet (HE, 0.15 or 0.3%), did not affect intramuscular fat (%), cooking loss (%), shear values, cold carcass weight (CCW) and weights of liver, heart, gizzard, and abdominal fat (% of CCW), neither compared with control nor with VitE (200 mg/kg) fed groups (Simitzis et al., 2011). No carcass' alterations were found in broilers supplemented with different herbal additives (rosemary leaves – RL-, rosehip, chokeberry pomace, and nettle at 2.5% each), rosemary powder (-RPO- 0.5, 1.0, 1.5% of the diet, or 1% RPO plus VitE at 100 mg/kg) (Rostami et al., 2017) and a commercial polyphenol product (at 0.2% of the diet) (Mazur-Kuśnirek et al., 2019a). Similar results were observed in broilers supplemented with various natural antioxidants (Marzoni et al., 2014) compared with VitE control group (100 or 200 mg/kg of the diet). Additionally, the carcass yield (CY) did not substantially diverge in a feeding trial where oregano oil (OO) (Basmacıoğlu H. and M, 2004), rosemary oil (RO- Basmacıoğlu H. and

M, 2004; Yeşilbag et al., 2011), and RL (Yeşilbag et al., 2011) were compared with VitE. At the same time, no significant effects were observed in a 20 days' trial in broilers fed with a basal diet including VitE (150 mg/kg), or thyme extract (TE), sage extract (SE), coneflower extracts (CE; at 0.056% of the diet), marigold xanthophylls (MX, 0.02% of the diet), a mix of synthetic antioxidants (at 0.0486% of the diet of butylated hydroxytoluene, ethoxyquin, butylated hydroxyanisol), or β-apo-8-carotenoic acid ethylester (0.0040% of the diet) alternatively (Koreleski and Światkiewicz, 2007), as Pitino et al., (2021) defined in his review.

The nutritional addition with a polyphenol product (PP, 0.01% of the diet) alone or in association with VitE (PPE, 0.01% of PP plus 100 mg of VitE/kg) in heat stressed (HS) broilers improved (by 7.4%) their breast muscle yield compared with the control or fed with VitE (100 mg/kg). Nevertheless, the color parameter was lower in both PPE (by 11.8%) and PP (by 13.2%) groups than in thermoneutral (TN) animals. Natural drip loss and water holding capacity (WHC) were reduced in PPE (by 39.9 and 29.7%, respectively) and PP (by 37.3 and 24.9%, respectively) than HS group. Conversely, crude ash content remained at an higher lever in PP than in TN (by 9.8%) and HS group (by 6.0%) (Mazur-Kuśnirek et al., 2019).

In a similar paper collected by the author Pitino et al., (2021), broilers were supplemented with the same PP (at 0.02% of the diet) and tended to increase their carcass dressing (by 3.4%) compared with the VitE (100 mg/kg) control group (Mazur-Kuśnirek et al., 2019a). The addition proved not to influence the weight of carcass and organ; only the gizzard weight was lower (by 15.6%) in PP than VitE fed broilers. Additionally, compared with those fed a control diet (NCD), only PP administration (at 0.02% of the diet) or in association with VitE (PPE) decreased their carcass dressing (by 3.8% and 2.6%, respectively). Broilers of PP compared with the NCD ones had also heavier liver (by 27.0%), even though both PPE and PP compared with NCD only, had longer small intestine (by 22.3 and 24.2%, respectively) but lower small intestine weight (by 20.7%, 25.5%, respectively). The PPE diet proved to be effective in reducing the weight of the small intestine (by 9.8%) and the length of the caeca (by 13.3%) respect the one fed VitE only (100 mg/kg) (Mazur-Kuśnirek et al., 2019a).

A substantial increase (by 3.9%) in hot carcass weight (CW) was noted, when broilers diet was supplemented with Thyme oil (TO, at 0.02%) compared with VitE (100 mg/kg), whereas a decrease of the CW by 4.7% was observed when in their diet lower TO (0.01% of the diet) or higher VitE (200 mg/kg) levels were included (Bölükbaşi et al., 2006). On the other hand, a VitE deficient dietary treatment proved to be less functional in reducing their hot carcass weight (by 4.1%) respect a TO inclusion (0.01% of the diet) (Bölükbaşi et al., 2006).

In line with the cited above assertions redacted by Pitino et al., (2021), liver weight of TO fed broilers, was averagely decrease (by 12.0%) than those receiving VitE (200 mg/kg) (Bölükbaşi et al., 2006).

Pancreas and liver weight were reported to be greater in RL than control (by 21.5 and 18.6%, in pancreas and liver, respectively) and VitE groups (by 27.6 and 18.2%, respectively) (Loetscher et al., 2013). The supplement of grape pomace (GP, 1.5%) in broilers as brought to an higher (by 18.4%) relative weight (RW) of the abdominal fat compared with those consuming a deficient VitE diet (Brenes et al., 2008): the same study brought to a noticeable higher spleen relative weight (RW; by 20%), duodenum (by 7.4%) and ceca (by 10.4%) relative length (RLE) compared with those consuming VitE (200 mg/kg of the diet) (Brenes et al., 2008).

Another study comprehended in Pitino et al., (2021)'s review pointed out a reduction of heart and liver RW (by an average of 31.3% and 3.5% respectively) in broiler fed with a dietary protocol supplemented with a low amount of grape polyphenols in combination with VitE (25 mg/kg of GPP + 75 mg/kg of VitE) compared with broilers supplemented with VitE (100 mg/kg) only (Iqbal et al., 2015).
Some years later, a study with GP supplementation (from 5% to 10%) seemed not to affect broilers' relative organ and carcass weight in analogy with VitE (200 mg/kg) (Ebrahimzadeh et al., 2018). If it is possible to draw some partial conclusions, a variable effect on carcass yield and quality and internal organs development appears to be present: it is all due to the nature and dose of the dietary inclusion PFA, in comparison to both basel or VitE supplemented diet; it is not to forget that the presence of stressors can possibly affect animals' performance (Tsiplakou et al., 2021).

## 3.10 Meat quality characteristics

**pH at 24h postmortem, color, shear force value, cooking loss, intramuscular fat, and sensory qualities, fatty acid composition.**

The final results of all sanitary control need to be focused onto the market demand: therefore, meat quality characteristic are of great importance from a consumer perspective, and thus for the working professionals.
The review displays the actual parameters, written by Pitino et al., (2021), collected a great number of articles from different authors, that have aimed at evaluating them all, but together with other traits, such as slaughter and intrinsic characteristic of meat.

As a start, it was noticed that meat color, meat pH at 24h postmortem (pH24) in broilers was not affected by the addition of different PFA at different dietary inclusion (Marzoni et al., 2014). Furthermore, pH, drip loss and electrical conductivity of broiler meat was not influenced by a dietary inclusion of sweet chestnut wood extract (SCW) (Voljč et al., 2013).

Hesperidine (HE; at 0.3% of the diet), supplemented in relation to the basal diet increased the pH24, without differences from the VitE (200 mg/kg of the diet) inclusion (Simitzis et al., 2011). Comparing dietary inclusion of PP alone or combined with VitE (PPE) or with unsupplemented or VitE supplemented animals or with those faced with or without heat stress under the parameters of dry matter, ether extract and saturated fatty acids (SFA) contents in HS broilers breast meat, no difference was noted (Mazur-Kuśnirek et al., 2019c).

Analyzing pectoralis major (PM) and ileotibialis (IL) under the Yellowness parameter of the meat, it was noted to be improved in broilers consuming oregano plant (OP) compared with basal (by 41.4 and 56.7%, respectively) and VitE (by 51.9 and 74.1%, respectively) enriched diets for 42 days (Young et al., 2003). Feeding He (0.15% of the diet) brought also to an increase in lightness (L) by 4.5% than VitE diets (Simitzis et al., 2011).

In the same vein of content, greater pH24 in breast (by1.2% on average) and thigh (on average, 0.68%) meat was described when supplementing broilers with RO (at 0.01,0.015, 0.02% and VitE at 200 mg/kg of the diet) compared with RL fed ones (Yeşilbag et al., 2011). Rearing animals under HS brought to a greater ash content in the polyphenols and VitE (200 mg/kg) supplemented groups than in the negative (no HS and no supplementation) and VitE (100 mg/kg) control groups (Mazur-Kuśnirek et al., 2019c). Furthermore, greater ash content was found in PP fed animals than the positive (by 5.7%) and negative (by 9.8%) control ones; likewise, the PP and low VitE (100 mg/kg) additioned animals showed and higher poly-unsaturated fatty acids (PUFA) content compared with the positive control group (only heat stress). Similarly, poorer natural drip loss was found in the PP and VitE (200 mg/kg) fed animals compared with the basal and low VitE (100 mg/kg) diets (Mazur-Kuśnirek et al., 2019c). In another similar paper collected by Pitino et al., (2021), a combination of VitE (100 mg/kg) and PP (at 0.01 and 0.02% respectively) supplemented to broilers augmented (by 5.1 and 3.8% respectively) their breast muscle pH at 15 h after slaughter in comparison with the control diet, but not compared with a second group fed with that containing VitE and low quality oil (Mazur-Kuśnirek et al., 2019b). On the contrary, the pH decreased (by 2.7%) 24h after slaughter, in broilers consuming the PP diet (at 0.02%) in comparison with the VitE (200 mg/kg) ones. The supplementation of PP had a remarkable effect on color parameters, specifically onto L, and in fact birds of PPE and PP groups improved the value compared with negative (by 3.5 and 5.0%, respectively), and positive control (by 3.7 and 5.2%, respectively) groups. Similarly, the same L value was greater in PP group than in VitE groups (by 4.8% on average): it is to be noted that the other color parameters were not affected. Changing line of work a analyzing what Pitino et al., (2021) collected on the fatty acid composition of breast muscle (BM), the dietary supplementation with PP alone or in association with VitE did not exert a positive effect on PUFAs, hypocholosterolemic fatty acids (DFAs), neutral and hypocholosterolemic/hypercholesterolemic fatty acids ratio, and on atherogenicity index as VitE did (Mazur-Kuśnirek et al., 2019b). Adding the same doses of antioxidants of the previous trial to broilers fed an experimental diet containing ochratoxin A, PP and VitE were not functional in attenuating the deleterious effect of the contaminated diet: to be noted is that the percentages of PUFA, n-3 FA, and DFA were lower in the ochratoxin A-fed animals compared with those consuming the not contaminated diet while the SFA and oxide fatty acids (OFA) were significantly higher (Mazur-Kuśnirek et al., 2019a).

A 42-day trial where a dietary inclusion of OP (3% of the diet) was used, resulted in a lower L (by 4%) of broilers PM than vitamin C (VitC; 1000 mg/kg of the diet) and VitE (200 mg/kg of the diet) (Young et al., 2003). The addition of HE (0.15% of the diet), commensurate with VitE, reduced the redness (by 15.9%,) (Simitzis et al., 2011): from the two previous studies, the author can define that color was not improved by feeding PFA (Pitino et al., 2021).

On the same line of work, the PFA addition on its own or in combination with VitE (0.02% of the diet, PP or 0.01% + 100 mg/kg of VitE, PPE) in HS broilers diets induced lower WHC compared with positive HS control treatment (Mazur-Kuśnirek et al., 2019c). Albeit the greater PUFA content observed in the supplemented groups, lower concentrations of eicosadienoic acid (C20:2), arachidonic acid (C20:4), eicosapentanoic acid (C20:5) and docosapentanoic acid (C22:5), and greater proportions of n-6 fatty acids were observed compared with the control HS broilers (Mazur-Kuśnirek et al., 2019c). On the other hand, OO addition in two different diets (CSB, crude soybean oil diet; ASO, acidulated soybean oil soapstock,) did not modify the percentages of SFA, MUFA, PUFA and PUFA:SFA ratio in broilers BM compared with the VitE (10 or 100 mg/kg) administration. Despite that, the proportion of linolenic acid (C18:3) was enhanced in OO compared with the VitE fed animals (by 38.4 and 44.4%, respectively) (Avila-Ramos et al., 2012).

A supplementation of aqueous extract in broiler (OAE, 0.02% of the diet) did not show any considerable effect on physicochemical, proximate, and fatty acid (including CLA isomers and PUFA n-3 content) composition of BM, in comparison with those reared with a negative (low VitE content) and the VitE diet (150 mg/kg) (Forte et al., 2018). After adding SE to the broilers diet, the proportions of stearic acid (C18:0) (by 20.1%), arachidonic acid (20:4) (by 44.72%), DHA (C22:6 n-3) (by 59.58%), and n-3 PUFA augmented, in comparison with those fed the basal diet, but not to the VitE fed group (Koreleski and Światkiewicz, 2007). Despite that, remaining in the cited above trial, the breast meat of SE group showed a lesser content of oleic acid (C18:1) (by 12.75%) compared with control group, and a lower alpha-linolenic acid (C18:3) (by 24.55%), PUFA (by 24.46%), and n-6/n-3 ratio (by 23.44%) commensurate with animals receiving a commercial antioxidant (Koreleski and Światkiewicz, 2007).

Generally speaking, when compared to either a basal diet or basal diet and VitE, it manifest that the PFA were able to: increase poultry meat pH, ash, lower drip loss; furthermore, depending on the author considered, they inconsistently improved yellowness and lightness and increased PUFA, depending, as cited before, from the product tested and the environmental stress factors (Pitino et al., (2021).

# Chapter 4: Experimental contribute

## Introduction

Antibiotic restrictions and the ban of growth promoters led to the need for the recruitments of new natural feed additives able to improve the health status and growth performances of the animals. Moreover, in the organic livestock farming the use of synthetic vitamins is considered as contentious inputs, thus several studies have been performed testing PFA as antibiotics or antioxidants alternatives. The literature reviews performed on the *in vivo* and *post-mortem* effects of PFA both on livestock animals and poultry concluded that PFA effects are mainly affected by the type of plant, part of plant tested, production process, interaction with other compounds, dose, time of supplementation and host interaction (Righi et al., 2021; Tsiplakou et al., 2021).

The first aim of this trial was to identify two potentially and commercially promising PFA, to refute their functionality in the broiler industry as alternative to synthetic vitamins.

Green tea inclusion in broiler diets is reported to have a positive effects on growth performance and lean meat production (Sarker et al., 2010), take active part in the regulation of lipid-metabolism-related genes and transcription factor expression (Huang et al., 2017) and are considered to be a good alternatives to antibiotic growth promoters (Afsharmanesh and Sadaghi, 2014). The latter author tested a first group with 1.2 g/kg diet Kombucha tea (20 % concentration) and a second with the dietary addition of 10 g/kg green tea powder.

Based on the results of Aditya et al., (2018) supplementation of grape pomace in the diet of broilers was effective in reducing serum cholesterol and improving meat quality parameters without affecting growth performance, nutrient digestibility, and carcass traits.

Another interesting compound is found in wood waste extracts, which appear to be a valuable bio-functional source of phenolic acid such as ferulic acid, caffeic acid, protocatechuic acid, gallic acid, p-coumaric acid and chlorogenic acid. All these extracts exhibit strong free radical scavenging properties antimicrobial activities (Smailagić et al., 2020).

Despite that, no thorough examination was conducted to evaluate the antioxidant activity of the latter two components and possible substitutions of synthetic vitamins.

Based on the conclusions reported in Righi et al., (2021), Tsiplakou et al., (2021) and Pitino et al., (2021), Manuelian et al. (2021), and based on the factors affecting the PFA effects, it is of importance to evaluate the antioxidant capacity of the PFA in order to build an experiment. The first aim of the present study was to compare the antioxidant capacity of several PFA in order to select the two more promising PFA to replace the vitamin E in poultry diets. The second aim was to compare diets containing a mixture of green tea and Resinox, hydrolysed polyphenols or vitamin E on poultry health status and performances.

## Materials and Methods

The present trial was conducted among the Organic Plus project (774340-2), which is a European Research and Innovation action for Sustainable Food Security – Resilient and resource-efficient value chain with the overall aim of providing high quality, transdisciplinary, scientifically informed decision to support all actors of the organic sector to promote the EU's organic actions and regulations.

## Herbal product tested

The products tested were a mixture of polyphenols in two different compositions: the product referred as Resinox 80Q-Green Thea (RT) is a combination of grape pomace skin extract and green Thea leaves extract; the product named Oxistim (OXI) is a commercial mixture of hydrolysed polyphenols of wood deriving from waste products of arboriculture.

Prior to build the experiment, the antioxidant capacity evaluation was performed through an ABTS test on the herbal extracts in order to select the doses able to mimic the dietary antioxidant ability induced by vitamin E, that represented the control treatment. The analyses were carried out according to the methods described by Pellegrini et al. (2003) and Dall'Asta et al. (2013) with minor modifications. The sample pre-treatment was adapted based on the chemical-physical characteristics of the product to be tested itself.

The results of the test are reported in Table 1.

**Table 1**. Antioxidant capacity of Resinox 80Q and Green Thea compared with Vit E at 50%.

| | FOLIN mg/g | | ABTS µmol Trolox/g | | DPPH µmol Trolox/g | |
|---|---|---|---|---|---|---|
| | average | sd | average | sd | average | sd |
| Resinox 80Q | 147.36 | 2.87 | 133.84 | 4.24 | 159.98 | 8.80 |
| Green thea | 125.91 | 21.11 | 199.00 | 10.29 | 170.79 | 23.24 |
| Vit E 50% | nd | nd | 12.88 | 0.92 | | |

1. **Birds and housing**

Two hundred fifty-two 1-d-old broiler chicks (Ross 308) were bought from a commercial hatchery and housed the 2nd of April 2021 in pens built inside the Large Animal Isolation Unit of the Veterinary Medicine Department of Parma University. The chicks were randomly divided into three homogeneous groups (84 birds/group) and each group was allocated in three pens (28 birds/pen) of identical size in compliance with the organic rearing maximum density recommendation of of 21 $kg/m^2$ (Regolamento di esecuzione UE 2020/464 della commissione del 26 marzo 2020, Allegato 1 parte 4). Indoor temperature and humidity were recorded twice a day and adjustment in heaters activity and air flow were made in order to accomplish with the requirements of the animals. During the first 4 weeks, animals had an UV bulb light of 250 W that kept the temperature into the proper parameter for the growth of the chicks. During the first growing phase the temperature was on average 40°C and then decreased to an average of 25 °C until the end of the trial. An intermittent lightening protocol was applied providing 16 hours of light. All animals had fresh flowing water provided at libitum in the bell-shaped thanks, which were weekly cleaned and daily checked for malfunctions.

## 2. Dietary treatments

A basal organic commercial diet was fed to all the animals, a starter diet was administered until 21 days of age while a grower diet was fed until the end of the trial (21 to 42 days of age). The feedstuff was integrated with 5 g/Kg of macro- and microminerals and trace elements premix. Three dietary treatments were applied on the basal diet: the first treatment was an addition of 0,10 g/Kg of vitamin e to the basal diet (Control), in the other dietary treatments the vitamin e was replaced by two herbal extracts in an amount selected to equalize its antioxidant activity. The second dietary treatment included a mixture of Resinox – Green thea at a dose of 3.65 g/Kg of the feedstuff (RT), the third dietary treatment was a mixture of polyphenols (Oxisteam) at a dose of 0.746 g/Kg of the feedstuff.

The feedstuff was provided *ad libitum* during the whole experiment. A sample of 1 kg was collected from each dietary treatment for each batch of feedstuff preparation. Feed samples were dried at 55 °C to constant weight, thus ground in a Cyclotec mill (Tecator, Herndon, VA, USA) to pass 1 mm screen as described in Simoni et all., (2020). The samples were chemically analysed for humidity, dry matter (DM), ash, nitrogen (N), crude protein (CP), ether extract (EE) and crude fibre (CF). Dry matter was determined drying a sub-sample at 103 °C overnight and ash content was measured gravimetrically by igniting samples in a muffle furnace at 550 °C (Simoni et al., 2020). Humidity was defined by calculation as 100 – DM. The N content was determined by the combustion digestion of the sample at 900 °C in excess of oxygen by Dumatherm® (Gerhardt GmbH

& Co, Königswinter, Germany) as described by (Mihaljev et al., 2015), and CP content was calculated as percentage of N × 6.25. According to the European Commission Regulation No. 152/2009 (European Commission, 2009), EE was determined using the Soxhlet extraction system and starch content was determined by polarimetric method. The CF was assessed using Fiber analysis (Fritted Glass Crucible), AOAC 978.10 (12); ANKOM, American OilChemists' Society (AOCS) Ba 6a-05 (13). The composition of the feedstuffs fed to the animals is presented in Table 2.

**Table 2**. Chemical composition of the feedstuffs fed in the two different breeding phases. The table outlines the chemical composition divided for the first feedstuff called "starter" given up to 21 days of age, and "grower" following 21d to the end of the trial – 42 days of age.

| | | Starter | | | Grower | | |
|---|---|---|---|---|---|---|---|
| [4] | | OXI[1] | RT[2] | VIT E[3] | OXI | RT | VIT E |
| Humidity | % as fed | 10.01 | 10.31 | 9.95 | 8.80 | 8.82 | 8.71 |
| DM | % as fed | 89.99 | 89.69 | 90.05 | 91.20 | 91.18 | 91.29 |
| Ash | %DM | 8.13 | 8.04 | 8.06 | 6.57 | 6.98 | 6.81 |
| N | %DM | 3.88 | 3.93 | 3.96 | 4.10 | 3.79 | 3.66 |
| CP | %DM | 24.25 | 24.59 | 24.73 | 25.62 | 23.69 | 22.84 |
| EE | %DM | 6.83 | 6.66 | 6.77 | 7.37 | 7.54 | 7.08 |
| CF | %DM | 4.90 | 3.82 | 4.61 | 4.53 | 4.03 | 4.42 |

[1]Oxistin (OXI), [2]Resinox-Green thea (RT), [3]Vitamin E (VIT E),

[4]Dry Matter (DM), Nitrogen (N), Crude Protein (CP), Ethereal extract (EE), Crude fibre (CF).

## 3. Health status, body weight, feed and water consumption

During the trial health status was evaluated twice a day and the number of dead animals was recorded. The dead animals were weighted.

Feed (FC) and water consumption (WC) where measured daily during the morning check, by subtracting the amount of feedstuff provided the previous day and the orts after 24 h. While the humidity index of the litter (3 points in each box), pH of the litter (3 point in each box) and temperature of the litter (1 point in each bow) were recorded during the evening check.

Birds body weight (BW) were individually recorded at arrival and then at weekly intervals by using a digital scale. Values of feed consumption and weight gain were used to calculate the feed to gain ratio (FG) and the feed efficiency (FE). FG was calculated dividing the feed consumed with weight of the animals, FE as its analytical opposite.

Mortality was recorded daily, as adjusting the total number of birds determines the total feed intake per bird.

## 4. Organ Weights, Carcass Yield and meat quality

At 42 days, 108 broilers were weighted and identified with a numbered label. They were brought in a commercial slaughterhouse.

The hot carcass, heart, liver, gizzard, proventricolus, duodenum-pancreas, jejunum, ileum, caecum, colon, hearth, spleen, kidneys, liver, bursa of Fabricius as well as the overall intestine were weighted individually with a digital scale. Eviscerated carcasses were weighed, then sectioned to separate thighs, wings, and breast. Each part was weighted individually.

Chicken breast was hand-deboned, and pectoralis major muscles were separated from the breasts to be subjected to meat quality analyses. Three measures of pH were taken at different points of the muscle using a model HD2107.2 Delta Ohm (Delta Ohm, Padova, Italy) pH-meter with a high precision (± 0.002 pH units) and the values were averaged to obtain the final pH. The L*, a*, and b* color indexes (CIE, 1976) were measured after the muscles have been exposed to air for an hour using a Minolta colorimeter (CM-600d, Konica-Minolta Sensing Inc. Ramsey, NJ). To obtain a single value for each sample 3 measurements were performed at different points of the muscle's surface and averaged. Subsequently, a muscle slice (1 × 7 × 3 cm) was cut, weighed, vacuum-packed and cooked in a water bath at 80° C for 1 hour. The sample was removed from the bag, drained from the excess liquid and weighed, thus cooking loss was calculated as difference between the weight of uncooked and cooked sample. Data was integrated using NEXYGEN PLUS 3 software (AMETEK Lloyd Instruments Ltd., West Sussex, UK). Afterwards, each raw sample was ground, and 150 g were used to collect the NIR spectra, whereas 2 falcons have been filled with 50 g of ground meat and stored at -20° C until the analyses for mineral and fatty acid (FA) content have been performed.

### 5. Blood and plasma samples

At the slaughterhouse on each animal after eiagulation 20 ml of whole blood were collected, using two 10 ml Lithium heparin (LH) tube. The LH vials were centrifugated at 3000 rpm for 10 min and the obtained plasma was analysed to obtain the metabolic profile. Hematobiochemical profile was analysed for total protein (colorimetric MP 13/007), albumin (colorimetric MP 13/007), globulin (calculated MP 13/007), albumin/globulin (A/G, calculated MP 13/007), urea (colorimetric MP

13/007), non esterificated fatty acid (NEFA, automatic colorimetric MP 13/003), glucose (colorimetric MP 13/007), total cholesterol (colorimetric MP 13/007), triglyceride (colorimetric MP 13/007), aspartate aminotransferase (AST, colorimetric MP 13/007), gamma glutamyl transferase (GGT, colorimetric MP 13/007), total bilirubin (colorimetric MP 13/007), creatine kinase (CK, colorimetric MP 13/007), calcium (colorimetric MP 13/007), phosphorus (colorimetric MP 13/007), magnesium (colorimetric MP 13/007), beta hydroxybutyrate (colorimetric MP 13/007), cholesterol HDL (colorimetric MP 13/007), creatinine (colorimetric MP 13/007), cholesterol LDL (calculated MP 13/007) and alanine aminotransferase (ALT-GPT, colorimetric MP 13/007).

Analysis were conducted using an ILab 650 automatic multiparametric analyser for clinical biochemistry (Instrumentation Laboratory Company, Lexington, MA, USA) with the reagent from the same industry. All the analysis were conducted at 37 °C.

On 27 selected animals (9 per dietary treatment) an additional 10 ml of blood was collected in an EDTA vials and analysed to obtain haematological profile, particularly: erythrocytes (RBC, flow cytometry MP 13/002), haemoglobin (HB, colorimetric 13/002), mean globular volume (MCV, flow cytometry MP 13/002), mean globular haemoglobin (MCH, flow cytometry 13/002), mean globular haemoglobin concentration (MCHC, flow cytometry MP 13/002), erythrocyte cell distribution width (RDW, flow cytometry 13/002), platelet (PLT, flow cytometry MP 13/002), neutrophil granulocytes (flow cytometry MP 13/002), lymphocytes (flow cytometry MP 13/002), eosinophil granulocytes (flow cytometry MP 13/002), basophil granulocytes (flow cytometry MP 13/002). All the upon cited parameters (RBC, HB, HCT, MCV, MCH, MCHC, RDW, PLT), except WBC and the relative granulocytic formula, were analysed and computed through the automatic multiparametric analyser Sysmex XN1000V with the appropriate IPU software for veterinary clinical haematology. WBC and the relative formula were calculated as percentage after 150 WBC blood smear coloured with hemacolor rapid staining of blood smear (Carlo Erba, Milano, Italy).

## 6. Statistical analysis

Statistical analyses were conducted with the Statistical Package for Social Science (IBM SPSS for Windows Version 26; SPSS GmbH, Munich, Germany). In order to analyze weekly individual cumulative FC, weekly FC, weekly FG and feed efficiency, cumulative feed consumption, cumulative FG and FE and individual cumulative weekly WC were analyzed as repeated measures using the General Linear Model (GLM). The LSD post-hoc test was used in order to compare the treatments. Data about metabolic profiles, hematological profiles, organs weight and carcass yield were analyzed through the multivariate procedures of the GLM. The weekly BW was analyzed with the multivariate procedures of the GLM using the BW at the beginning of the experiment as a covariate. The dietary treatment was used as a fixed effect, while the box replicate was used as random variables. The statically difference was set at $P < 0.05$, while the tendency was set at $0.1 < P < 0.05$.

## Results and Discussion

### Feed consumption

Weekly individual cumulative FC (Table 1), weekly group FC (Table 2) and weekly group cumulative FC (Table 3) were not affected by the treatments during the whole experiment.

Generally, we found a lower FC compared to the weekly individual cumulative FC reported by the Ross 308 breed management handbook (Aviagen, 2014). In fact, our data resulted slightly decreased in the experiment at 7 days of age (97.4 g vs 154 g expected) continuing onto this trend also at 14 days of age (313 g vs 378 g expected), at 21 days of age (619 g vs 634 g expected), at 28 days of age (847 g vs 919 g expected), at 35 days of age (1083 g vs 1189 g expected) and at 42 days of age (1411 g expected – 868.5 g vs). The FC at 42 days of age (868.5 g) is calculated considering that the animals have been under feed restriction 12 hours before the slaughter. This trend is in agreement with the literature (Bakhshalinejad et al., 2018; Scott, 2005) although it has to be considered slightly imprecise due to the fact that, respectively, use different samples intervals (at day 10 – 24 – 42) and do not specifies the broiler breed. Moreover, the fact that no differences in FC has been observed, between the control and the two treatments, may suggest that the latter two did not affected the palatability of the feedstuff (Alenier and Combs, 1981).

**Table 1**. Individual cumulative feed consumption (g) as affected by treatments. 1 (VIT E), 2 (RT) and 3 (OXI) during 42 days of experiment.

| Interval (days) | Treatments 1 | 2 | 3 | SEM | Sig. |
|---|---|---|---|---|---|
| 0-7 | 95.4 | 101.0 | 95.8 | 3.58 | 0.821 |
| 8-14 | 309.3 | 313.7 | 317.0 | 5.81 | 0.891 |
| 15-21 | 601.9 | 638.8 | 617.0 | 8.69 | 0.240 |
| 22-28 | 846.0 | 849.4 | 847.7 | 9.06 | 0.991 |
| 29-35 | 1068.8 | 1077.6 | 1101.4 | 15.25 | 0.723 |
| 36-42 | 865.3 | 864.9 | 875.5 | 14.26 | 0.957 |
| Cumulative | 3786.7 | 3845.4 | 3854.4 | 25.09 | 0.553 |

**Table 2.** Weekly feed consumption (g) as affected by treatments. 1 (VIT E), 2 (RT) and 3 (OXI) during 42 days of experiment.

| Interval (days) | Treatements 1 | 2 | 3 | SEM | Sig. |
|---|---|---|---|---|---|
| 0-7 | 3064.7 | 2717.7 | 2817.7 | 95.31 | 0.354 |
| 8-14 | 8722.0 | 8609.7 | 8987.0 | 162.78 | 0.686 |
| 15-21 | 16951.7 | 17397.7 | 17505.7 | 238.42 | 0.667 |
| 22-28 | 23039.0 | 23538.7 | 23440.0 | 164.31 | 0.482 |
| 29-35 | 29243.7 | 30013.3 | 28279.7 | 417.05 | 0.260 |
| 26-42 | 23589.7 | 23414.7 | 21733.7 | 407.20 | 0.105 |

**Table 3.** Cumulative feed consumption (g) as affected by treatments. 1 (VIT E), 2 (RT) and 3 (OXI) during 42 days of experiment.

| Interval (days) | Treatements 1 | 2 | 3 | SEM | Sig. |
|---|---|---|---|---|---|
| 0-7 | 3064.7 | 2717.7 | 2817.7 | 95.31 | 0.354 |
| 0-14 | 11786.7 | 11327.3 | 11804.7 | 153.74 | 0.408 |
| 0-21 | 25673.7 | 26007.3 | 26492.7 | 360.65 | 0.708 |
| 0-28 | 39990.7 | 40936.3 | 40945.7 | 375.81 | 0.556 |
| 0-35 | 52282.7 | 53552.0 | 51719.7 | 381.93 | 0.123 |
| 0-42 | 52833.3 | 53428.0 | 50013.3 | 769.60 | 0.150 |

**Water consumption**

Water consumption (WC) was not affected by treatments during the experiment as highlighted by Table 6. The ideal WC, at 21 °C, is between 1.6:1/1.8:1 litre/kg of feed consumed, depending on the type of drinking system (Aviagen, 2014). In agreement with the breed management guidelines the WC can be averagely comprehended between the ideal consumption ratio of 1.6:1/1.8:1. However, as stated by Manning et al. (2007), which evaluated the ability of using WC as a welfare parameter, it is difficult to use this parameter to compare site performance from different production systems. In fact the WC is also affected by the losses of the drinking troughs. Therefore, we can define how the trial's WC is comprehended in the literature ranges.

Moreover, the WC reflected also the FC, due to the fact that no differences were observed in the FC there was any effects on WC (Glista and Scott, 1949).

**Table 4.** Individual cumulative weekly water consumption (ml) as affected by treatments. 1 (VIT E), 2 (RT) and 3 (OXI) during 42 days of experiment.

| Interval (days) | Treatments 1 | 2 | 3 | SEM | Sig. |
|---|---|---|---|---|---|
| 0-7 | 418.3 | 326.3 | 334.9 | 39.15 | 0.636 |
| 8-14 | 809.8 | 710.7 | 733.3 | 19.99 | 0.084 |
| 15-21 | 1510.3 | 1668.7 | 1742.4 | 67.10 | 0.405 |
| 22-28 | 1979.0 | 2058.0 | 2087.5 | 65.81 | 0.829 |
| 29-35 | 2235.7 | 2452.0 | 2386.5 | 74.72 | 0.544 |
| 36-42 | 2711.2 | 2909.3 | 3014.0 | 130.96 | 0.693 |
| Cumulative | 9664.3 | 10125.0 | 10298.6 | 166.77 | 0.312 |

**Body weight**

No differences were detected for body weight (BW) as highlighted in Table 5-6-7, with exception for the BW at 21 days of age (Table 7) in which the RT treatment showed lower weight compared with OXI (844.1 and 889.4 respectively; P=0.022) even if they were not different from the VIT E group. Overall, we detected a lower BW (Table 5) than indicated by Ross 308 breed management handbook (Aviagen, 2014). In our, experiment there was a trend at each interval for a lower BW compared to the upon cited literature both at 7 days of age (177.76 g vs 208 g expected), 14 days of age (454.7 g vs 519 g expected), 21 days of age (869.36 g vs 985 g expected), 28 days of age (1385.06 g vs 1573 g expected), 35 days of age (1989.93 g vs 2235g expected) and at 42 days of age (2472.43 g vs 2918 g expected). It is contemplated in literature that high levels of polyphenols could decrease FC or decrease nutrient utilisation (Farahat et al., 2016): being FG and FE at optimal levels, it is conceivable that the treatments did decrease the FC, affecting consequently also the BW. Similarly Md. Abdul Hai Biswas and Masaaki Wakita., (2001), testing Japanese green tea powder (GTP) at 52-d on Shaver red broiler, confirmed that FC on a group basis tended to decrease with GTP feeding (1%), and on the contrary GTP feeding improves feed conversion ratio. The latter author suggested that GTP contain high catechin and may have an inhibitory effect on intestinal absorption of lipid, and therefore prevent an excessive accumulation of lipid reducing the absolute weight.

**Table 5.** Individual weekly body weight (g) as affected by treatments. 1 (VIT E), 2 (RT) and 3 (OXI) during 42 days of experiment.

| | Treatments | | | | |
|---|---|---|---|---|---|
| Days of life | 1 | 2 | 3 | SEM | Sig. |
| 7 | 180 | 174 | 178 | 1.514 | 0.306 |
| 14 | 460 | 441 | 462 | 4.035 | 0.073 |
| 21 | 874 ab | 844 a | 889 b | 6.666 | 0.022 |
| 28 | 1374 | 1376 | 1404 | 10.743 | 0.479 |
| 35 | 1991 | 2000 | 1977 | 17.464 | 0.868 |
| 42 | 2479 | 2487 | 2450 | 26.081 | 0.838 |

BW0=41.8475 used to covariate

**Table 6.** Weekly group body weight gain as affected by treatments. 1 (VIT E), 2 (RT) and 3 (OXI) during 42 days of experiment.

| Interval (days of life) | Treatments | | | SEM | Sig. |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | | |
| 0-7 | 5034 | 4880 | 4947 | 46.36 | 0.451 |
| 8-14 | 7834 | 7566 | 8274 | 225.23 | 0.494 |
| 15-21 | 16621 | 16192 | 16272 | 159.04 | 0.570 |
| 22-28 | 21745 | 21675 | 21250 | 315.84 | 0.831 |
| 29-35 | 33424 | 33798 | 31211 | 690.05 | 0.285 |
| 26-42 | 33078 | 32226 | 32773 | 480.33 | 0.812 |

**Table 7**. Cumulative body weight gain as affected by treatments. 1 (VIT E), 2 (RT) and 3 (OXI) during 42 days of experiment.

| Interval (days of life) | Treatments 1 | 2 | 3 | SEM | Sig. |
|---|---|---|---|---|---|
| 0-7 | 5034 | 4880 | 4947 | 46.36 | 0.451 |
| 0-14 | 12869 | 12446 | 13222 | 211.78 | 0.373 |
| 0-21 | 24455 | 23759 | 24546 | 218.31 | 0.309 |
| 0-28 | 38366 | 37868 | 37522 | 212.58 | 0.298 |
| 0-35 | 55169 | 55473 | 52461 | 748.67 | 0.206 |
| 0-42 | 66502 | 66024 | 63984 | 752.92 | 0.400 |

**Feed to gain and feed efficiency**

Weekly FG and FE were not affected by the treatments (Table 8). The FG ratios were found to be lower, and therefore positive for the feed conversion, compared to those reported both by Esteve-Garcia et al., (2007), confirmed also by Rahimi et al., (2020): in both cases the comparison has been done with the control group and the breed tested was the same used in the present study. Furthermore, Ebrahimzadeh et al., (2018) reported proportional higher FG supplying different increasing doses of grape pomace (5% - 7,5% - 10%) to Ross 308 compared to a vitamin e control. In general, we found a better FG ratio compared to the literature which reported a ratio higher than 1.7 at 0-42 days interval (Farahat et al., 2016; Yeşilbag et al., 2011). Similarly, Md. Abdul Hai Biswas and Masaaki Wakita. (2001) found a tendency for improved FG in GTP feed broilers.

At 42 days of age, cumulative FG ratio resulted increased by RT, as shown in Table 9, demonstrating that the latter group had the lowest FC compared to both VIT E and OXI groups (0.79 vs 0.78 and 0.81, respectively). Indeed, cumulative FE was not affected by the treatments until 35 days of age, while considering the FE of the whole experiment OXI group showed a higher FE (1.28) compared to the RT group (1.24) even if both treatments did not differ from the VIT E (1.26). It appears that the OXI treatment improved the ability in converting feed into body mass during the whole experiment.

**Table 8**. Weekly Feed Gain ratio and Feed efficiency as affected by treatments. 1 (VIT E), 2 (RT) and 3 (OXI) during 42 days of experiment.

| Item | Interval (days of life) | Treatments | | | SEM | Sig. |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | | |
| Feed:gain ratio | 0-7 | 0.61 | 0.56 | 0.57 | 0.016 | 0.443 |
| | 8-14 | 1.11 | 1.14 | 1.09 | 0.016 | 0.557 |
| | 15-21 | 1.02 | 1.08 | 1.08 | 0.021 | 0.508 |
| | 22-28 | 1.06 | 1.09 | 1.11 | 0.017 | 0.573 |
| | 29-35 | 0.88 | 0.89 | 0.91 | 0.009 | 0.521 |
| | 26-42 | 0.71 | 0.73 | 0.66 | 0.014 | 0.143 |
| Feed efficiency | 0-7 | 1.66 | 1.80 | 1.76 | 0.042 | 0.475 |
| | 8-14 | 0.90 | 0.88 | 0.92 | 0.013 | 0.543 |
| | 15-21 | 0.98 | 0.93 | 0.93 | 0.018 | 0.525 |
| | 22-28 | 0.94 | 0.92 | 0.91 | 0.013 | 0.575 |
| | 29-35 | 1.14 | 1.13 | 1.10 | 0.012 | 0.512 |
| | 26-42 | 1.40 | 1.38 | 1.51 | 0.029 | 0.130 |

It has been demonstrated that in broilers the phenotypic expression of low FE and high FG is probably correlated to higher mitochondrial ROS production and protein oxidation (Bottje et al., 2006). The latter author explained that even if it is identified higher ROS production and protein oxidation in broilers with low feed efficiency, it is not exactly known the aetiology of the problem. The higher FE found in the OXI group at 42 days of age may be induced by the hepatoprotective effect of the treatment, which is proven also by the higher ALT values found in plasma (2.92 IU/l), that indicate a higher hepatic mitochondrial activity (Baradaran et al., 2019).

Another positive effects of PFAs and their volatile oils in the digestive system, was confirmed by Yeşilbag et al., (2011), which reported their ability in improving the enzymatic activity helping the feed digestion, and therefore increasing FE and FG. On the contrary, it is reported that including high quantities of dietary polyphenols could decrease FG (Jansman et al., 1994; Sáyago-Ayerdi et al., 2009).

**Table 9.** Cumulative Feed Gain ratio and Feed efficiency as affected by treatments. 1 (VIT E), 2 (RT) and 3 (OXI) during 42 days of experiment.

| Item | Interval (days of life) | Treatments 1 | | 2 | | 3 | | SEM | Sig. |
|---|---|---|---|---|---|---|---|---|---|
| Feed:gain ratio | 0-7 | 0.61 | | 0.56 | | 0.57 | | 0.016 | 0.443 |
| | 0-14 | 0.92 | | 0.91 | | 0.89 | | 0.007 | 0.420 |
| | 0-21 | 1.05 | | 1.10 | | 1.08 | | 0.013 | 0.441 |
| | 0-28 | 1.04 | | 1.08 | | 1.09 | | 0.012 | 0.251 |
| | 0-35 | 0.95 | | 0.97 | | 0.99 | | 0.009 | 0.231 |
| | 0-42 | 0.79 | a | 0.81 | b | 0.78 | a | 0.005 | 0.036 |
| Feed efficiency | 0-7 | 1.66 | | 1.80 | | 1.76 | | 0.042 | 0.475 |
| | 0-14 | 1.09 | | 1.10 | | 1.12 | | 0.008 | 0.408 |
| | 0-21 | 0.95 | | 0.92 | | 0.93 | | 0.011 | 0.461 |
| | 0-28 | 0.96 | | 0.93 | | 0.92 | | 0.011 | 0.257 |
| | 0-35 | 1.06 | | 1.04 | | 1.01 | | 0.009 | 0.232 |
| | 0-42 | 1.26 | ab | 1.24 | a | 1.28 | b | 0.008 | 0.036 |

**Coelomic content weight**

The natural antioxidants did not affect the organs development as reported in Table 10. The relative weight of the coelomic organs, related to BW, of VIT E, RT and OXI respectively are 0.631 % for the heart and 1.724 % for the liver, 0.599 % for the heart and 1.577 % for the liver and 0.614 % for the heart and 1.655 % for the liver. Comparing the upon cited relative weight with the relative organs weight of Ross 308 at 42 days of age of the control diet, Begum et al., (2015) encountered that the liver weight as 2.73% of the BW, while Kokoszyński et al., (2017) report 2.1% for the liver and 0.46% for the heart. Moreover, Çabuk et al., (2006) measured the relative liver weight of the control group (1.83%), of a group with an addition of a mixture of essential oils at 24 mg/kg (1.87%) and group with addition of mixture of essential oils at 48 mg/kg (1.83%), which highlight how the treatment did not affect the hepatic morphology. In addition, Md. Abdul Hai Biswas and Masaaki Wakita,. (2001) tested Japanese green tea powder (GTP) in a group of thirty shaver red broiler breed (52 days of age) relative organs weight: the relative weight of the liver was 2% for the control group, 1.79 % in the group with and addition of 0.5 % GTP, 1.90% in the 0.75 % GTP addition, 1.72% in the 1% GTP addition and 2.14 % in the 1.5 % addition. In the latter study the higher inclusion of GTP determined the lower relative weight of the liver, which is considered as a positive parameter in opposition to hepatomegaly, which could be a sign of adverse reaction to the dietary supplements. In conclusion we can confirm that groups' relative liver weight remained into the literature ranges, which it shows to be the most considered organ for relevance.

Spleen relative weight (0.1 %), compared to Sáyago-Ayerdi et al., (2009) who tested grape pomace (GP) at different dosages (15 g/kg – 30 g/kg – 60 g/kg) on Cobb broilers, is considered into the ranges, respectively 0.11

% control, 0.10 % vitamin E, 0.12 % with the inclusion of 15 g/kg, and 0.11 % both for the 30 g/kg and 60 g/kg. The latter author report also, respect to the trials results respectively, the relative length of jejunum (2.66 % vitamin e vs 2.66 %), ileum (2.64 % vitamin e vs 2.3%) and caecum (0.77 % vitamin e vs 1.25 %) which compared to the trail's relative length, as showed, are considered physiological.

**Table 10.** Coelomic content weight and organs weight (g) after slaughter (42-d of age) as affected by treatments. 1 (VIT E), 2 (RT) and 3 (OXI).

| | Treatments | | | | |
|---|---|---|---|---|---|
| Organs | 1 | 2 | 3 | SEM | Sig. |
| Coelomic content weight (g) | 193.09 | 187.41 | 192.35 | 3.570 | 0.847 |
| Proventriculus (g) | 9.21 | 8.95 | 8.89 | 0.248 | 0.105 |
| Gizzard (g) | 31.37 | 34.38 | 33.32 | 0.732 | 0.641 |
| Duodenum-Pancreas (g) | 14.12 | 14.44 | 14.02 | 0.305 | 0.824 |
| Jejunum (g) | 23.26 | 22.25 | 21.14 | 0.577 | 0.937 |
| Ileum (g) | 19.88 | 19.14 | 20.22 | 0.518 | 0.627 |
| Ciecum (g) | 11.03 | 10.08 | 10.14 | 0.251 | 0.696 |
| Colon (g) | 2.35 | 2.5 | 2.33 | 0.076 | 0.804 |
| Heart (g) | 15.65 | 14.92 | 15.06 | 0.303 | 0.574 |
| Spleen (g) | 2.18 | 2.45 | 2.76 | 0.120 | 0.526 |
| Kidneys (g) | 4.04 | 4.8 | 4.43 | 0.252 | 0.526 |
| Liver (g) | 42.76 | 39.24 | 40.55 | 0.880 | 0.754 |
| Fabricius Bursa (g) | 1.98 | 1.99 | 2.63 | 0.287 | 0.573 |
| Carcass (kg) | 1.56 | 1.55 | 1.56 | 0.025 | 0.672 |
| Total weight after slaughter (kg) | 1.76 | 1.73 | 1.75 | 0.028 | 0.690 |

**Carcass yield**

The natural antioxidants did not affect the carcass yield parameters as defined in Table 11, which included total weight of the carcass, thigh weight, breast weight, pH of the meat, cooking losses of the meat and colour definition.

The pH values agreed with the ranges of values reported by Yeşilbag et al. (2011) which tested the effects of rosemary, rosemary volatile oil and vitamin E at different dosages on Ross 308 at 42 days of age. The upon cited values ranges resulted statistically different between the treatments, specifically ranging between 5.87 on breast meat and 5.90 on the tight meat for rosemary; rosemary oil ranged between 5.89 on breast meat and 5.93 on thigh meat; for the last addition, vitamin e, pH ranges are considered between 5.89 for breast meat and 5.95 for tight meat. Also Starčević et al., (2015) confirm similar values, considering that in his trail were tested different phenolic compounds (thymol, tannic acid and gallic acid) on Ross 308 after 45 min the slaughter, which the tight values were ranging between 5.32 – 5.45 and breast between 5.23 – 5.41.

The colorimetric parameters L*, a and b resulted lower compared to the results reported by Yeşilbag et al. (2011), which described the rosemary addition with L at 64.21, a* -2.27 and b* 12.05. Rosemary oil addition influenced numerically the upon cited parameters L* 61.24, a* -1.08 b* 13.30 while vitamin E led to L* 61.59, a* -3.08 and b* 11.11. It is reported that L* values in meat could be influenced by lipid oxidation (Fernández-López et al., 2003). The inhibitory effect upon lipid oxidation of polyphenols could explain the lower L* values obtained in these samples. Concerning the relative redness, authors have studied the effect of different antioxidants on the colour of meat and meat products (Higgins et al., 1998) and have reported that meat oxidation provokes a decrease in a* values. Furthermore, radicals generated by lipid oxidation promote the accumulation of metamyoglobin (Fernández-López et al., 2003). The increase in b* values observed in the samples could be related to the increase in metamyoglobin, occurred after stocking.

**Table 11.** Carcass yield, meat characteristics and colour as affected by treatments. 1 (VIT E), 2 (RT) and 3 (OXI) during 42 days of experiment.

| | Treatments | | | | |
|---|---|---|---|---|---|
| Item | 1 | 2 | 3 | SEM | Sig. |
| Total weight (g) | 1538.87 | 1528.85 | 1539.47 | 24.99551 | 0.687 |
| Leg-tight weight (g) | 439.39 | 446.71 | 441.48 | 7.49932 | 0.753 |
| Bust weight (g) | 503.55 | 476.81 | 496.22 | 9.45913 | 0.481 |
| pH | 5.8 | 5.76 | 5.79 | 0.00955 | 0.672 |
| Cooking loss | 23.87 | 21.84 | 23.36 | 0.46311 | 0.5 |
| L*[1] | 51.72 | 52.06 | 51.81 | 0.26291 | 0.473 |
| a*[2] | 2 | 2.01 | 1.89 | 0.08215 | 0.628 |
| b*[3] | 9.12 | 9.56 | 8.82 | 0.12452 | 0.097 |

[1]L=lightness. [2]a=relative redness. [3]b=relative yellowness

**Metabolic Profile**

The treatments with both natural antioxidants did not affect most of the analysed parameters, as displayed in Table 12: ranges do not vary from literature, as confirmed by Sugiharto et al., (2021) which evaluated total proteins, albumin, globulin, total cholesterol, HDL, LDL on Loham meat broilers at 35 days of age. Another trial evaluating the same parameters on Ross 308 at 21 days of age gave similar ranges of values (Basmacioglu et al., 2005).

Plasmatic VIT E content was higher in the C group, as expected, compared to both the natural antioxidants treatments (7.29 versus 5.01 and 5.17 mg/l, $P \leq 0.001$).

**Table 12**. Metabolic profile as affected by treatments. 1 (VIT E). 2 (RT) and 3 (OXI) at 42 days of age.

| | Treatments | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | | 2 | | 3 | | SEM | Sig. |
| Vitamin E (mg/l) | 7.29 | b | 5.01 | a | 5.17 | a | 0.159 | <0.001 |
| Total proteins (g/l) | 28.59 | | 29.25 | | 29.37 | | 0.568 | 0.622 |
| Albumin (g/l) | 12.06 | | 12.25 | | 12.16 | | 0.207 | 0.754 |
| Globulins (g/l) | 16.52 | | 17.02 | | 16.78 | | 0.416 | 0.833 |
| Albumin:Globulin | 0.74 | | 2.22 | | 0.72 | | 0.498 | 0.400 |
| Urea (mmol/l) | 0.46 | | 0.41 | | 0.41 | | 0.017 | 0.432 |
| Glucose (mmol/l) | 12.7 | | 12.71 | | 12.14 | | 0.209 | 0.261 |
| Cholesterol (mmol/l) | 3.88 | | 3.86 | | 3.82 | | 0.068 | 0.967 |
| Triglicerid (mmol/l) | 0.32 | | 0.31 | | 0.41 | | 0.031 | 0.337 |
| AST (IU/l) | 318.58 | | 308.14 | | 326.43 | | 10.077 | 0.629 |
| Bilirubin (µmol/l) | 2.38 | | 2.38 | | 2.42 | | 0.061 | 0.964 |
| CreatinK (IU/l) | 9585.72 | | 9474.47 | | 10537.77 | | 609.368 | 0.713 |
| Ca (mmol/l) | 2.27 | | 2.21 | | 2.31 | | 0.030 | 0.462 |
| P (mmol/l) | 2.27 | | 2.28 | | 2.27 | | 0.038 | 0.918 |
| HDL (mmol/l) | 2.62 | | 2.62 | | 2.61 | | 0.036 | 0.960 |
| Creatinin (µmol/l) | 91.98 | b | 63.0 | a | 88.62 | b | 4.073 | <0.001 |
| LDL (mmol/l) | 1.18 | | 1.18 | | 1.16 | | 0.038 | 0.998 |
| ALT (IU/l) | 3.78 | b | 3.17 | ab | 2.92 | a | 0.127 | 0.012 |

Creatinine content was significantly reduced by RT treatment (63.0 µmol/l – 0.7 x $10^{-2}$ g/l) compared to VIT E (91.98 µmol/l – 1.04 x $10^{-2}$ g/l) and OXI (88.62 µmol/l – 1 x $10^{-2}$ g/l). Creatinine ranges are variables in the literature moving from 0.7 x $10^{-2}$ g/l (Ologhobo. 1992) in the control group of a trail on Hubbard, with increasing levels supplying tropical legumes into the diet, to 0.07 x $10^{-2}$ g/l (Sugiharto et al.. 2021), for the control group of Lohmann meat broiler at 35 days of age. Two trials with Ross 308, sampled at respectively at 21 and 42 days of age, reported creatinine to be 0.3 x $10^{-2}$ g/l (Basmacioglu et al., 2005) and 0.2 x $10^{-2}$ g/l (Polat et al., 2011). In human medicine is reported that antioxidants are substances that remove, delay or prevent oxidative damage to target molecules and is strongly demonstrated their protective and curative properties including kidney protection or even reno-prevention effects (Panizo et al.. 2015; Tamadon et al.. 2013). Confirming the same trend also in animal medicine, Khafaga et al. (2019)**,** stated that the consumption of green tea extract by rats can effectively prevent the cell injury in kidneys. We can therefore hypothesize that the inclusion of RT had a reno-protective effect, decreasing the creatinine levels respect the other two treatments. Furthermore, in general, the VIT E and OXI treatment had a worst impact onto the renal district compared to the literature (Basmacioglu et al., 2005; Ologhobo, 1992; Polat et al., 2011; Sugiharto et al., 2021).

The last exception was found in the ALT value where OXI group showed lower values (2.92 IU/l) compared to VIT E (3.78 IU/l). The average level of the latter parameter are widely variables for Ross 308 broilers, going from really high levels of respectively 278 IU/l and 68 IU/l (Chand et al.. 2018; Ologhobo. 1992), to medium levels -respectively 7.3 U/l and 2 U/l (Andretta et al.. 2012; Sugiharto et al., 2021)- or low levels -0.07 U/l (Senanayake et al., 2015)-. The ALT value found in the present trial may be considered within the reported ranges. As stated by Baradaran et al., (2019) antioxidants have the potential to alleviate the adverse effects of oxidative stress in the liver, and therefore it is clear how OXI have an higher hepatoprotective activity respect the other natural treatment and the control group.

**Hematological profile**

Haemoglobin (VIT E 6.55, RT 6.42, OXI 6.49 g/dL) results, are slightly lower than reported in bibliography (Ewa Sosnóka - Czajka. Renata Muchacka. 2005; Etim. 2014) even if any difference was observed among the treatments, while (FI et al., 2018) consider it on the lower cut off of the parameter, as shown in Table 13. On the same trend, the haematocrit (HCT) results were on average 10% points lower than the literature (Andretta et al., 2012; Sugiharto et al., 2021), while it does not show a statistical difference between the treatments: as reported by Scanes (2016) it is nowadays researched in the ratio with Packed Cell Volume (PCV) as indicator of stress. The latter author report that the lesser the ratio is, the more the animal are to be considered stressed: in this line of thought, and coherent with HL parameter, HCT can find its lower levels in the animal’s stress.

**Table 13**. Haematologic profile as affected by treatments. 1 (VIT E). 2 (RT) and 3 (OXI) during 42 days of experiment. 1 (VIT E). 2 (RT) and 3 (OXI) are the three chosen treatments.

| | Treatments | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | SEM | Sig. |
| RBC (M/µL) | 1.81 | 2.74 | 1.73 | 0.32673 | 0.451 |
| Haemoglobin (g/dL) | 6.55 | 6.42 | 6.49 | 0.36468 | 0.899 |
| HCT (%) | 22.63 | 21.88 | 21.43 | 1.30335 | 0.956 |
| MCV (fL) | 125.3 | 125.7 | 125.41 | 0.74713 | 0.907 |
| MCH (pg) | 36.86 | 36.86 | 39.34 | 1.04576 | 0.593 |
| MCHC (g/dL) | 29.34 | 29.28 | 31.23 | 0.67082 | 0.431 |
| RDW (%) | 8.48 | 8.44 | 8.55 | 0.16556 | 0.974 |
| Platelet (K/µL) | 4.87 | 4.56 | 6.37 | 0.87613 | 0.984 |
| Heterophile (%) | 62.88 | 57.89 | 56.43 | 1.64439 | 0.320 |
| Lymphocytes (%) | 33.13 | 37.67 | 40 | 1.65467 | 0.302 |
| Monocytes (%) | 1 | 1.11 | 1 | 0.2724 | 0.995 |
| Eosinophil Granulocytes (%) | 2.75 | 3.22 | 1.71 | 0.5241 | 0.494 |
| Basophil Granulocytes (%) | 0.13 | 0.11 | 1.43 | 0.3759 | 0.183 |

The parameters evaluated were Erythrocytes (RBC), Haemoglobin (HB). Mean globular volume (MCV), Mean globular haemoglobin (MCH), Mean globular haemoglobin concentration (MCHC), Erythrocytic cell distribution width (RDW), Platelet (PLT), Heterophiles, Lymphocytes, Monocyte, Eosinophil granulocytes and Basophil granulocytes.

Concerning MCV, MCH, MCHC and RDW no significant alterations where detected and are within the bibliography ranges (Sugiharto et al., 2021). Heterophile (VIT E: 62.88 %, RT: 57.89%, OXI: 6.43%) and Lymphocyte (VIT E: 33.13 %, RT: 37.67 %, OXI: 40%) ratio is used a precise parameter of poultry stress (Gross WB. Siegel HS. 1983). The stress induced increase in the H:L ratio can be attributed, at least partially, to effects of stress increasing cortisol (Scanes, 2016) and it is influenced by *E.coli* endotoxin challenge and fasting (Gross and Siegel. 1983), feed restriction (Najafi et al., 2015), heat stress (Prieto and Campo., 2010), lighting sources (Huth and Archer., 2015), shackling (Bedánová et al., 2006a) and transportation stress (Maxwell. 1993; Al-Murrani et al., 1997; Al-Aqil et al., 2013). It is reported to be normal a ratio of $0.33 < H:L < 0.35$ *in vivo* between heterophiles (25.0 ± 2.3) and lymphocytes (65.8 ± 2.6) (Scanes, 2016). Another author reports H:L to be = 0.39 ± 0.02 at 42 days of age in Ross 308 (Gross and Siegel, 1983). In our trial HL ratio is reported to be 1.89. However, high level of stress may be due to the sampling at the slaughterhouse after the transport of the animals and therefore the feed restriction and shackling. The other haematological parameters did not differ from the control groups in literature (Andretta et al., 2012; Talebi et al., 2005).

**Conclusions**

Plant Feed Additives as natural alternatives to synthetic Vitamin E in organic broiler farming appear as an innovative and functional way to accomplish with the organic farming regulations, in order to substitute the use of synthetic vitamins, which are considered contentious inputs allowed by the EU organic disciplinary.

No major variation in health status, feed and water consumption, performances, organs development, carcass yield/quality, haematological profile, were observed in broilers in the present trial, comparing the effect of hydrolysed polyphenols, a mixture of natural antioxidants and vitamin e. However, the hydrolysed polyphenols improved the overall efficiency of feed conversion in the whole trial.

From a metabolic point of view, decreased creatinine levels suggested a possible reno-protective effect of hydrolysed polyphenols from wood. Furthermore, statistically increased ALT values allow to hypothesize a hepato-protective activity of the mixture of natural antioxidant. It can be concluded that the trial confirmed the results obtained through *in vitro* studies and set “stones” for further usage of the natural extracts in livestock farming.

It is of importance that the general evaluations performed in the present trial must be completed by specific *in vivo* and *post-mortem* tests on the antioxidant status of blood and tissues are to be addressed, and the use these natural antioxidants must be preceded by an accurate evaluation of their antioxidant capacity/activity, especially in the case of not standardised natural extracts.

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