

## Prebiotics, probiotics and thyme (*Thymus vulgaris*) for broilers: performance, carcass traits and blood variables<sup>□</sup>

*Prebióticos, probióticos y tomillo (*Thymus vulgaris*) para pollo de engorde: rendimiento, características de la canal y variables sanguíneas*

*Prebióticos, probióticos e tomillo (*Thymus vulgaris*) para frangos de corte: desempenho, características da carcaça e variáveis sanguíneas*

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### Abstract

**Background:** a stressful environment predisposes broilers to various diseases. Probiotics, prebiotics and essential oils can be used as an alternative to antibiotics in poultry production. **Objective:** to evaluate the effects of the Fermacto (prebiotic), Bioplus 2B (probiotic), and thyme essential oil on growth, organs and carcass traits, and hematology of “Ross” broiler chicks during 42 days. **Methods:** five-hundred sixty broilers were randomly assigned to seven dietary treatment groups (with 20 broilers in each group), with four repetitions. The diets contained 1 or 2 g/Kg Fermacto, 1 or 2 g/Kg Bioplus 2B, and 0.5 or 1 g/Kg thyme essential oil. A control group was also included (without additives). **Results:** when compared with the control group, Fermacto, Bioplus 2B, and thyme essential oil resulted in an increase in body weight gain and feed intake. The feed conversion ratio (FCR) was enhanced by 2 g/Kg Fermacto. Thyme essential oil at 1 g/Kg also improved FCR but it led to a reduction in the relative weight of thighs and wings. Limited differences were observed in blood parameters between groups. **Conclusion:** Fermacto, Bioplus 2B and thyme essential oil improved the average daily gain of broilers and had a limited effect on carcass, organs and plasma constituents.

**Keywords:** *feed additive, feed conversion, poultry nutrition, weight gain.*

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## Resumen

**Antecedentes:** un ambiente estresante predispone las aves de engorde a diferentes enfermedades. Los probióticos, prebióticos y aceites esenciales pueden ser una alternativa a los antibióticos en la producción de pollos de engorde. **Objetivo:** evaluar el efecto del Fermacto (prebiótico), del Bioplus 2B (probiótico) y del aceite esencial de tomillo sobre el crecimiento, las características de la canal y de los órganos, y parámetros hematológicos de pollos de engorde “Ross” durante 42 días. **Métodos:** quinientos sesenta pollos de engorde fueron asignados aleatoriamente a siete grupos dietarios (con 20 pollos en cada grupo), con cuatro repeticiones. Las dietas contenían 1 o 2 g/Kg de Fermacto, 1 o 2 g/Kg de Bioplus 2B y 0.5 o 1 g/Kg de aceite esencial de tomillo. También se incluyó un grupo control (sin aditivos). **Resultados:** en comparación con el grupo control, el Fermacto, el Bioplus 2B y el aceite esencial de tomillo aumentaron la ganancia de peso corporal y la ingesta de alimento. El índice de conversión alimenticia (FCR) se vio mejorado con 2 g/Kg de Fermacto. La suplementación con 1 g/Kg de aceite esencial de tomillo causó el aumento del FCR, pero al mismo tiempo redujo el peso relativo de los muslos y de las alas. Se observaron pocas diferencias en las variables sanguíneas entre los grupos. **Conclusión:** Fermacto, Bioplus 2B y el aceite esencial de tomillo mejoraron la ganancia diaria de peso de los pollos de engorde, pero con un efecto limitado sobre las características de la canal, de los órganos y de los constituyentes del plasma.

**Palabras clave:** *aditivo alimenticio, conversión alimenticia, ganancia de peso, nutrición de pollos.*

## Resumo

**Antecedentes:** o stresse ambiental é um fator que predispõe a numerosas doenças em frangos de corte. Os probióticos, prebióticos e óleos essenciais podem ser uma alternativa viável ao uso de antibióticos na produção de frangos. **Objetivo:** avaliar o efeito do Fermacto (prebiótico), Bioplus 2B (probiótico) e óleo essencial de tomillo no desempenho zootécnico e nos parâmetros hematológicos em frangos “Ross”, durante 42 dias. **Métodos:** quinhentos e sessenta pintos foram aleatoriamente distribuídos por sete grupos (com 20 frangos em cada grupo), com quatro repetições. Foram usadas dietas contendo 1 ou 2 g/Kg de Fermacto, 1 ou 2 g/Kg de Bioplus 2B e 0.5 ou 1 g/Kg de óleo essencial de tomillo. Um grupo controle também foi incluído (sem aditivos). **Resultados:** em comparação com o grupo controle o Fermacto, o Bioplus 2B e o óleo essencial de tomillo aumentaram o ganho de peso e o consumo da ração. O índice de conversão alimentar (FCR) foi influenciado pela adição de 2 g/Kg de Fermacto. A suplementação da ração com 1 g/Kg de óleo essencial de tomillo aumentou o FCR e reduziu o peso relativo das coxas e das asas na carcaça. Não se observaram diferenças significativas entre grupos para os parâmetros da bioquímica sérica. **Conclusão:** as suplementações da dieta com Fermacto, Bioplus 2B e óleo essencial de tomillo melhoram o ganho de peso diário, mas seu efeito sobre as características da carcaça e os parâmetros bioquímicos do plasma sanguíneo foi limitado.

**Palavras chave:** *aditivo alimentar, ganancia peso, nutrição de aves, taxa de conversão.*

## Introduction

In the broiler industry, birds are often reared in confinement, which can predispose them to various diseases. Feed additives such as antibiotics, growth promoters and anti-coccidial drugs are often used to treat diseases, enhance performance and reduce losses. However, the routine use of antibiotics in the diet of broilers is now considered to cause an increase in antimicrobial resistance of human and animal bacteria. Hence, there is a growing trend to use probiotics and prebiotics as an alternative to growth promoting antibiotics (Griggs and Jacobs, 2005; Dhama et al., 2015). Unfortunately, contradictory results have been reported on the effect of these supplements on broiler performance (Cross et al., 2007; Nayebpor et al.,

2007; Willis et al., 2007; Aami-Azghadi et al., 2010; Rahimi et al., 2011; Amirdahri et al., 2012).

The purpose of our study was to simultaneously study the effect of two doses each of a prebiotic (Fermacto), a probiotic (Bioplus 2B) and thyme essential oil on growth, carcass, organ characteristics, and hematology parameters of broiler chicks. Fermacto (PetAg Inc., Hampshire, IL, USA) is derived from the fermentation of *Aspergillus orizae*. Fermacto enhances digestive efficiency and beneficial microflora of the gastrointestinal tract of broilers, and is, therefore, considered as a prebiotic (Ghyasi et al., 2008; Navidshad et al., 2010). Bioplus 2B is a probiotic (Chr. Hansen A/S, Boege Alle, Horsholm, Denmark) containing both *Bacillus licheniformis* and

*B. subtilis* and is thought to increase the amounts of digestive enzymes (such as proteases, lipases and amylases) in the small intestine of broilers leading to better digestion of nutrients (Šabatková *et al.*, 2008; Fallah *et al.*, 2013). The essential oil of *Thymus vulgaris* (Thymol) is also considered to increase the production of digestive enzymes (Khan *et al.*, 2012; Feizi *et al.*, 2013).

## Materials and methods

### Ethical considerations

All procedures used in this study were approved by the Animal Ethics Committee of the Islamic Azad University, Rasht Branch, Iran, and were in agreement with Directive 2010/63/EU.

### Location

The experiment was conducted at a commercial poultry farm (Somesara city, Guilan, Iran, latitude 37°18'15"N, longitude 49°19'2"E).

### Birds and management

A total of 140 mixed Ross 308 broilers with similar body weight ( $44.0 \pm 1.3$  g) were randomly assigned to seven treatment groups of 20 birds/group. The experiment was repeated four times and hence the total number of birds used in the study was 560. The broilers were caged in land cages ( $2.0 \times 1.0 \times 0.5$  m) and treatment groups were equally distributed between first, middle, and end positions to minimize the cage effect. All broilers were housed in a windowless and environmentally controlled room, with room temperature kept between 32 to 22 °C as appropriate for the age of the bird. The light cycle was maintained for 23 h/d during the rearing period. Each cage was equipped with an individual feeder and nipple drinker. Broilers in a cage were not able to consume feed assigned to the adjoining cage. Dietary treatments were fed from day 1 to day 42 of age. The birds were vaccinated against infectious bronchitis (1 and 7 days of age), Newcastle disease (1, 7, 20, and 30 days of age), avian influenza (1 day of age), and Gumboro disease (16 days of age).

### Feed

A two-phase feeding program was used; grower feed was used from 1 to 21 days and finisher feed from 22 to 42 days. Ingredient and nutrient composition of diets are shown in Table 1. Feed and water were supplied *ad libitum* throughout the experimental period. The diets met or exceeded Ross 308 catalogue recommendations (Ross, 2007). The birds in the control group were given a basal diet while those in treatment groups were supplemented with the additives, as follows:

Treatment 1: basal diet (control).

Treatment 2: basal diet + Fermacto powder (1 g/Kg).

Treatment 3: basal diet + Fermacto powder (2 g/Kg).

Treatment 4: basal diet + Bioplus 2B powder (1 g/Kg).

Treatment 5: basal diet + Bioplus 2B powder (2 g/Kg).

Treatment 6: basal diet + thyme essential oil (0.5 g/Kg).

Treatment 7: basal diet + thyme essential oil (1 g/Kg).

**Table 1.** Feed ingredients and nutrient analysis of grower (1 to 21 days of age) and finisher (22 to 42 days of age) diets.

	Grower diet	Finisher diet
<i>Ingredient (g/Kg)</i>		
Corn	543.2	586.9
Soybean meal	394.3	318.7
Mineral oysters	9.0	7.9
Corn oil	21.6	58.3
Dicalcium phosphae	20.5	16.8
Sodium chloride	3.7	3.7
DL-methionine 98%	2.0	2.2
Lysine-hydro-chloride	0.7	0.5
Vitamin mixture <sup>1</sup>	2.5	2.5
Mineral mixture <sup>2</sup>	2.5	2.5
<i>Calculated chemical composition</i>		
Metabolisable energy (MJ/Kg)	12.12	13.38
Crude protein (g/Kg)	221.6	192.0
Calcium (g/Kg)	10.0	8.5
Available phosphorus (g/Kg)	5.0	4.2
Lysine (g/Kg)	11.5	9.6
Methionine (g/Kg)	5.0	4.8
Methionine + Cystine (g/Kg)	8.3	7.8
Threonine (g/Kg)	7.9	7.1

<sup>1</sup> Supplied per Kg of mixture: 1,081 mg *trans*-retinol; 20 mg cholecalciferol; 4 mg  $\alpha$ -tocopherol acetate; 800 mg menadione; 720 mg thiamine; 2,640 mg riboflavin; 4,000 mg niacin; 12,000 mg calcium pantothenate acid; 1,200 mg pyridoxine; 400 mg folic acid; 6 mg cyanocobalamin; 40 mg biotin; 100,000 mg choline; 40,000 mg antioxidant.

<sup>2</sup> Supplied per Kg of mixture: 39,680 mg manganese; 20,000 mg iron; 33,880 mg zinc; 4,000 mg copper; 400 mg iodine; 80 mg selenium; 1 mg excipient.

### Carcass and blood characteristics

The amount of feed consumed and weight gain were recorded by pen, followed by calculation of feed conversion ratios. At day 42, one bird per group was selected for blood collection and carcass evaluation; thus, a total of four birds per treatment (one bird/treatment/replicate) were selected for this purpose. Blood samples (1 mL/bird) were collected into EDTA tubes from the wing veins. After centrifugation (3000 g x 10 min at room temperature), plasma was harvested and stored at -20 °C until assayed. Glucose, cholesterol, triglycerides, and high-density lipoproteins (HDL) were determined as reported by Shabani *et al.* (2015). These selected birds were then euthanized and eviscerated, and the weight of carcass, organs and body parts was recorded.

### Statistical analysis

A cage was the experimental unit for performance traits while the individual bird was the experimental unit for carcass and organ characteristics, and hematological traits. The model assumptions of normality and homogeneity of variance were tested using Shapiro Wilk and Levene tests, respectively. Data were analyzed by the ANOVA option of the general linear model of SAS/STAT software (SAS Institute Inc., Cary, NC, USA) as a completely randomized design with dietary additive as the main effects. The statistical model used was:

$$Y_{ijk} = \mu + T_i + R_{ij} + \varepsilon_{ijk}$$

Where:

$Y_{ijk}$  = response variables from each individual replication or pen.

$\mu$  = overall mean.

$T_i$  = effect of dietary additive.

$R_{ij}$  = inter-experimental unit (replications) error term.

$\varepsilon_{ijk}$  = intra-experimental unit error term.

Means were compared for significant differences using the LSMEANS option of SAS version 8 (SAS

Institute Inc., Cary, NC, USA). Statistical significance was established at  $p \leq 0.05$ .

### Results

The effects of feed additives on growth performance are shown in Table 2. During the entire experimental period, the broilers fed diets supplemented with Fermacto, Bioplus 2B and thyme essential oil presented higher daily weight gain than broilers fed the control diet ( $p < 0.05$ ), reflecting the results obtained during the finisher period. During the entire experimental period, the broiler fed diets supplemented with additives showed higher feed intake than broilers fed the control diet ( $p < 0.05$ ). Considering the different treatments, the highest feed intake was recorded in animals fed diets supplemented with thyme essential oil at 1 g/Kg ( $p < 0.05$ ). Also in this case, these results reflect those obtained during the finisher period. Conversely, during the grower period, broilers fed control diet had higher feed intake than broilers fed diets supplemented with Fermacto 2 g/Kg, Bioplus 2B and thyme essential oil ( $p < 0.05$ ). During the entire experimental period, the feeding of thyme essential oil and Fermacto at the highest level, 1 and 2 g/Kg, respectively, improved the feed conversion ratio compared to animals fed the control diet ( $p < 0.05$ ). However, feed additives did not affect live body weights at slaughter (Mean  $\pm$  SE; 2,627.1  $\pm$  91.90 g;  $p > 0.05$ ) nor did they affect the empty abdomen carcass weight (1,647.5  $\pm$  63.76 g;  $p > 0.05$ ) or the eviscerated carcass percentage (62.4  $\pm$  0.01%;  $p > 0.05$ ; data not shown).

The relative weights of organs and body parts are shown in Table 3. Feeding of thyme essential oil at 1 g/Kg reduced the relative weight of thighs, wings and notarium ( $p < 0.05$ ) of carcasses compared to the control group. The relative weight of thighs and wings increased, reducing the level of thyme essential oil from 1 to 0.5 g/Kg ( $p < 0.05$ ). Compared with the control group, an effect of dietary additives on relative weight of breast, pancreas and gizzard was not found ( $p > 0.05$ ). However, broiler chickens fed thyme essential oil at 1 g/Kg had lower weight of pancreas and gizzard than those fed with Bioplus 2B at 1 and 2 g/Kg, respectively ( $p < 0.05$ ). Broiler chickens fed thyme essential oil at 1 g/Kg showed

**Table 2.** Effect of different levels of prebiotic (PRE), probiotic (PRO), and thyme (THY) essential oil supplementation on daily weight gain (BWG; g/chick/day), feed intake (FI, g/chick/duration), and feed conversion ratio (FCR) of Ross 308 broilers.

	Age (day)	Dietary treatment						SEM <sup>2</sup>	
		CON <sup>1</sup>	PRE 1 g/Kg	PRE 2 g/Kg	PRO 1 g/Kg	PRO 2 g/Kg	THY 0.5 g/Kg		THY 1 g/Kg
BWG	1-21	33.67 <sup>a</sup>	33.28 <sup>ab</sup>	31.87 <sup>b</sup>	30.07 <sup>c</sup>	32.56 <sup>ab</sup>	32.39 <sup>ab</sup>	32.31 <sup>ab</sup>	0.48
	22-42	83.37 <sup>b</sup>	97.49 <sup>a</sup>	102.51 <sup>a</sup>	98.07 <sup>a</sup>	98.81 <sup>a</sup>	101.34 <sup>a</sup>	106.71 <sup>a</sup>	3.51
	1-42	58.52 <sup>b</sup>	65.38 <sup>a</sup>	67.19 <sup>a</sup>	64.07 <sup>a</sup>	65.69 <sup>a</sup>	66.86 <sup>a</sup>	69.51 <sup>a</sup>	1.83
FI	1-21	1072.3 <sup>a</sup>	1036.5 <sup>ab</sup>	1006.0 <sup>b</sup>	946.0 <sup>c</sup>	1027.5 <sup>b</sup>	1006.3 <sup>b</sup>	1015.0 <sup>b</sup>	12.09
	22-42	3650.0 <sup>d</sup>	3912.8 <sup>c</sup>	3996.3 <sup>bc</sup>	3916.3 <sup>c</sup>	3986.3 <sup>bc</sup>	4038.5 <sup>ab</sup>	4140.0 <sup>a</sup>	41.40
	1-42	4722.3 <sup>d</sup>	4949.3 <sup>bc</sup>	5002.3 <sup>bc</sup>	4865.3 <sup>c</sup>	5013.8 <sup>b</sup>	5090.0 <sup>ab</sup>	5155.0 <sup>a</sup>	44.89
FCR <sup>3</sup>	1-21	1.52 <sup>a</sup>	1.48 <sup>b</sup>	1.50 <sup>ab</sup>	1.50 <sup>ab</sup>	1.50 <sup>ab</sup>	1.47 <sup>b</sup>	1.49 <sup>ab</sup>	0.008
	22-42	2.09 <sup>a</sup>	1.91 <sup>ab</sup>	1.86 <sup>b</sup>	1.90 <sup>b</sup>	1.92 <sup>ab</sup>	1.92 <sup>ab</sup>	1.86 <sup>b</sup>	0.054
	1-42	1.92 <sup>a</sup>	1.80 <sup>ab</sup>	1.77 <sup>b</sup>	1.81 <sup>ab</sup>	1.82 <sup>ab</sup>	1.81 <sup>ab</sup>	1.77 <sup>b</sup>	0.039

<sup>1</sup> CON = control diet.

<sup>2</sup> SEM = standard error of the mean.

<sup>3</sup> FCR values were obtained as FI/BWG of the period considered.

Means with different superscript letters (<sup>a, b, c, d</sup>) within the same row differ significantly ( $p < 0.05$ ).

lower relative weight of breast than animals fed with 1 g/Kg of Fermacto, Bioplus 2B at 2 g/Kg and thyme essential oil at 0.5 g/Kg ( $p > 0.05$ ). Considering the relative weight of liver and bile, broilers fed 1 g/Kg of Fermacto presented lower value than those showed by broilers fed the control diet ( $p < 0.05$ ). There were no differences among experimental groups in relative weight of spleen, hearth and abdominal fat ( $p > 0.05$ ).

There was no significant difference among groups in the plasma levels of glucose, cholesterol, triglyceride, alkaline phosphatase and uric acid (Table 4). However, high-density lipoprotein (HDL) cholesterol was significantly ( $p < 0.05$ ) lower in the diet containing Fermacto 1 g/Kg as compared to the control diet and the diet containing thyme essential oil at 1 g/Kg.

## Discussion

Several authors have argued that the effect of additives on broiler performance will increase if rearing conditions, such as hygiene and dietary energy are not appropriate (Rahimi and Khaksefidi,

2006; Feizi *et al.*, 2013). However, in the present trial, the mean daily weight gain recorded was in line with the Ross 308 performance objective (Ross, 2012) and with that reported by Ebrahimi *et al.* (2013). It is interesting to note that, despite all three additives affect digestion and health status of broilers, the birds in all experimental treatments achieved similar average daily weight gain at the end of the experimental period, which were higher than that of the control group. The different effect of additives during the grower and finisher periods could be due to different diet, especially in terms of protein, and to the development of the digestive tract, enhancing feed digestibility during the second part of the trial (Ocak *et al.*, 2008). In agreement with the present study, Falaki *et al.* (2011) reported a 9% increase in slaughter weight and improvement in feed conversion of broilers fed 2 g/Kg Fermacto. However, they did not find these effects when Fermacto was added at 1 g/Kg level. Šabatková *et al.* (2008) reported that Bioplus 2B, even at a level lower than 1 g/Kg, resulted in a 4 to 5% improvement of weight gain and feed conversion. Our results are also in agreement with those by Feizi *et al.* (2013) who suggested that thyme extract promoted weight gain of broilers.

**Table 3.** Effect of different levels of prebiotic (PRE), probiotic (PRO), and thyme (THY) essential oil supplementation on relative weights of carcass cut (%) and organs (%) of Ross 308 broilers.

	Dietary treatment							SEM <sup>2</sup>
	CON <sup>1</sup>	PRE 1 g/Kg	PRE 2 g/Kg	PRO 1 g/Kg	PRO 2 g/Kg	THY 0.5 g/Kg	THY 1 g/Kg	
Breast	21.70 <sup>ab</sup>	22.38 <sup>a</sup>	21.68 <sup>ab</sup>	21.06 <sup>ab</sup>	23.22 <sup>a</sup>	23.91 <sup>a</sup>	18.91 <sup>b</sup>	0.006
Drumsticks (thighs)	18.92 <sup>a</sup>	18.58 <sup>a</sup>	18.09 <sup>a</sup>	18.45 <sup>a</sup>	17.53 <sup>ab</sup>	18.62 <sup>a</sup>	15.59 <sup>b</sup>	0.002
Wings	5.64 <sup>ab</sup>	6.15 <sup>a</sup>	5.61 <sup>ab</sup>	5.50 <sup>abc</sup>	5.32 <sup>bc</sup>	5.86 <sup>ab</sup>	4.86 <sup>c</sup>	0.006
Pancreas	0.22 <sup>ab</sup>	0.25 <sup>ab</sup>	0.20 <sup>ab</sup>	0.27 <sup>a</sup>	0.24 <sup>ab</sup>	0.21 <sup>ab</sup>	0.19 <sup>b</sup>	0.00011
Liver and bile	2.76 <sup>ab</sup>	2.28 <sup>c</sup>	2.93 <sup>a</sup>	2.60 <sup>abc</sup>	2.46 <sup>bc</sup>	2.59 <sup>abc</sup>	2.35 <sup>bc</sup>	0.001
Spleen	0.14	0.15	0.13	0.13	0.11	0.13	0.14	0.0001
Hearth	0.62	0.56	0.57	0.59	0.63	0.55	0.52	0.004
Gizzard	2.66 <sup>ab</sup>	2.61 <sup>ab</sup>	2.56 <sup>ab</sup>	2.40 <sup>ab</sup>	2.75 <sup>a</sup>	2.46 <sup>ab</sup>	2.14 <sup>b</sup>	0.001
Back thoracic vertebrae (notarium)	13.92 <sup>a</sup>	13.59 <sup>a</sup>	13.34 <sup>ab</sup>	13.96 <sup>a</sup>	12.50 <sup>ab</sup>	13.20 <sup>ab</sup>	11.41 <sup>b</sup>	0.006
Abdominal fat	1.65	1.48	1.41	1.77	1.38	1.41	1.31	0.002

<sup>1</sup> CON = control diet.<sup>2</sup> SEM = standard error of the means.Means with different superscript letters (<sup>a, b, c</sup>) within the same row differ significantly (p<0.05).**Table 4.** Effect of different levels of prebiotic (PRE), probiotic (PRO), and thyme (THY) essential oil supplementation on plasma constituents of Ross 308 broilers.

	Dietary treatment							SEM <sup>2</sup>
	CON <sup>1</sup>	PRE 1 g/Kg	PRE 2 g/Kg	PRO 1 g/Kg	PRO 2 g/Kg	THY 0.5 g/Kg	THY 1 g/Kg	
Glucose (mg/dl)	170.5	156.3	139.3	131.1	131.1	131.1	131.2	16.01
Total cholesterol (mg/dl)	137.9	131.1	123.1	126.5	126.7	126.7	127.0	6.86
Triglycerides (mg/dl)	108.3	102.2	88.3	77.9	78.2	78.2	78.5	15.06
Alkaline phosphatase (U/dl)	559.4	556.2	609.3	523.0	523.0	523.0	523.0	36.11
Uric acid (mg/dl)	6.23	5.70	5.77	4.71	4.71	4.71	4.71	0.88
HDL Cholesterol (mg/dl)	77.23 <sup>a</sup>	57.91 <sup>b</sup>	68.38 <sup>ab</sup>	67.76 <sup>ab</sup>	65.62 <sup>ab</sup>	69.37 <sup>ab</sup>	75.21 <sup>a</sup>	4.47

<sup>1</sup> CON = control diet.<sup>2</sup> SEM = standard error of the mean.Means with different superscript letters (<sup>a, b</sup>) within the same row differ significantly (p<0.05).

On the other hand, our results differ from those by Cross *et al.* (2011), who did not find any effect of thyme oil on broiler performance.

The control group presented similar relative weight of breast as the experimental groups. This is in agreement with the findings by Aami-Azghadi *et al.* (2010) and Navidshad *et al.* (2010); similar amino acids uptake could be hypothesized between control and experimental groups (Rostagno *et al.*, 1995). In the present study, feeding thyme essential oil at 1 g/Kg reduced relative weight of thighs, wings and back thoracic vertebrae, but did not influence the relative weight of internal organs, which is in agreement with the results obtained by Najafi and Toriki (2010) and Pourmahmoud *et al.* (2013). These authors found that supplementation of broilers diets with thyme essential oil (0.2 g/Kg) or thyme extract (up to 6 g/Kg) had no effect on the relative weight of internal organs. Ayoola *et al.* (2014) reported that up to 30 g/Kg thyme leaf inclusion in broiler diets did not influence the weight of thighs and wings.

As expected, a limited effect of feed additives on relative weight of liver, bile and spleen was observed. The overgrowth of liver and spleen may indicate a health problem; in general, our results indicate that the probiotic, prebiotic and essential oil used are not deleterious to broiler health. The relative weight of abdominal fat was similar among experimental groups indicating that, in agreement with plasma results, the additives did not influence fat accumulation. Consequently, the lack of difference in the relative weight of abdominal fat among experimental groups could reflect a similar live weight at slaughter. Abdollahi *et al.* (2003), Falaki *et al.* (2011), and Sadeghi *et al.* (2012) also observed similar results.

A limited effect of dietary additives on plasma constituents was observed. Lee *et al.* (2004) discussed that essential oils could inhibit hepatic 3-hydroxy-3-methylglutaryl coenzyme A -which is involved in cholesterol synthesis- while Rahimi and Khaksefidi (2006) reported that probiotics are able to reduce cholesterol absorption and synthesis in the gastrointestinal tract of broilers. However, these hypotheses do not seem to be confirmed in our study, which is in agreement with results by Sedghi *et al.*

(2010), who supplemented broiler diet with 2 g/Kg Fermacto. Our results differ with those by Bölükbaşı *et al.* (2006), who showed an increase of serum triglyceride and HDL in broilers fed 1 g/Kg thyme oil, and with those by Ebrahimi *et al.* (2012), who showed that probiotics reduce serum cholesterol in broilers.

In conclusion, prebiotic (Fermacto), probiotic (Bioplus 2B) and thyme essential oil improved the average daily weight gain of broilers from 1 to 42 days of age with a limited effect on carcass characteristics and plasma constituents.

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### Conflicts of interest

The authors declare they have no conflict of interest with regard to the work presented in this report.

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