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Edible mushroom powder (*Agaricus bisporus*) and flavophospholipol improve performance and blood parameters of broilers¤

Champiñón en polvo (Agaricus bisporus) y flavofosfolipol mejoran el desarrollo y parámetros sanguíneos en pollos de engorde

O uso de champignon em pó (Agaricus bisporus) e flavofosfolipol melhoram o desenvolvimento e parâmetros sanguíneos em frangos de corte

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Summary

Background: flavophospholipol is an antibiotic growth promoter (AGP). The current ban of AGP in some countries is controversial because their benefits on the environment and economy by saving feed and reducing nitrogen excretion have been overlooked. White button mushrooms have important nutritional properties and the industry discards large quantities of waste that could be fed to animals. **Objective:** to evaluate the effect of dietary inclusion of five levels of edible mushroom powder (EMP) and flavophospholipol on the performance and blood serum metabolites of broilers. **Methods:** a total of 300 one-day-old male broiler chicks were randomly distributed into 10 treatments with three replicates of 10 chicks per pen. The experiment consisted of a factorial arrangement (2x5 treatments) with five inclusion levels of EMP supplementation (0, 0.5, 1.0, 1.5, and 2.0 g/kg of diet) and the addition of 0 or 5 mg/kg of flavophospholipol. **Results:** supplementation with EMP and flavophospholipol, as individual factors, had a negative effect on feed intake, but positively affected broiler weight gains and feed conversion ratio. Antibiotic supplementation increased uric acid concentration and, as an interaction with mushroom powder, reduced serum triglycerides, and very low density lipoprotein cholesterol (VLDL). The EMP also affected serum concentration of total cholesterol. **Conclusion:** the two substances studied, but not their combination, had a positive effect on growth performance of chickens that could be translated into economic benefits.

Keywords: *antibiotic, blood chemical analysis, fungi, poultry.*

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Resumen

Antecedentes: el flavofosfolipol es un antibiótico promotor del crecimiento (AGP). La actual prohibición de los AGP en muchos países es polémica porque sus beneficios económicos y medioambientales a través del ahorro en pienso y la disminución en la producción de heces y nitrógeno son pasados por alto. El champiñón común tiene importantes propiedades nutricionales y la industria desecha grandes cantidades de subproducto que podrían ser aprovechables en la alimentación de animales. **Objetivo:** evaluar el efecto de cinco niveles de champiñón en polvo (EMP) y flavofosfolipol sobre el desarrollo y metabolitos sanguíneos en suero de pollos de engorde. **Métodos:** trescientos pollos de engorde machos de un día de vida fueron distribuidos aleatoriamente en 10 grupos en función del tratamiento con tres réplicas de 10 animales. El diseño experimental consistió en un arreglo factorial (2x5 tratamientos), incluyendo cinco concentraciones de champiñón en polvo (0, 0,5, 1,0, 1,5 2,0 g/kg de alimento) y la adición de 0 o 5 mg/kg de flavofosfolipol. **Resultados:** la suplementación con EMP y flavofosfolipol, individualmente, tiene un efecto negativo en la ingestión diaria de alimento, pero positivo sobre la ganancia de peso vivo y el índice de conversión. La suplementación con antibióticos aumenta la concentración de ácido úrico en sangre e, interaccionando con el champiñón en polvo, disminuye la concentración sérica de triglicéridos y de colesterol de muy baja densidad (VLDL). El EMP también afectó la concentración sérica de colesterol total. **Conclusiones:** las dos sustancias estudiadas, pero no su combinación, tuvieron un efecto positivo sobre el desarrollo de los pollos de engorde que se puede traducir en un beneficio económico.

Palabras clave: *análisis químico sanguíneo, antibiótico*, *aves de corral, hongo*.

Resumo

Antecedentes: o flavofosfolipol é um antibiótico promotor de crescimento (AGP). A proibição de AGP em muitos países é controversa pois os seus benefícios económicos e ambientais a través da poupança de subministro de alimento e diminuição da produção de fezes e nitrogênio tendem a ser esquecidos. A respeito disso, o cogumelo tem propriedades nutricionais interessantes e a indústria alimentícia rejeita grandes quantidades deste produto que poderiam ser de utilidade para ser aproveitadas na alimentação animal. **Objetivo:** avaliar o efeito de cinco níveis diferentes do cogumelo em pó (EMP) e da presença ou não de flavofosfolipol sobre o desenvolvimento e a concentração sérica de vários metabolitos em frangos de corte. **Métodos:** trezentos frangos de um dia foram aleatoriamente divididos em dez grupos de acordo com o tratamento, com três repetições de dez animais. Usou-se um delineamento experimental fatorial (2x5 tratamentos), em que os tratamentos realizados incluíram cinco concentrações de champignon em pó (0, 0,5, 1,0, 1,5 e 2,0 g/kg de ração) e da adição da 0 ou 5 mg/kg flavofosfolipol. **Resultados:** adicionar flavofosfolipol e cogumelo em pó às dietas exerce, independentemente, uma diminuição sobre a ingestão diária da ração, mas com resultados positivos no ganho de peso e na taxa de conversão alimentar. O antibiótico aumenta a concentração de ácido úrico no sangue; quando está associado com o pó de cogumelo, diminui a concentração sérica de triglicerídeos e colesterol de baixa densidade (VLDL). O consumo do pó de cogumelo afetou a concentração sérica do colesterol total. **Conclusões:** as duas substâncias estudadas, mas consumidas independentemente, têm um efeito positivo sobre o desenvolvimento com o intuito de ter além, um benefício econômico na produção de frangos de corte.

Palavras chave: *antibiótico, composição química sanguínea, frangos de corte, fungo.*

Introduction

The increase in bacterial antibiotic resistance could be directly linked to the abuse of antibiotic growth promoters (AGP) in livestock production (De Barros *et al.,* 2012). This has resulted in banning their use in many countries, mainly within the European Union. Others, including Iran, following the recommendations of the international office of epizootics (OIE) have relied on prudent use guidelines and programs that reduce total microbial loads, rather than banning AGP. According to the available data, the use of antibiotics for livestock and poultry in Iran is higher than that in developed countries with the exception of South Korea (Aalipour *et al.,* 2014).

Until today, a direct and clear link between AGP use and increased antibiotic resistance has not been established (Bywater, 2005). Moreover, for some researches, the strategy of banning or restricting the use of antibiotics in animals has had limited success: it has been followed in many cases by deterioration of animal health and increase in human illnesses and resistance rates (Cox Jr and Ricci, 2008). Therefore,

the use of antimicrobial feed additives of different class from the antibiotics used in humans, and hence not putatively cause of resistance, seems reasonable. Flavophospholipol is a phosphoglycolipid antimicrobial produced by various strains of *Streptomyces* (Pfaller, 2006), has no therapeutic use in human or veterinary medicine, and can still be legally added to poultry diets in many countries (Butaye *et al.,* 2003).

Additionally, several medicinal mushrooms have demonstrated potent antioxidant activities and, as a consequence, have potential application as natural antioxidants (Barros *et al.,* 2006; Minareci *et al.,* 2011; Liu *et al*., 2013). *Agaricus bisporus*, commonly known as white button mushroom or champignon, is one of the most commonly and widely consumed mushrooms in the world. It is considered a valuable healthy food with high content of polyphenols, ergothioneine, vitamins, minerals, and polysaccharides (Tian *et al.,* 2012). Moreover, *A. bisporus* has demonstrated various valuable biological properties including antitumor, anti-aromatase, antimicrobial, immune-modulatory, anti-inflammatory, and antioxidant activities (Chen *et al.,* 2006; Tsai *et al.,* 2009; Moro *et al.,* 2012; Liu *et al.,* 2013). During the mushroom production process, high quantities of this mushroom are wasted, which could be recycled as a feed additive for broiler production.

Therefore, the aim of this study was to determine the effects of edible mushroom powder (EMP) on performance and haematology parameters of broiler chickens. Simultaneously, the use of flavophospholipol as an antimicrobial feed additive and its interaction with EMP was also evaluated.

Materials and methods

Ethical considerations

The experiments were approved by the Scientific Board of the Islamic Azad University and were conducted according to the International Guidelines for research involving animals (Directive 2010/63/EU).

Location

This study was conducted at the Poultry Farm Facilities in the city of Ramsar (latitude 50°40' N and longitude 36°54' E) and the Agriculture Faculty of the Islamic Azad University, Rasht Branch, Iran, between July-September, 2013 (summer, warm season), during a total of 42 days.

Animals and housing

Three hundred one-day-old male Ross 308 broilers (Aviagen, 2007) were randomly assigned to 10 treatments with three replicates per treatment. Each replicate consisted of 10 chicks housed in pens of 1.2 m^2. The pens were equipped with electrical heaters. The room temperature was adjusted to approximately 33 ºC on day 1, and was then gradually reduced to 24 ºC. Lighting was provided with a 23L:1D (from 19:00 to 20:00) program. Humidity was maintained between 55 and 65% in the early growing period by spraying water on the floor. The chicks were vaccinated against Newcastle and Gumboro. Within 24 h after vaccination, a multiple-vitamin and electrolyte solution (1:1000) was offered via drinking water to reduce stress.

Diets and experimental design

Feed and water were supplied *ad libitum* during the entire experimental period. The nutritional requirements were met according with the Ross rearing catalogue (Aviagen, 2007). Feeds were formulated to be iso-proteic and iso-energetic for all treatments. The composition of diets and their nutrient content are presented in Table 1. The feed remaining in the feeders (orts) was weighed and weekly recorded. Mushrooms (*A. bispours*) were obtained from a national mushroom producer (Pars Company, Ramsar, Iran). Mushroom composition was provided by the same company and is presented in Table 1. Whole mushrooms were dried at 60 ºC and added to the experimental diets after grinding. The treatments consisted of five levels of EMP (0, 0.5, 1.0, 1.5, and 2.0 g/kg) and 0 or 5.0 mg/kg flavophospholipol (Teif Azmoon Pars, Co., Tehran, Iran). According to De Barros *et al.* (2012), the best performance is obtained with 10 mg/kg flavophospholipol. Regarding mushroom powder, Giannenas *et al.* (2011) reported positive results on turkey poults when it was added up to 20 g/kg feed. Dietary treatments used in the present study are detailed in Table 2.

Table 1. Feed ingredients and nutrient content of diets used during the starter (1st to14th days of age), grower (15th to 28th days of age), and finisher periods (29th to 42nd days of age).

Ingredient (g/kg)	Starter period	Grower period	Finisher period
Corn	474	454	420
Soybean meal	395	330	264
Wheat	70	150	250
Soybean oil	15	25	28
Ca22%P18%	21.5	18	15.7
Sodium bicarbonate ($NaHCO_3$)	1	1.4	1.9
$CaCO_3$	9	8.7	8.5
NaCl	2.6	2.4	2
Mineral mixture[1]	2.5	2.5	2.5
Vitamin mixture[2]	2.5	2.5	2.5
DL-methionine	2.9	2.5	2.3
Threonine	1	0.8	0.5
Lysine-hydrochloride	2.5	1.7	1.6
Avizyme multienzyme	0.5	0.5	0.5
Total	1000	1000	1000
	Calculated nutrient composition of the diet		
Metabolizable energy (kcal/kg)	2900	2950	3000
Crude protein (%)	22	20	18
Digestible protein SID[3] (%)	20	18	16
Calcium (%)	1.05	0.9	0.85
Available phosphorus (%)	0.5	0.45	0.42
Sodium (%)	0.16	0.16	0.16
DCAB[4] (mEq/kg)	240	230	210
Lysine (%)	1.4	1.18	1.04
Lysine SID (%)	1.28	1.08	0.95
Methionine (%)	0.63	0.57	0.5
Methionine SID (%)	0.6	0.55	0.48
Methionine + Cysteine (%)	0.99	0.91	0.82
Methionine + Cysteine SID (%)	0.9	0.83	0.75
Threonine (%)	0.88	0.85	0.74
Threonine SID (%)	0.76	0.74	0.65
	Mushroom (*Agaricus bisporus*) **composition**[5]		
Moisture (g/kg)		900	
Energy (kcal/kg)		1050	
Carbohydrates (in dried mushrooms; g/kg)		248.8	
Crude protein (in dried mushrooms; g/kg)		350.0	
Cysteine (g/kg)		0.18	
Lysine (g/kg)		0.002	
Methionine (g/kg)		0.004	
Ca (g/kg)		0.71	
P (g/kg)		9.12	
Fe (g/kg)		0.09	
Na (g/kg)		1.06	
K (g/kg)		28.5	

[1]Cu: 3 mg/g; Zn: 15 mg/g; Mn: 20 mg/g; Fe: 10 mg/g; K: 0.3 mg/g.

[2]Calcium pantothenate: 4 mg/g; niacin: 15 mg/g; vitamin B6: 13 mg/g; vitamin A: 5000 IU/g; vitamin D3: 500 IU/g; vitamin E: 3 mg/g; vitamin K3: 1.5 mg/g; vitamin B2: 1 mg/g.

[3]SID (standardized ileal digestible).

[4]DCAB (dietary cation-anion balance).

[5]Composition provided by the producer company.

Table 2. Treatment groups.

Treatment	EMP[1] (%)	Ab[2] (mg/kg)
1	0	0
2	0	5
3	0.5	0
4	0.5	5
5	1.0	0
6	1.0	5
7	1.5	0
8	1.5	5
9	2.0	0
10	2.0	5

[1]EMP = edible mushroom powder; [2]Ab = antimicrobial flavophospholipol.

Body weight at birth was 43.5 ± 1.2 g. Weight gain was weekly recorded by pen. At the end of the study (42 days of age) the mean body weight of birds (commercial weight) was 2,715.3 ± 31.7 g. At this day one bird per group, totalling three birds per treatment, were selected for blood collection. Care was taken to choose the most representative birds with respect to body weight compared to the mean body weight of the group. Blood samples (1 mL/bird) were collected into EDTA tubes from the wing veins. Samples were transferred to the laboratory for analysis within 2 hours of collection. After centrifugation (3,000 g x 10 min at room temperature) serum was harvested and stored in Eppendorf tubes at -20 ºC until analysis.

Chemical analysis

Serum cholesterol and triglyceride levels were determined using enzymatic methods (Teif Azmoon Pars Co., Tehran, Iran). High-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol were measured directly with HDL-C and LDL-C diagnostic kits (Teif Azmoon Pars Co., Tehran, Iran). Colorimetric determination of cholesterol in blood serum samples was done by the cholesterol oxidase procedure by Barham and Trinder (1972), which is based on the formation of a red-purple quinoneimine produced by oxidative condensation of a phenolic compound with 4-aminoantipyrine in the presence of hydrogen peroxide. Quinoneimine absorbance, measured spectrophotometrically, has a direct relationship with the amount of cholesterol in the sample.

Serum triglycerides were measured using a series of coupled reactions in which triglycerides are hydrolyzed to produce glycerol. Glycerol is then converted to pyruvate and then to lactate. Decreased absorbance, measured spectrophotometrically, is proportional to the triglyceride concentration in the sample (Schmid and von Forstner, 1986).

A glucose oxidase kit (Teif Azmoon Pars Co., Tehran, Iran), based on an oxidase-peroxidase procedure, was used to measure serum glucose. In this assay, glucose is oxidized in the presence of the glucose oxidase catalyst into H_2O_2 and gluconic acid. The reactions involving gluconic acid, hydrogen peroxide, a phenolic compound and 4-aminoantipyrine form a red-violet quinoneimine, and the absorbance of the quinoneimine chromagen, measured by spectrophotometry, is directly related to the amount of glucose in the sample.

A uric acid-uricase enzyme kit (Teif Azmoon Pars Co., Tehran, Iran), based on an oxidase-peroxidase procedure (Trinder, 1969), was used to measure serum uric acid. In this procedure uric acid is oxidized with uricase and, in the presence of the generated hydrogen peroxide, a phenolic compound and 4-aminoantipyrine forms a red-colored quinoneimine. The absorbance of quinoneimine chromagen, measured by spectrophotometry, is directly associated with the amount of uric acid in the sample (Thomas, 1998).

Serum alkaline phosphatase (ALP) was determined enzymatically using a commercial kit (Teif Azmoon Pars Co., Tehran, Iran). In this procedure, ALP activity was determined colorimetrically by a modification of the Bessey *et al.* (1964) method, using p-nitrophenol phosphate as the enzyme substrate, which is converted to phosphate and p-nitrophenol by the ALP. The released p-nitrophenol is proportional to the ALP activity.

Albumin (Alb) was determined based on the bromocresol green method (Doumas *et al.,* 1971) using a commercial kit (Teif Azmoon Pars Co., Tehran, Iran), while total protein (TP) was assayed by the Biuret method (Gornall *et al.,* 1949; Teif Azmoon Pars Co., Tehran, Iran). Total protein determination was based on the principle that protein forms an intense violet-blue complex with copper salts in

alkaline media. Iodine was included as an antioxidant, the intensively color formed is proportional to the total protein concentration in the sample. The globulin values were calculated by subtracting albumin values from the corresponding total protein.

Statistical analysis

General lineal models (GLM, SPSS 15.0, Chicago, IL, USA) were used to analyse the effects of EMP and flavophospholipol on feed intake (FI), weight gain (WG), feed conversion ratio (FCR), body weight at 42 d, and blood metabolites in a 2x5 factorial arrangement. Tukey's test was used to determine disparities among the groups.

Results

Growth performance

The effects of dietary EMP and antibiotic supplementation on bird performance are detailed in Tables 3 and 4, respectively. Results from the current work show that dietary EMP as well as the addition of flavophospholipol had a negative effect on FI. These effects were absente in the starter period, but during the grower period both variables reached significance (see GLM on Table 3). Similarly, both EMP and flavophospholipol affected FI during the finisher period. Finally, the GLM showed a negative effect of these variables on FI for the whole growth period (from birth to 42 days; Table 3). Regarding WG, results showed a positive effect of EMP and antibiotic supplementation, but showed a negative effect when combined. This effect was not found in the starter period, while only EMP effects reached significance during the grower period. In the finisher period, as well as in the GLM analysing the whole rearing period, both variables were significant. Results similar to those for FI were observed for FCR, i.e., EMP and antibiotic supplementation decreased FCR for all rearing periods except for the first one. Finally, results showed that both flavophospholipol and EMP addition positively affected final body weight (model $F_{3,26} = 23.1$, $p<0.001$, $R^2= 72.7\%$), coefficients were: EMP = 99.3 ± 34.9, $p<0.001$; antibiotic = 185.7 ± 60.5, $p<0.01$; interaction = -173.6 ± 49.4, $p<0.01$.

Blood serum metabolites

Values for selected serum metabolites in birds fed the 10 experimental diets are given in Table 4. The antibiotic supplementation did not affect serum concentration of total cholesterol, HDL, LDL, glucose, or ALP. However, dietary flavophospholipol linearly increased uric acid concentration ($p = 0.040$) and, as an interaction with EMP, decreased triglycerides serum concentration ($F_{1,28} = 5.04$, $p<0.01$, $R^2 = 27.2\%$) and VLDL ($F_{1,28} = 5.29$, $p<0.05$, $R^2 = 28.2\%$). EMP affected, individually or combined with antibiotic, the serum concentration of triglycerides, total cholesterol ($F_{1,28} = 6.38$, $p<0.05$, $R^2 = 18.6\%$; coefficient = -12.27 ± 4.86, $p<0.05$), and VLDL.

No statistically significant differences were found for total protein concentration at day 42 between treatment groups and control (Table 5). Similarly, no significant differences were observed for total albumin or globulin concentrations in treatment groups in comparison to controls.

Discussion

In general, adding flavophospholipol and EMP to diets caused positive effects on broiler performance. The results for flavophospholipol addition are similar to those reported by De Barros *et al.* (2012), who also found improvements in body weight gains and feed conversion ratio. However, there are two main differences between their study and ours. First, they found these benefits throughout the whole rearing period while we found improvements only from the third week of rearing onward. This difference can be explained by the different doses of antibiotic employed in both studies; in our work we used 5 mg/kg of the antibiotic while De Barros *et al.* (2012) used 10 mg/kg of flavophospholipol.

The second difference is that they did not find a decrease in feed intake like the one found in the present study. A possible explanation for this negative effect of flavophospholipol and EMP on feed intake could be that supplementation of both products reduces feed palatability. To our knowledge, there are no studies concluding that EMP or flavophospholipol addition could affect feed intake by changing its palatability.

Table 3. General linear models (GLM) examining the effects of flavophospholipol[1], EMP[2] and their interaction on FI, WG, and FCR[3] of male chickens[4] during three rearing periods[5].

Dependent variable	Intercept	Ab	EMP	Ab x EMP	$F_{3,26}$	R^2
FI						
Starter						
Grower	126.5 ± 0.8***	-2.8 ± 1.1*	-3.5 ± 0.6***	2.5 ± 0.9**	11.4***	56.8
Finisher	190.7 ± 1.4***	-6.0 ± 1.9**	-7.5 ± 1.1***	5.0 ± 1.6**	16.9***	66.1
Whole	118.7 ± 0.5***	-3.1 ± 0.7***	-3.7 ± 0.4***	2.6 ± 0.6***	31.6***	78.5
WG						
Starter						
Grower	68.9 ± 1.0***		5.3 ± 0.8***		42.7***	60.4
Finisher	86.2 ± 2.1***	7.8 ± 3.0**	3.2 ± 1.7***	-7.4 ± 2.4*	14.0***	61.8
Whole	62.5 ± 0.7***	3.7 ± 1.0***	2.7 ± 0.6***	-3.1 ± 0.8***	46.1***	84.2
FCR						
Starter						
Grower	1.76 ± 0.04***	-0.12 ± 0.06*	-0.09 ± 0.03***	0.11 ± 0.05*	14.51***	62.6
Finisher	2.16 ± 0.06***	-0.26 ± 0.08**	-0.10 ± 0.05***	0.21 ± 0.07**	16.50***	65.6
Whole	1.73 ± 0.02***	-0.13 ± 0.03***	-0.08 ± 0.02***	0.10 ± 0.03**	39.84***	82.1

[1]Ab = antibiotic (0 or 5.0 mg/kg).

[2]Edible mushroom powder (0, 0.5, 1.0, 1.5, or 2.0 g/kg).

[3]FI = feed intake (g/chicken/day); WG = weight gain (g/chicken/day); FCR = feed conversion ratio (g/g).

[4]Averages of three replicate pens of 10 chickens each.

[5]Starter: 1-14 d, grower: 15-28 d, finisher: 29-42 d. Whole rearing period: 1-42 d.

Each row corresponds to each GLM. Blanks indicate non significant variables and empty rows indicate non significant GLM. Statistical significance at $p<0.05$, 0.01, and 0.001 are indicated by *, ** and ***, respectively.

Table 4. Effect of flavophospholipol[1] added to diets containing EMP[2] on performance of male broilers[3] at three rearing periods[4].

Treatment	Feed intake (g/chicken/day)				Body weight gain (g/chicken/day)				Feed conversión ratio (g:g)			
	starter	grower	finisher	whole	starter	grower	finisher	whole	starter	grower	finisher	whole
Antibiotic addition x mushroom waste level												
EMP0	38.7	127.4	189.4	118.5	30.5	63.9	80.8	58.3	1.29	1.99	2.35	1.88
EMP0Ab	37.8	122.0	184.8	114.9	30.5	69.4	89.4	63.1	1.27	1.75	2.10	1.70
EMP0.5	38.7	124.2	186.8	116.6	30.6	74.5	79.1	61.4	1.28	1.67	2.39	1.78
EMP0.5Ab	39.1	124.3	183.6	115.7	30.6	75.7	84.3	63.5	1.29	1.66	2.18	1.71
EMP1.0	38.8	121.9	186.3	115.7	30.7	75.8	91.4	66.0	1.28	1.62	2.06	1.65
EMP1.0Ab	38.0	124.8	181.6	114.8	30.9	71.4	88.5	63.6	1.25	1.81	2.06	1.71
EMP1.5	38.9	121.2	178.7	112.9	30.7	76.5	93.6	66.9	1.29	1.59	1.94	1.61
EMP1.5Ab	38.8	121.1	181.3	113.7	35.7	77.8	90.9	68.1	1.08	1.58	2.00	1.56
EMP2.0	38.4	120.3	174.9	111.2	30.9	79.4	100.1	70.2	1.26	1.53	1.79	1.53
EMP2.0Ab	38.9	121.2	179.7	113.3	30.6	78.2	94.1	67.6	1.28	1.58	1.92	1.59
Pooled SEM	0.13	0.45	0.91	0.40	0.35	0.90	1.31	0.66	0.01	0.03	0.04	0.02

Continues

Table 4. Continued

Treatment	Feed intake (g/chicken/day)				Body weight gain (g/chicken/day)				Feed conversión ratio (g:g)			
	starter	grower	finisher	whole	starter	grower	finisher	whole	starter	grower	finisher	whole
Main effect												
Antibiotic												
0	38.7	123.0	183.2	115.0	30.7	74.0	89.0	64.5	1.28	1.68	2.11	1.69
5.0	38.5	122.7	182.2	114.5	31.6	74.5	89.5	65.2	1.23	1.67	2.05	1.65
Mushroom waste level												
0.0	38.2	124.7	187.1	116.7	30.5	66.5	85.1	60.7	1.28	1.87	2.22	1.79
0.5	38.9	124.2	185.2	116.1	30.6	75.1	81.7	62.5	1.28	1.66	2.29	1.74
1.0	38.4	123.4	184.0	115.3	30.8	73.6	89.9	64.8	1.26	1.71	2.06	1.68
1.5	38.8	121.1	180.0	113.3	33.2	77.1	92.3	67.5	1.18	1.59	1.97	1.58
2.0	38.6	120.7	177.3	112.2	30.7	78.8	97.1	68.9	1.27	1.55	1.86	1.56
Significance of ANOVA												
Antibiotic	NS	0.015	0.005	<0.001	NS	NS	0.014	<0.001	NS	0.044	0.004	<0.001
EMP	NS	<0.001	<0.001	<0.001	NS	<0.001	<0.001	<0.001	NS	<0.001	<0.001	<0.001
Ab x EMP	NS	0.009	0.004	<0.001	0.036	NS	0.005	<0.001	NS	0.022	0.004	<0.001

[1]Ab = antibiotic (0 or 5.0 mg/kg).

[2]Edible mushroom powder (0, 0.5, 1.0, 1.5, or 2.0 g/kg).

[3]Data are from averages of three replicate pens of 10 chickens.

[4]Rearing periods: starter: 1-14 d, grower: 15-28 d and finisher: 29-42 d. Whole rearing period: 1-42 d.

NS = no significant.

Table 5. Effect of flavophospholipol[1] added to diets containing EMP[2] on blood serum metabolites of male broiler chickens at 42 d of age[3].

Lipoprotein treatment	Tryglycerides	Total colesterol	HDL-C[4]	LDL-C[4]	VLDL-C[4]	Glucose	Uric acid	ALP[4]	Total protein	Albumen	Globulin
Antibiotic addition x mushroom waste level											
EMP0	89.3	143.0	80.7	44.3	18.0	241.0	2.53	4823.0	2.57	1.30	1.27
EMP0Ab	97.0	115.3	72.0	24.0	19.3	280.0	3.10	6403.3	2.43	1.23	1.20
EMP0.5	84.0	114.0	60.3	36.7	17.0	223.3	1.97	5153.0	2.37	1.27	1.10
EMP0.5Ab	105.3	110.7	67.3	22.3	21.0	226.7	3.10	4276.0	2.60	1.40	1.20
EMP1.0	79.7	110.0	54.3	39.7	16.0	228.3	1.80	4996.0	2.33	1.20	1.13
EMP1.0Ab	99.0	113.3	62.7	31.0	19.7	216.7	2.40	5788.0	2.63	1.33	1.30
EMP1.5	108.7	100.0	65.0	13.3	21.7	243.0	2.13	3562.3	2.80	1.43	1.37
EMP1.5Ab	59.7	94.7	67.3	15.7	11.7	219.3	2.87	3675.7	2.27	1.17	1.10
EMP2.0	91.3	108.3	61.7	28.3	18.3	223.0	3.07	6172.0	2.53	1.37	1.17
EMP2.0Ab	52.3	103.7	69.0	24.0	10.7	215.0	2.33	5801.3	2.47	1.23	1.23
Pooled SEM	4.43	3.7	2.2	3.9	0.9	7.3	0.11	278.0	0.05	0.03	0.03
Main effect											
Antibiotic											
0.0	90.6	115.1	64.4	32.5	18.2	231.7	2.30	4941.3	2.52	1.31	1.21
5.0	82.7	107.5	67.7	23.4	16.5	231.5	2.76	5188.9	2.48	1.27	1.21

Continues

Table 5. Continued

Lipoprotein treatment	Tryglycerides	Total colesterol	HDL-C[4]	LDL-C[4]	VLDL-C[4]	Glucose	Uric acid	ALP[4]	Total protein	Albumen	Globulin
Mushroom waste level											
0.0	93.2	129.2	76.3	34.2	18.7	260.5	2.82	5613.2	2.50	1.27	1.23
0.5	94.7	112.3	63.8	29.5	19.0	225.0	2.53	4714.5	2.48	1.33	1.15
1.0	89.3	111.7	58.5	35.3	17.8	222.5	2.10	5392.0	2.48	1.27	1.22
1.5	84.2	97.3	66.2	14.5	16.7	231.2	2.50	3619.0	2.53	1.30	1.23
2.0	71.8	106.0	65.3	26.2	14.5	219.0	2.70	5986.7	2.50	1.30	1.20
Significance of ANOVA											
Antibiotic	NS	NS	NS	NS	NS	NS	0.040	NS	NS	NS	NS
EMP	NS	0.017	NS	NS	NS	NS	NS	NS	NS	NS	NS
Ab x EMP	0.003	NS	NS	NS	0.003	NS	NS	NS	NS	NS	NS

[1]Ab = antibiotic (0 or 5.0 mg/kg).

[2]Edible mushroom powder (0, 0.5, 1.0, 1.5, or 2.0 g/kg, respectively).

[3]Data are means of three birds (one per pen) per treatment.

NS = no significant.

However, it has been demonstrated that mushrooms have a quite intense taste (Tsai *et al.,* 2009) so it can affect feed flavour (Perdok *et al.,* 2003; Isabel and Santos, 2009).

The positive impact of growth promoting antibiotics is well known in the poultry industry. Antibiotics suppress harmful microorganisms and the intestinal bacterial population comes into balance as a more beneficial microbiota. In this sense, a well-balanced gut microbe population is considered an effective barrier against pathogenic bacteria, which positively influences animal growth through increased nutrient absorption (Pfaller, 2006). Inhibition of inflammatory effects has also been proposed as a means of action for this type of compound (Niewold, 2007).

It is not surprising that mushroom powder had a positive effect on broiler performance; it is acknowledged that mushrooms have a good effect on human and animal health. Giannenas *et al.* (2010) reported that *A. bisporus* has a prebiotic-like effect on turkeys since fermentable polysaccharide content in mushrooms may improve growth of *Lactobacilli* and *Bifidobacteria* populations and inhibit *E. coli* leading to a more balanced biota population in the gastrointestinal tract of poultry, as a consequence, to a greater efficiency in digestibility and feed utilization. Furthermore, *Lactobacilli* may produce organic acids such as lactic acid (Rehman *et al.,* 2007) and bactericidal substances (Neal-McKinney *et al.,* 2012) that may improve gastrointestinal function and feed digestibility, resulting in enhanced growth and improved FCR (Ferket, 2004).

An important result of the present work is the combined negative effect of the antibiotic and EMP on weight gain, FCR and body weight at 42-d. Since flavophospholipol promotes proliferation of a bacterial population capable of profiting in a more effective way the mushroom nutritional properties, including sugar composition as polysaccharide fractions, we expected to find the opposite results. In contrast, the present study showed that the combination of the antibiotic and EMP decreases the positive effect on growth produced by the two substances added separately.

Limited information is available on changes in blood metabolites associated with the addition of flavophospholipol or EMP. In the present study, the addition of EMP showed an overall decrease on blood lipid metabolite profiles. In this sense, results show that EMP added individually or with flavophospholipol decreases serum triglycerides, total cholesterol and VLDL concentration but no LDL or HDL. So, the decrease in serum total cholesterol may be a consequence of the decrease in VLDL.

Abdel-Fatah *et al.* (2008) showed that total blood lipids and cholesterol decreased significantly with dietary acidifiers. Panda *et al.* (2006) discovered that the addition of probiotics in diets reduced total serum cholesterol and triglycerides. They attributed this to a reduced absorption and/or synthesis of cholesterol in the gastro-intestinal tract of probiotic-supplemented chickens (Mohan *et al.,* 1995; Mohan *et al.,* 1996). Similarly, *Lactobacillus acidophilus* and other acidophilic bacteria lower the pH of the environment it occupies. Bile acids are less soluble at low pH, are absorbed less in the intestine, and are more likely to be excreted in the faeces (Klaver and van der Meer, 1993).

Since fermentable polysaccharides in mushrooms may improve *Lactobacilli* growth, this could explain part of the results in the present study. Furthermore, flavophospholipol inhibits transglycosylase, an enzyme necessary for the formation of bacterial cell wall, and its consequent intestinal microbiota modulation promotes the development of *Lactobacilli* and *Bifidobacteria,* which are generally considered beneficial bacteria (Bolder *et al.,* 1999). Another possible explanation, or at least partially, is that the observed lower feed consumption and consequently lower fat intake may also contribute to reducing blood lipid content. This increment in beneficial bacterial populations would lead to a more efficient assimilation of the feed throughout a better intestinal absorption that could explain the observed reduction in FI and FCR increment.

Flavophospholipol treatment showed an increment in serum concentration of uric acid. Uric acid is the major end product of protein metabolism in poultry (Griminger and Scames, 1986) so increased serum concentration of uric acid could indicate increased growth potential in broilers treated with the antibiotic, which would be reflected in the increased weight gain observed in our results. Supporting this idea, Darsi *et al.* (2012) observed that uric acid plasma levels were reduced in parallel with dietary crude protein. On the other hand, increased plasma uric acid has anti-oxidant activity in chickens (Klandorf *et al.,* 2001; Carro *et al.,* 2010), which suggest an anti-oxidative effect of flavophospholipol.

Finally, treatments failed to induce any significant effect on total protein, albumin or globulin serum concentrations. Higher serum globulin is an indicator of better immune response and source of antibody production, and low albumin to globulin ratio signifies better disease resistance and immune response (Griminger, 1986), so the good health status of birds in the present study can explain why there was no evident influence of mushrooms on these blood compounds. Similarly, Guo *et al.* (2003) suggested that the mushrooms effect was more pronounced under infectious conditions than under normal ones.

In conclusion, the use of flavophospholipol and EMP has a positive effect on growth performance and these benefits can be translated into important amounts of feed saved in animal production. Moreover, results showed an overall decrease in blood lipid metabolite profiles and uric acid content of chicks.

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Conflicts of interest

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

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