PHYSIOLOGICAL AND IMMUNOLOGICAL RESPONSES OF JAPANESE QUAILS TO OLEOBIOTIC

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ABSTRACT

This study was conducted to investigate the effect of the administration of oleobiotic to the drinking water of Japanese quails. Three different concentrations ofoleobiotic (0, 30 or 50 ml/1000L) were administrated in the drinking water and provided to the birds for 5 weeks. Throughout the five weeks experimental period, there was no significant effect of oelobiotic on the performance parameters including (feed intake, body weight, weight gain and FCR) of Japanese quail. The liver was heavier and there was a tendency of spleen to be heavier by rising levels of oleobiotic. Among the measured biochemical parameters of serum, the concentration of albumin increased in the birds that received the medium level of oleobiotic. The concentration of thyroid stimulating hormone in the serum decreased by rising level of oleobiotic in the drinking water of Japanese quails. Furthermore, the concentration of amylase in the serum decreased at the highest administration level of oleobiotic to the drinking water. As an essential oil, offering of oleobioticwas not promising regarding to the performance and physiology of Japanese quails when birds were raised in a relatively hygienic environment.Further researches are required to examine the efficacy of oleobiotic for poultry. Oleobiotic may expresses its beneficial effects during the bird exposure to highly pathogenic conditions.

Keywords: Oleobiotic, quails, physiology, growth

الاستجابة الفسلجية والمناعية لطيور السمان الى تاثيرات اضافة الاوليوبيوتيك لمياة الشرب

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مدرس

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المستخلص

أجريت هذه الدراسة لبيان تأثير إضافة الأوليوبيوتيك إلى مياه الشرب لطيور السمان الياباني. تم خلط ثلاث مستويات مختلفة من الأوليبيوتيك (0، 30 أو 50 مل / 1000لتر) مع مياه الشرب وقدمت للطيور لمدة خمس أسابيع. لم يكن هناك أي تأثير معنوي للاوليوبيوتيك على الأداء الانتاجي (معدل استهلاك العلف، وزن الجسم، الزيادة الوزنية) للسمان الياباني. كان وزن الكبد أثقل وكان هناك ميل من الطحال لتكون أثقل مع ارتفاع مستويات الأولوبيوتيك. من بين المقاييس البيوكيميائية المقاسة في مصل الدم، زاد تركيز الألبومين في مصل دم الطيور التي تلقت المستوى المتوسط من الأولوبيوتيك. انخفض تركيز الهرمون المحفز للغدة الدرقية في المصل وكذلك انخفض تركيز الأميليز في المصل مع ارتفاع مستوى الأولوبيوتيك في مياه الشرب للسمان. كزيت أساسي، تاثيراضافة الاوليوبيوتيك لم يكن ايجابيا فيما يتعلق بالأداء الانتاجي والفسلجيلطيورالسمان الياباني عندما تم تربية الطيور في بيئة صحية نسبيا. هناك حاجة لاجراء المزيد من البحوث لدراسة فعالية الأولوبيوتيك على الدواجن. قد يعبر الأوليوبيوتيك تاثيرات ايجابيةاذا تعرضت الطيور الى ظروف بيئية او صحيةغير مثالية

كلمات مفتاحية: oleobioticK، **ودجاج السمان والنمو.**

***Received:20/4/2017, Accepted:14/9/2017**

INTRODUCTION

The fundamental growth and health promoting effects of antibiotics in animal production are non-doubtable confirmation. In parallel, antibiotics residues and bacterial resistance to it are considered scientific facts. Therefore, replacement of antibiotic growth promoters with the other natural alternatives is an important objective of poultry industry. Subsequently following the restriction of the use of antibiotic, feed additives particularly that of plant origin, including aromatic plants and essential oils, have gained the interest to be used in poultry nutrition (6).Essential oils (EO) could have a vital role and act as growth and health promotors in birds (8, 14, 10) due to its content of biological active compounds. The positive effects of these active compounds could be mediated via activation of the appetite sensors of birds which may intern increase the feed intake and subsequently improve the secretion and activity of digestive enzymes. In addition, essential oils were found to have antimicrobial and antioxidant properties that may promote the immune response of birds (12, 9, 19). Thus, essential oils can be used as growth stimulators in animal production (3, 11) . Based on this, products that contain blend of essential oils and spices have been developed to enhance and secure poultry digestive functions. The present experiment was designed to assess some physiological responses of Japanese quails to a novel commercial prepared blend of natural herbal oils and spices (Oleobiotic) that delivered to the birds via drinking water.

MATERIALS AND METHODS

Description of the product (Oleobiotic): Oleobiotic is a French produced plant based complex which contains essential oils (oregano, thyme, cinnamon) and spices (ginger, turmeric, pepper) that are able to act at various levels of the digestive process.

Experimental design and bird management

In a completely randomized design, a total of 135-one weeks-old Japanese quails were randomly assigned to three treatments, each with 3 replicates, 15 birds per replicate. The birds were reared in floor pens. Three different levels of Oleobiotic (0, 30 and or 50 ml) were administrated to the 1000 litters of drinking water and offered to the birds for five weeks. Feed and water were provided ad libitum. At the end of week six of bird's age, the birds and feed refusal were weighed to measure the body weight, feed intake and feed conversion ratio (FCR: feed intake/weight gain). Mortalities were recorded as they occurred, and feed per gain values were corrected for mortality.

Sample collection and processing

All samples were collected at the end of week six of birds' age. Three birds from each cage were randomly selected and killed. The abdominal cavity was opened and visceral organs removed. The weights of immune-related organs (liver, spleen and bursa of Fabricius) heart and gizzards were recorded and calculated as mass per unit of live bodyweight (g/100 of live body weight). Blood samples were collected and serum was harvested for serum biochemistry, enzymes and hormones analyses

Serum biochemical parameters

Blood samples (approximately 5 ml) were collected from the jugular vein into non-heparinized tubes. Subsequently, serum was harvested after centrifuging the blood for 15 min and stored in the refrigerator for analyses.Serum biochemical parameters including total protein, albumin, cholesterol, triglycerides and high density lipoprotein were determined by colorimetric enzymatic methods following the procedures provided in the used corresponding commercial kits provided by (Randox laboratories limited, United Kingdom).

The activity of enzymes in the serum

The concentration of lipase and amylase were determined in the serum using special commercial kit for each parameter. Aspartate-aminotransferase (AST) and Alanine-aminotransferase (ALT) concentrations in the serum were spectrophotometrically determined following the procedure described in the used commercial kit (Biolabo, Maizy, France)

Concentration of hormones in the serum

Enzyme linked fluorescent assay (ELFA) was used to determine the concentration Thyroid stimulating hormone (TSH), thyroxin (T4) and triiodothyronine (T3) in the serum by (mini VIDAS-France) using commercial kits.

Statistical analysis of data

In a complete randomize analysis, all data collected were analyzed by one way ANOVA

of Minitab 17 (17). Differences between mean values were determined using Duncan's multiple range test.

RESULTS AND DISCUSSION

Growth response: The weight of birds was almost same when they are distributed to the experimental treatment groups at week 2 (Table 1). Birds that received water containing oleobiotic exhibited similar feed intake to those that received pure water throughout the 5 weeks experimental period. Nevertheless, in this experiment neither body weight nor weight gain were affected by the administration of oleobiotic to the drinking water of quails. Same trend was also found for the FCR of the birds. This was in contrast with the finding of Bertrandet al. (3) who found a significant improvement in broiler performance due to dietary oleobiotic.Alciceket al. (1) also reported a significant effects of a dietary mixture of essential oils on broiler performance. The principle aim behind the supplementation of essential oils to poultry, is to be used as natural alternative to antibiotics and may have potential effects as growth and health promoter to enhance the productivity (7).It was reported that EOs having phenols or aldehydes, for example, cinnamaldehyde, carvacrol, citral, thymol or eugenol as major components could show considerable antibacterial activity (5). Thus its effects may be more pronounced in the conventional and disease challenged condition. Therefore, the non-significant effects of oleobiotic on the growth performance of quails in this study could be attribute to the fact that the birds were raised in a disease free conditions, giving no chance to the oleobiotic to exert its biological effects.

Table 1. Feed intake (FI), Body weight (BW) and Feed conversion ratio (FCR) of Japanese quails received different levels of oleobiotic in their drinking water

Oleobiotic Level	Response				
	Initial BW	FI	BW	WG	FCR
0	28.6	528.6	169.3	140.8	3.77
30	28.7	526.7	166.2	137.6	3.83
50	28.3	515.2	163.7	135.3	3.82
SEM	0.15	5.36	2.36	2.36	0.069
P Value	0.71	0.61	0.68	0.70	0.94

Each value represents the mean of three replicates; SEM= Standard error of means

The relative weight of visceral organs

Inclusion of oleobiotic to the drinking water significantly increased ($P< 0.05$) the relative weight of the liver in birds that were received the higher level of oleobiotic (Table 2). The relative weight of spleen tended ($P = 0.06$) to increase, arising from a relatively higher spleen weight in birds that were offered the oleobiotic supplemented water. The same trend has been found for the relative weight of the heart, indicating the higher heart weight in birds that received the medium level of oleobiotic in their drinking water. In this study, neither bursa nor gizzard weights were affected by oleobiotic inclusion to the drinking water of Japanese quails. The effect of oleobiotic on the liver and spleen could be physical more than functional effects.

Table 2. The relative weight of visceral organs of Japanese quails received different level of oleobiotic in their drinking water

Oleobiotic level	Response				
	Liver	Spleen	Bursa	Heart	Gizzard
0	1.85	0.031	0.09	0.63	2.08
30	2.63	0.057	0.11	0.76	2.58
50	3.79	0.063	0.06	0.64	1.95
SEM	0.371	0.006	0.009	0.030	0.142
P Value	0.05	0.06	0.122	0.06	0.15

Each value represents the mean of three replicates; SEM= Standard error of means

Serum biochemistry

Among the measured biochemical contents only serum albumin and cholesterol were affected by the treatment (Table 3). Serum albumin content was higher ($P< 0.05$) in birds that received the medium level (30ml) of oleobiotic than the other experimental groups. The same trend was found for the serum

cholesterol level by rising level of oleobiotic, indicating the higher (*P< 0.05*) cholesterol level in birds that received the medium level of oleobiotic in their drinking water.The results were in contrast with the finding of Srisudaet al. (18) who reported that there was a significant decrease in the serum lipid profile due to the administration of a mixture of essential oils and herbal extract (AroLief) in the broiler drinking water. In contrast to the expected hypocholersterolemic effect of essential oils, oleobiotic showed to behypercholesterolemic in this study. This may explain that oleobiotic retard the metabolism of lipids and altered its levels in the serum as a cholesterol. The effects of oleobiotic on the serum biochemistry were dose related effects, as it was only significant in birds that were received the medium level of oleobiotic, however the serum biochemistry was almost similar in the control birds and those that received the highest level of oleobiotic

Table 3. Serum biochemical content of Japanese quails received different levels of oleobiotic in their drinking water

						Response	
Oleobiotic level	TP[1] g/L	Albumin g/L	Globulin g/L	A/G[2]	Cholesterol mg/dL	TRG3 mg/dL	HDL4 mg/Dl
0	31.3	16.7[b]	14.7	1.2	211[b]	187.3	140.4
30	33.0	20.0[a]	13.0	1.5	313[a]	387.0	213.4
50	34.0	16.5[b]	17.5	1.0	250[ab]	425.0	131.2
SEM	1.04	0.65	1.26	0.14	18.00	61.7	19.4
P Value	0.64	0.01	0.38	0.22	0.03	0.23	0.16

Each value represents the mean of three replicates; a, b, c – mean values on the same row not sharing a superscript are significantly different. [1]TP= Total protein; [2]A/G= Albumin/globulin ratio; [3]TRG = Triglycerides; [4]HDL= high density lipoprotein; SEM= Standard error of means

The concentration of thyroid hormones in the serum

The effects of oleobiotic on the serum thyroid hormones are presented in table 4. The level of TSH was decreased (P< 0.002) by rising level of oleobiotic. However the level of T3, T4 and the ratio of T3 to T4 were not affected by treatments. The magnitude of the hormonal response to oleobiotic is difficult to explain from a purely nutritional effect. It may be mediated throughinfluences in the hypothalamic-pituitary axes function. As the product contains a diversity of elements some of which could have negative impacts or work as hormone binding materials which in turn may decrease the secretion and the activity of TSH. It was not possible to compare the obtained results with other researchers' findings due to the unavailability of such results in the literature on poultry.

Table 4. Thyroid hormones concentration (nmol/m) of Japanese quails received different level of oleobiotic in their drinking water

				Response
Oleobiotic level	TSH	T4	T3	T3/T4
0	0.051[a]	5.93	0.57	0.095
30	0.048[b]	5.90	0.40	0.068
50	0.048[b]	5.93	0.58	0.098
SEM	0.001	0.052	0.049	0.008
P Value	0.002	0.97	0.27	0.26

Each value represents the mean of three replicates; a, b, c – mean values on the same row not sharing a superscript are significantly different. SEM=Standard error of means

The concentration of digestive and immune related enzymes in the serum

The effects of oleobiotic on the serum enzymes are presented in table 5 and 6. The level of amylase was decreased (P< 0.001) to half by rising level of oleobiotic. However there was no significant differences among the experimental groups regarding to the level of lipase in the serum of Japanese quails.The results were in line with the finding of Lee et al. (13) who didn't found any effect of essential oils on the concentration and activity of digestive enzymes. Contrary with the finding of the current study, it was suggested that essential oils have the ability to improve digestion (2, 14, 15). It might be reasoned that spices and herbs, from which essential oils are derived, will positively affect food digestion

(Pradeep *et al*. 1991; Pradeep and Geervani, 1994). Decreasing the level of amylase in the serum in this study could be dose dependent effect and may confirm the negative impacts of oleobiotic on the carbohydrate digestion. High doses of thymol and cinnamaldehyde, when introduced into the chickens' diet, could inhibit the digestion process (13). Same effect is expected for Japanese quails. However, the inhibitory level of both principles in diet or water has not been established yet. Neither AST nor ALT level in the serum were affected by the administration ofoleobioticto the drinking water of Japanese quails (Table 6).

Table 5. concentration of amylase and lipase in the serum of Japanese quails received different levels of oleobiotic in the drinking water

	Response	
Oleobiotic level	Amylase	Lipase
0	760.5[a]	32.7
30	708.0[a]	29.01
50	379.5[b]	35.81
SEM	61.8	4.03
P Value	0.001	0.83

Each value represents the mean of three replicates; SEM=Standard error of mean

Table 6. concentration of amylase and lipase in the serum of Japanese quails received different levels of oleobiotic in the drinking water

	Response	
Oleobiotic level	AST	ALT
0	260.7	9.5
30	273.0	9.5
50	290.5	7.5
SEM	18.9	1.74
P Value	0.11	0.91

Each value represents the mean of three replicates; SEM=Standard error of mean

CONCLUSION

It could be concluded, that the essential oils could be considered as potential growth promoters for poultry. However, the results of the current study provided some evidence that was opposite to what was expected about the beneficial effects of oleobiotic as an essential oil. In terms of performance, the administration of oleobiotic to the drinking water could not improve the performance of Japanese quails. Serum biochemistry was not affected by oleobiotic. The effect of oleobiotic was dose depended and was negative to some extent on the level of some digestive enzymes and metabolism related hormone such as TSH in the serum. Using of oleobiotic was not promising in this study and it could economically not recommended for poultry producers to use this product. As a new product, the knowledge about the use of oleobiotic in poultry nutrition is insufficient. Thus there is a demand for further trials to extensively examine its chemical properties and biological active compounds as well as its exact supplementation level prior it's introducing to poultry industry. As an essential oil, the effect of oleobiotic could be more pronounced during birds' exposure to environmental or immunological challenges.

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