Modulation of broilers’ productivity and blood biochemical parameters by Citrus-elements dietary supplementation

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Abstract
The present study was conducted to evaluate the effects of a commercial lemon extract (Nor-Spice AB), dried sweet orange peel, dried lemon peel and avilamycin on broilers’ live weight and carcass traits, and to investigate their effects on some blood biochemical parameters and intestinal morphometry.

A total number of 650 one day-old unsexed Arbor Acres broiler chicks were randomly assigned to 5 floor pens with 130 each and subjected to one of five different experimental diets: non-supplemented basal diet (control), basal diet with 100 mg/kg of avilamycin, basal diet with 250 mg/kg of a commercial lemon extract (Nor-Spice AB), basal diet with 250 mg/kg of dried sweet orange peel and basal diet with 250 mg/kg of dried lemon peel. The commercial-based feed additive improved significantly (P < 0.01) the live weight and carcass yield of broilers. Plasma glucose was very significantly (P < 0.0001) decreased in all supplemented groups, whereas plasma cholesterol was significantly (P < 0.05) increased in the group fed on avilamycin. Both lemon extract and lemon peel induced a significant increase in plasma triglycerides. Only avilamycin induced a significant decrease in the intestinal length.

Keywords: Citrus, avilamycin, carcass yield, biochemical components

Introduction
Antibiotic growth promoters (AGP) were used for up to 50 years as feed additives in all food animals in order to promote growth, improve feed efficiency and to prevent diseases (Katsunuma et al., 2007; Vidanarachchi et al., 2010).

The great worldwide overuse of antibiotics at subtherapeutic levels in poultry industry had led to the emergence and spread of antibiotic-resistance (ABR) which is considered as a major public health problem because of the transfer of resistance bacteria to human directly through food chain or indirectly by contact with animals (Vuuren, 2001; Founou et al., 2016). Consequently, the use of AGP in poultry feed was restricted in many countries in the European Union to preserve food safety and subsequently to protect the public health from the harmful impact of these substances (Sharifi et al., 2013).

However, in developing countries such as Algeria where poultry production is intensified, antibiotics are largely used as therapeutic agents, in metaphylaxy or to prevent infectious diseases resulting from stress. Moreover, the misuse of antibiotics and/or the non-respect of withdrawal period resulted in an imbalance between beneficial and harmful intestinal flora and had led to selection and amplification of multi-drug resistant bacteria (Dibner and Richards, 2005). This emerging threat on animal and human health led to the research of effective feed supplements alternatives to antibiotics (Vieira et al., 2008). So, many alternatives such as exogenous enzymes, organic acids, direct-fed microbial (probiotic) and prebiotics have been used as growth enhancers but a significant attention is now attributed to medicinal herbs and plant extracts as growth promoters (Huyghebaert et al., 2011). Several plant extracts were studied for their effects on growth performance in broilers. It was reported that some natural products mainly phenolic compounds (PC) may exhibit a potential antimicrobial activity and an antioxidant activity which lead to enhance the gut health and consequently increase feed efficiency (Akbarian et al., 2013).

These bioactive compounds are found in large amounts in some fruits and vegetables. Citrus fruits are rich in valuable substances such as phenolic compounds including flavonoids and carotenoids which possess antimicrobial and antioxidant properties (Akbarian et al., 2013).

Nowadays, the industrialization of food production has led to the generation of large amounts of “wastes” and residue materials that have a deleterious impact on the environment. Several approaches were applied to minimize the rate of pollution and to avoid the loss of valuable nutrients. One of these approaches is to use some of these by-products as natural feed additives in farm animals to get a food product with improved quality.

Sweet orange and lemon are very important horticulture...
products in Algeria and their fruit’s peels represent the major by-products of juice extraction industry. Citrus peels are considered a potential source of many nutritional components such as pectin, flavonoids (hespeirin and naringin), phenolic compounds, vitamin C and essential oils (Ebrahim et al., 2013). Many studies have focused on the effect of orange peels as feed additives in cattle, pig and poultry feeds (Oluemu et al., 2010; Abbasi et al., 2015). However, the effects of lemon peels in poultry dietary supplementation have not been extensively explored.

In this regard, the present study was conducted to investigate and to compare the effects of a standardized commercial lemon extract based feed additive (Nor-Spice AB), non standardized crude products consisting of dried sweet orange peel and dried lemon peel and an antibiotic growth promoter on broiler chicken productivity with a particular focus on carcass traits and blood biochemistry.

**Material and methods**

**Experimental design**

The study was conducted in the Algerian Animal Research Center in Sougueur (Tiaret, Algeria) and the experimental protocol was approved by the Ethical Committee of Veterinary Institute. A total number of six hundred and fifty (650) one-day-old unsexed Arbor Acres broiler chicks with an average body weight of 49 g were obtained from a commercial hatchery and randomly assigned to five floor pens with 130 birds per pen. One manual feeder and one bell drinker were placed in each pen to provide feed and water ad libitum. The floor was removed then visceral organs (proventriculus, gizzard, and the floor was open to provide feed and water ad libitum. The room temperature was adjusted to 31°C on day one and was then gradually reduced to 21°C by the end of the experiment.

**Dietary treatments**

Broilers were reared to 42 days of age and during this period they received two phase-diets: a basic starter feed between placement and 3 weeks and a grower feed from 22 days to 42 days. Five dietary treatments containing a natural coccidiostat Norponin XO2 (Nor-Feed Beaucouzé, France) in drinking water 24 hours before and after vaccination. A lighting program of 23h light and 1-hour dark was applied during the first week followed by 20 h lighting program was for the remaining experimental feeding period. The temperature was reduced to 21°C by the end of the experiment.

Twelve birds from each experimental treatment were wing-banded and weighted twice a week throughout the experimental period. Mortality was daily recorded and dead birds were weighted. At the end of the rearing period, twelve birds from each group were selected and weighted then the average live weight for each group was recorded.

**Live body weight measurement**

Twelve birds from each experimental treatment were wing-banded and weighted twice a week throughout the experimental period. Mortality was daily recorded and dead birds were weighted. At the end of the rearing period, twelve birds from each group were selected and weighted then the average live weight for each group was recorded.

**Carcass characteristics**

At the last day of the experimental period (d 42), twelve (12) birds per treatment group were randomly selected and individually weighed. After slaughter, abdominal cavity was opened and the total gastro-intestinal tract was removed then visceral organs (proventriculus, gizzard,
lungs, heart, liver, pancreas, spleen and kidney) were weighed and the carcass yield was concluded.

**Blood collection and biochemical analysis**

During slaughter, blood samples were collected into heparin tubes and immediately centrifuged at 2000 g for 15 minutes at room temperature. Plasma was then harvested and stored in eppendorf tubes at -20°C for subsequent analysis. Serum concentrations of total cholesterol, triglycerides and glucose were measured by enzymatic colorimetric method using commercial kits (SPINREACT, S.A /S. A. U, SPAIN).

**Gut length measurement**

Total intestinal tract was removed and transferred into individual sterile containers, sealed and transported to the laboratory for total intestinal length measurement.

**Statistical analysis**

Data collected were subjected to the analysis of variance (ANOVA) with Tukey’s post hoc test for multiple comparisons using Prism Graph PAD 6 (GraphPad software, Inc, USA). All statements of significance were based on probability of less than 0.05 ($P<0.05$).

## Results

### Live weight and carcass yield

The effects of experimental diets on live body weight and carcass yield are shown in Fig. 1.

Among the different dietary treatments, Nor-Spice AB, orange peel and avilamycin showed an increase of broiler’s live weight, which was only significant ($P<0.01$) for Nor-Spice AB - supplemented group compared with the negative control group. In contrast, dietary supplementation with lemon peel induced a slight but no significant decrease of live weight. Carcass yield was significantly ($P<0.01$) improved in the group fed diet supplemented with Nor-Spice AB. Both, avilamycin and lemon peel showed a mild carcass yield decrease.

### Blood biochemical parameters

The effects of dietary treatments on mean plasma concentrations of selected components including total cholesterol, triglycerides and glucose of broiler chickens at 42 days are given in Fig. 2.

The results showed that feeding avilamycin supplemented diet induced a significant increase ($P<0.05$) of plasma cholesterol compared to the negative control group.

Triglycerides concentration increased significantly ($P<0.05$) in Nor-Spice AB and lemon peels fed broilers compared to the negative control group. In contrast, the other experimental diets did not affect the plasma concentration of triglycerides in comparison to the control group. The mean concentration of plasma glucose was very significantly ($P<0.0001$) reduced in all the experimental diets groups compared to the negative control group.

### Intestinal length

Results related to the intestinal length are shown in Fig. 3. Only avilamycin supplemented groups group showed a significant ($P<0.05$) decrease of intestine length when compared with the negative control group.

### Internal organ weights

The effects of dietary supplementation on visceral

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**Figure 1:** Effects of dietary supplementation on (A) live weight, (B) carcass yield of broiler chickens at 42 days of age. Negative controls (CTRL), Nor-Spice AB (NSAB), avilamycin (AVMY), lemon peel (LP) and orange peel (OP).
organs weights are illustrated in Fig. 4. No significant differences were observed among the groups for gizzard, pancreas and liver mean weights. However, the weight of proventriculus decreased significantly ($P < 0.05$) in the avilamycin-supplemented group compared to the negative control. Likewise, there was a highly significant decrease ($P < 0.001$) of spleen weight in the group fed orange peel-supplemented diet compared to the negative control group. Moreover, heart weight was significantly ($P < 0.05$) decreased in all dietary treatments.

**Figure 2:** Effects of dietary supplementation on mean concentrations of selected plasma constituents (A) cholesterol, (B) triglycerides and (C) glucose of broiler chickens at 42 days of age. Negative control (CTRL), Nor-Spice AB (NSAB), avilamycin (AVMY), lemon peel (LP) and orange peel (OP).

**Figure 3:** Effects of dietary supplementation on intestinal length of broiler chickens at 42 days of age. Negative control (CTRL), Nor-Spice AB (NSAB), avilamycin (AVMY), lemon peel (LP) and orange peel (OP).
**Discussion and conclusions**

Various plants, herbs and spices have been used either as crude products or as plant extracts in poultry feed in order to improve performances and productivity of chickens. However, many investigations in this field showed contradicting results.

These variable results have been attributed to several factors related to the plant including: dose of feed additive, type and concentration of the bioactive components in the pure compound or in its extracts, extraction process, collection time of the herb materials, physiological development period of herbs and seasonal changes (Kirbaşlar et al., 2009). Other factors include the composition of basal diet, management practices, and health status of birds, environmental conditions, and duration of rearing period, age, sex and breed of birds (Nunes et al., 2012). The present experiment was therefore conducted to investigate the effects of dietary supplementation with different citrus byproducts (standardized and not standardized) as alternative to antibiotic growth promoters on quality traits of broilers.

The experimental diets caused a significant variation in the final mean body weight among the groups. Broilers fed diet supplemented with a commercial standardized lemon extract (Nor-Spice AB) achieved the highest body weight and the highest carcass yield. In fact, it is well known that there is a close relationship between body weight and carcass weight as reported by Saleh (1992). The growth promoting effect of this commercial additive obtained in this study may be attributed to its antibacterial activity against pathogenic microflora, which leads to improve digestibility and nutrient absorption in the intestinal epithelium, increasing thereby the weight gain and the carcass dressing percentage.

In the present study, feeding orange peel did not affect significantly live weight and carcass yield of broilers. These results are in agreement with those of Ebrahimi et al. (2013) who reported that feeding diet supplemented with dried sweet orange peel (DSOP) had no significant effect on final body weight and carcass yield percentage of broiler chickens. Moreover, Ebrahimi et al. (2014) stated that the effect of diet supplemented with orange (*Citrus sinensis*) peel extract (CSPE) on final body weight and carcass yield of broilers was not significantly different from the control group.
In the current study, neither the live weight nor the carcass yield of broilers fed on lemon peel was improved. This result partly agreed with the finding of Nobakht (2013) who reported that inclusion of dried lemon pulp (DLP) increased live weight of broilers but it had no significant effect on carcass yield. Recently, Behboudi et al. (2016) showed that inclusion of lemon juice in drinking water had no significant effect on body weight of broilers.

Although it was expected that supplementing diet with an antibiotic growth promoter would improve the growth of broilers, the results obtained from this study showed that avilamycin did not have any significant effect on live weight or carcass yield in comparison with other dietary treatments. These results are in agreement with those of other researchers who reported dietary supplementation of avilamycin had no effect on broiler’s body weight (Akbari, 2014; Riyazi et al., 2015).

In contrast to our results, Wellenreiter et al. (1999) reported that broilers fed avilamycin were heavier and had high carcass weight and dressing percentage than the control birds. Zhou et al. (2009) also reported that the body weight of birds fed avilamycin significantly increased compared to the control group. According to our results and many previous research studies, the efficacy of antibiotic growth promoters on broiler performance was inconsistent and this may be attributed to several factors including the health status of chicks, quality of basal diet, environmental conditions, management practices and dose of feed additive (Nunes et al., 2012).

Serum metabolites showed that cholesterol was significantly increased in the group fed on avilamycin compared to the negative control. Similar to our result, Sharifi et al. (2013) reported that the addition of an antibiotic growth promoter, flavomycin increased the concentration of total cholesterol. According to Gunan et al. (2006), this hypercholesteremic effect may be attributed to a reduction in the growth and activity of the intestinal microflora responsible for bile salt deconjugation, reducing thereby the hepatic fat emulsification and lipid absorption and leading consequently to total cholesterol increment.

On the other hand, dietary supplementation of dried citrus (orange and lemon) peels decreased serum cholesterol level compared to antibiotic growth promoter-fed group. This result is consistent with the finding of Ebrahimi et al. (2016) and Abbasi et al. (2015). Moreover, Chaudry et al. (2004) reported that blood cholesterol significantly decreased with the incorporation of high levels of citrus peel in the ration and they attributed this lowering effect to pectin which is present according to Baker (1994) in both the edible parts of fruits as well as the inedible residue like peel. Several mechanisms have been proposed for the cholesterol lowering of pectin and one of the major mechanisms is that pectin decreased absorption of bile acids and bile salts and increased the fecal bile acids excretion which leads in turn to decrease serum cholesterol levels (Gallagher et al., 1993). Further, the hypcholesterolemic effect of citrus was attributed according to Bok et al. (1999) to the flavonoids present in the peel which inhibit the active hepatic enzyme 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase which is responsible for cholesterol biosynthesis in liver.

Plasma triglyceride concentration in the commercial standardized lemon extract (Nor-Spice AB) and lemon peel fed groups was significantly higher than negative control group. This increase may be attributed to enhanced hepatic lipogenesis (Herzberg and Rogerson, 1988). In contrast to this finding, feeding citrus peel-supplemented diets had no significant effect on blood triglycerides of broilers (Ebrahimi et al., 2016). Moreover, adding avilamycin to broiler diet resulted in a marked increase in the concentration of the triglyceride compared to the control group (Riyazi et al., 2015).

Mean plasma glucose concentration was significantly reduced in all dietary supplemented groups in comparison with the negative control group. Similarly, plasma glucose concentration was significantly decreased in 42 days broilers fed dried sweet orange peel supplemented diet (Ebrahimi et al., 2016). Additionally, blood glucose level decreased significantly in diabetic rats fed different levels of orange peel (Elhardi et al., 2015).

Abbasi et al. (2015) reported that the use of dried citrus pulp had no effect on glucose level. However, using dried citrus pulp at more than 16% increased significantly the serum glucose level and reduced the triglyceride concentration in laying hens (Nazok et al., 2010).

The hypoglycemic mechanism was not explored in this study and need to be addressed but according to Hazelwood and Langslow (1978), it might be due to increased insulin secretion from beta cells or decreased glucagon secretion from alpha cells in the pancreas. In another study conducted by Parmar and Kar (2007), a concomitant decrease of serum glucose was associated with an increase of insulin level in rats given aqueous extracts of Citrus sinensis.

A significant decrease of intestinal length was noted for the group fed avilamycin in comparison to the control group. This is in accordance with the finding of Miles et al. (2006) who reported that birds fed either virginamycin or bacitracin methylene disalicylate had shorter gastrointestinal lengths than birds given the control diet. It has been reported that antibiotic growth promoters induce thinning and shortening of gut (Modi et al., 2011; David et al., 2016). It is well known that antibiotic growth promoters modify microflora and their metabolites within the gastrointestinal lumen, which lead in turn to early gut morphological changes since microflora play an important role in morphogenesis and gut development during the first days post-hatch. These elements taking together may explain the effect of avilamycin on intestinal length.

None of the dietary treatments in the present study showed significant effect on the mean weight of gizzard, pancreas and liver corroborating thus other findings (Akbari et al., 2013; Ebrahimi et al., 2014).

Mean heart weight was decreased in all supplemented groups compared with the control group. Contrary to our
results, heart mean weight was not influenced by citrus supplemented feed (Akbarian et al., 2013; Ebrahimi et al., 2014). We observed that avilamycin supplemented diet induced a significant decrease of proventriculus weight whereas supplementation of orange peel resulted in significant decrease of spleen weight. These results are not in agreement with those of Akbarian et al. (2013) and Orayaga et al. (2016) who observed that dietary supplementation of either citrus peel or avilamycin had no significant effect on both proventriculus and spleen weights.

In contrast, the relative weights of proventriculus and gizzard increased in a dose dependent way after feeding diet supplemented of sweet orange peel meal (Agu et al., 2010). Even though, the incorporation of orange peel powder in the broiler’s feed had significantly increased liver, heart and spleen weights (Siyal et al., 2016), it had no significant effect on the same organs in another study conducted by (Alizadeh et al., 2016).

In a recent study, a significant increase in the weight of proventriculus, gizzard, liver, spleen and heart was noticed for broilers fed on sweet orange peel meal (Ahaotu et al., 2017).

In conclusion, dietary supplementation of Citrus extract improved the live weight and carcass yield of broilers. Although, dried orange and dried lemon peels had not improve the final broiler’s live weight, they did not alter the carcass quality traits and their effects were similar to those of the antibiotic growth promoter. Based on the results mentioned above, citrus supplemented diet could be an alternative to antibiotic growth promoters in poultry feeding. Data from the present study show the importance of phytochemical analysis, quality control and standardization of botanicals when these later are assigned for dietary supplementation. Further studies are required to screen the phytochemical components of citrus peels and to explore their mode of action in modulating blood biochemical parameters.

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Conflict of interest: none

References


Sažetak:
Modulacija produktivnosti i biohemijskih parametara kod brojlera uzgojenih sa citrusnim dodacima prehrani

Cilj studije je procijeniti učinke komercijalnog ekstrakta limuna (Nor-Spice AB), suhe kore slatke narandže, suhe kore limuna i avilamicinana težinu brojlera i karakteristike trupova, kao i na pojedine biohemijske parametre i morfometriju crijeva.

Ukupno 650 jedinki brojlera tipa Arbor Acres starih jedandan, čiji spol nije određivan, su randomizirane u 5 grupa sa po 130 jedinki, a svaka grupa je imala svoj tip eksperimentalne prehrane: osnovna prehrana bez suplemenata (kontrolnagrupa), osnovna prehrana uz 100 mg/kg avilamycina, osnovna prehrana uz 250 mg/kg komercijalnog ekstrakta limuna (Nor-Spice AB), osnovna prehrana uz 250 mg/kg suhe kore slatke narandže i osnovna prehrana uz 250 mg/kg suhe kore limuna. Komercijalni dodatak prehrani je sa statističkom signifikantnošću (P<0.01) povećao težinu živih brojlera i trupova. U svim grupama brojlera koje su primale dodatke prehrani, postojala je statistički značajna razlika u koncentraciji glukoze koja je bila snižena(P<0,0001), dok je holesterol bio povećan (P<0,05) kod grupe čijoj je prehrani dodan avilamicin. Ekstrakt limuna i kora od limuna su izazvali signifikantno povećanje triglicerida u plazmi. Samo je avilamicin uzrokovao signifikantno smanjenje dužine crijeva.