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# Effect of *Abies numidica* Extracts on Performance, Blood Parameters and Caecal Microflora of Broiler Chicks.

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**ABSTRACT**

The aim of this study was to investigate the effects of hydroalcoholic and aqueous extracts of *Abies numidica* on performance, caecal microflora and some blood parameters. Fifty, one-day-old mixed sex broiler chicks were used in the experiment. The animals were allocated into five dietary treatments groups in a complete randomized design and fed for 21 days. The experimental groups were as follow: the control group (CG)-basal diet without plant extract addition, the first experimental group (EG1) with the addition of 2 g of aqueous extract per 1 kg of basal diet, followed by the second experimental group (EG2) 4 g of aqueous extract $kg^{-1}$ and we used basal diet with the addition of 2 and 4g $kg^{-1}$ of hydroalcoholic extract in the third (EG3) and the fourth (EG4) experimental groups respectively. The results showed that body weight gain in the first week was significantly increased when the diets were supplemented with 4g hydroalcoholic extract. Feed intake and feed conversion ratio were not affected in birds fed diets with supplements in comparison with the control group. Glucose and cholesterol concentrations were not affected by different treatments but triglyceride and total protein concentrations were significantly increased. In addition, inclusion of 2 levels of both extracts had no effect on Lactic acid bacteria count, *Enterobacteriaceae* and total aerobes counts. These findings suggest that more studies are needed to investigate beneficial effects of *Abies numidica* extracts in broiler production.

**Keywords:** Broiler chicks, *Abies numidica*, performance, blood parameters, caecal microflora.

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## INTRODUCTION

Antibiotic feed additives as growth promoters have long been supplemented to poultry feed to stabilize the intestinal microbial flora and improve the general performances and prevent some specific intestinal pathologies [1-3]. However, the antibiotic growth promoters have been under scrutiny for many years and have been removed from the market in many countries [4]. Their usefulness has seldom been contested, it is their relatedness with similar antibiotics used in human medicine and the possibility that their use may contribute to the pool of antibiotic resistant bacteria that causes concerns [5].

In Algeria, the use of antibiotics in livestock farming is not regulated and control of the presence of maximum residue limits in foodstuffs of animal origin is not applied, posing a potential risk for the consumers. Few scientific studies and data on this topic are available [6]. Because of that it was necessary to find an efficacious alternative to antibiotic growth promoters.

Recently, plant extracts have received increased attention as possible alternatives of antibiotic growth promoters [7]. Plants contain a large variety of phytochemical compounds with antimicrobial activity [8]. These compounds could have beneficial or detrimental effects in animals [9], depending on the used concentration.

*Abies numidica* is a conifer species endemic solely to Algeria. This tree has a pyramidal port becoming conical with age. The needles are dense, short, 1 to 2cm long and rounded at the apex [10]. The objective of the current study was to determine the effect of two extracts obtained from the needles of *Abies numidica* on growth performance, blood factors and caecal microflora of broiler chicks.

## MATERIAL AND METHODS

### Plant collection

The plant was collected from Setif, Algeria, during the month of November, 2013. The needles were washed, dried at room temperature and ground into a powder. The powder was kept in a cool and dry place.

### Plant extracts preparation

**Hydroalcoholic extract:** *Abies numidica* needles powder was macerated in 80% methanol (MeOH) for 24h, 48h and 72h at room temperature. After maceration, the extracts were collected, filtered and evaporated [11].

**Water extract:** 10 g of the herb was extracted with 250 ml of boiling water for 10 min. The extract remained in the warm water for 15 min and then it was filtered [12, 13].

### Animals and dietary treatments

A total of fifty, 1-day-old mixed sex broiler chicks (ISA HUBBARD F15) were divided into five treatment groups of 10 birds each and randomly assigned to five treatment diets so that initial body weight and weight distributions were similar among different dietary treatments. Lighting was continuously for 16 h. Temperature was 32°C during the first week of age and was reduced by 2°C/week until the birds were 3 weeks old.

The commercial basal diets were formulated to meet the nutritional requirement of broiler chicks in starter phase. The dietary treatments were: Control group (CG) the basal diet without the addition of extract was used. We used basal diet with the addition of 2g aqueous extract per 1 kg in the first experimental group (EG1), basal diet with the addition of 4g aqueous extract per 1 kg in the second one (EG2), basal diet with the addition of 2g hydroalcoholic extract per 1 kg in the third one (EG3) and basal diet with the addition of 4g hydroalcoholic extract per 1 kg of feed in the fourth one (EG4). The composition of basal diets is given in table 1. The chicks were vaccinated against Newcastle disease and infectious bursal disease on day 1 by spray vaccination.

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**Table 1: Composition of the basal diet.**

| Ingredients | Amount in diet (g/kg) |
|---|---|
| Maize | 590 |
| Soybean meal | 350 |
| Wheat bran | 15 |
| Dicalcium phosphate | 20 |
| Limestone | 15 |
| Vitamin–mineral premix[1,2] | 10 |

**Vitamin premix per kg of premix**: 1000000 IU Vitamin A. 180000 IU Vitamin D3. 3295 mg Vitamin E. 200 mg Vitamin K. 120 mg Vitamin B1. 450 mg Vitamin B2. 900 mg Vitamin B3 240 mg Vitamin B6. 1,5 mg Vitamin B12. 60 g Folic Acid. 6 mg Biotin. 2000 mg Vitamin PP. 35000 mg Choline Chloride. **Mineral premix per kg**: 9590 mg manganese. 4920 mg iron. 7500 mg zinc. 2250 mg cuivre. 132620 mg calcium. 120 mg iode. 36 mg selenium. 332000 mg sodium chloride. **Other**: 180000 mg/ kg DL méthionine; 2500mg/kg anti-oxydant.

**Performance parameters**

The chickens were weighed individually on days 1, 7, 14 and 21. Feed intake for each pen was recorded weekly and feed conversion ratio (FCR) was calculated.

**Microbiological analysis**

**Sample collection**: At 21 d of age, two chicks from each treatment were slaughtered and their intestinal tracts were removed. Samples of fresh digesta (1g) from caeca were collected aseptically in preweighed 15-mL sterilized plastic tubes. The samples were weighed and diluted in saline solution to an initial $10^{-1}$ dilution. Microbial populations were determined by serial dilution ($10^{-1}$ to $10^{-7}$) of samples in saline solution before inoculation onto Petri dishes of sterile agar.

**Culturing and viable counts**: The media and culture conditions used were as follows: Plate count agar for total bacteria count (aerobic, 37°C for 24 to 48 h) and *Enterobacteriaceae* genera was determined and Mac Conkey agar (aerobic, 37°C for 24) respectively, whereas, Lactic acid bacteria was enumerated on Man, Rogosa and Sharpe agar (anaerobic, 37°C for 48 h).

**Blood sample and some organs weight**

On d 21, five chicks whose body weights were similar to the group average were selected from each experimental group and slaughtered by severing the jugular vein. Serum biochemistry parameters (total protein, glucose, cholesterol and triglyceride) were measured spectrophotometrically using commercial kits. Liver, heart and spleen were removed from the body of each bird and weighed separately.

**Statistical analysis**

Data were expressed as means ± SEM using Graphpad. Analysis of variance was determined by one-way ANOVA. The Tukey test was used to determine the significance of differences between the mean values of the treatment groups at the level of $p < 0.05$. Bacterial numbers were logarithmically transformed to secure a normal distribution of the data.

## RESULTS

**Performance parameters**

The effects of dietary treatment on averaged weekly weight gain, feed intake and feed conversion ratio are shown in table 2.

**Average weekly weight gain:** Broilers receiving 4g of hydroalcoholic extract had higher body weight gain compared to broilers in control group during the first week (P<0.05), whereas, broilers receiving 4g of aqueous extract had lower body weight gain in the second week.

**Feed intake:** For the variable feed intake, no statistical differences among treatments were observed during the all periods of age, but chicks fed on diet supplemented with 2g of aqueous extract showed numerically more in feed intake.

**Feed conversion ratio:** During all periods of measures, all treatments showed no significant effect on feed conversion ratio compared to the control group (p>0.05).

**Mortality**: In the current study, no influence of tested feed additives on mortality was detected.

**Table 2: Effect of treatments on growth performance, when included in broiler chick diets from 1 to 21 d of age (means±SEM).**

| Criteria | CG | EG1 | EG2 | EG3 | EG4 | P value |
|---|---|---|---|---|---|---|
| **Initial body weight, g** | | | | | | |
| 1 d | 35.22±0.75 | 35.1±1.001 | 35.1±0.93 | 36.37±0.83 | 36.34±0.91 | 0.7662 |
| **Body weight gain, g** | | | | | | |
| 1-7 d | 44.49±2.5$^{ab}$ | 53.89±2.3$^{bc}$ | 40.98±3.3$^{a}$ | 52 .06±2.3$^{abc}$ | 60.85±2.9$^{c}$ | P<0.0001 |
| 8-14 d | 108.85±4.3$^{a}$ | 94.26±3.04$^{ab}$ | 90.34±5.4$^{b}$ | 108.38±3.4$^{a}$ | 105.58±3.7$^{ab}$ | 0.004 |
| 15-21 d | 125.58±7.48 | 100.89±1.72 | 127.14±5.69 | 109.09±7.83 | 124.78±9.32 | 0.0284 |
| **Feed intake, g** | | | | | | |
| 1-21 d | 591.37 | 611.26 | 571.04 | 575.98 | 597.36 | 0.9984 |
| **FCR, g:g** | | | | | | |
| 1- 21d | 2.12 | 2.45 | 2.21 | 2.14 | 2.05 | 0.9596 |

$^{a,b,c}$ Means in the same row with different superscripts differ significantly (P< 0.05). CG-control group; EG1- 2g aqueous extract; EG2- 4g aqueous extract; EG3- 2g hydroalcoholic extract; EG4- 4g hydroalcoholic extract.

**Microbiological analysis**

The effects of the experimental treatments on bacterial counts are presented in table 3. As shown in table 3, there were no significant effects of dietary treatments on the number of total aerobic bacteria, *Enterobacteriaceae* genera and lactic acid bacteria in caecum of broilers.

**Table 3: Bacterial population counts in samples of caecal material taken from broilers aged 21 d (mean±SEM).**

| Treatments | Total aerobes | *Enterobacteriaceae* | Lactic acid bacteria |
|---|---|---|---|
| CG | 7.06±0.36 | 6.33±0.06 | 8.37±0.16 |
| EG1 | 7.09±0.16 | 5.59±0.24 | 8.29±0.11 |
| EG2 | 6.81±0.06 | 5.71±0.31 | 8.36±0.17 |
| EG3 | 6.86±0.2 | 5.77±0.18 | 7.98±0.46 |
| EG4 | 6.75±0.15 | 6.11±0.2 | 8.02±0.09 |
| P value | 0.5916 | 0.2292 | 0.6704 |

CG-control group; EG1- 2g aqueous extract; EG2- 4g aqueous extract; EG3- 2g hydroalcoholic extract; EG4- 4g hydroalcoholic extract.

**Blood parameters**

Effects of dietary supplemental plant extracts on blood parameters of broiler chicks are given in table 4. It was observed that cholesterol and glucose concentrations in all diets were similar to those found in the

control diet, however, triglyceride concentration was elevated (P<0.05) in EG1 and protein concentration was increased in EG2.

**Table 4: Effect of treatments on blood parameters of broiler chicks aged 21 d (mean±SEM).**

| Treatments | Blood parameters | | | |
|---|---|---|---|---|
| | Glucose (g/l) | Cholesterol (g/l) | Triglyceride (g/l) | Total Protein(g/l) |
| CG | 2.41 ±0.061 | 1.72±0.06$^{ab}$ | 0.38±0.02$^{a}$ | 13.6±1.94$^{ab}$ |
| EG1 | 2.46 ±0.093 | 1.49±0.045$^{a}$ | 0.96±0.150$^{b}$ | 12.2±1.356$^{a}$ |
| EG2 | 2.47±0.052 | 1.76±0.104$^{b}$ | 0.46±0.024$^{a}$ | 18.8±1.463$^{b}$ |
| EG3 | 2.43±0.032 | 1.73±0.034$^{ab}$ | 0.46±0.024$^{a}$ | 12±1.095$^{a}$ |
| EG4 | 2.31±0.176 | 1.71±0.039$^{ab}$ | 0.4±0.032$^{a}$ | 14±1.643$^{ab}$ |
| P value | 0.8006 | 0.0461 | P<0.0001 | 0.0326 |

$^{a,b,c}$ Means in the same column with different superscripts differ significantly (P< 0.05). CG-control group; EG1- 2g aqueous extract; EG2- 4g aqueous extract; EG3- 2g hydroalcoholic extract; EG4- 4g hydroalcoholic extract.

**Organs weight**

As can be seen in table 5, the incorporation of *Abies numidica* extracts to diet did not affect the weight of selected organs at day 21.

**Table 5: Effect of *Abies numidica* extracts on organs weight at the end of rearing period (mean±SEM).**

| Treatments | Organs | | |
|---|---|---|---|
| | Liver (g) | Heart (g) | Spleen (g) |
| CG | 9.33±0.82 | 2.99±0.31 | 0.22±0.04 |
| EG1 | 9.40±0.32 | 2.50±0.1 | 0.2±0.02 |
| EG2 | 8.57±0.72 | 2.71±0.29 | 0.26±0.05 |
| EG3 | 9.61±0.58 | 2.68±0.12 | 0.28±0.03 |
| EG4 | 9.26±0.43 | 2.87±0.27 | 0.23±0.04 |
| P value | 0.7849 | 0.6490 | 0.6191 |

CG-control group; EG1- 2g aqueous extract; EG2- 4g aqueous extract; EG3- 2g hydroalcoholic extract; EG4- 4g hydroalcoholic extract.

## DISCUSSION

This study showed that the use of 4g of hydroalcoholic extract of *Abies numidica* in broiler feeds has a beneficial effect on body weight gain during the overall rearing period and resulted significantly better weight gain in the first week of age compared to basal diet. The feed intake and feed conversion ratio were not affected (P>0.05) by dietary treatments, the lack of significant difference in relation to the control diet could be explained by the fact that the poultry may not acutely respond to flavor as previously suggested by Moran *et al.*[14].

Few studies have been performed on the effect of phytobiotics on microbial counts in broiler chicken [15, 9]. The main mechanism regulating the microbial ecology in the gut of chickens and the importance that changes in the intestinal microflora play in birds are still poorly understood [16]. There has been an upsurge in interest in the role that the normal intestinal flora, both aerobic and anaerobic plays in protecting against Salmonella infection [17-19].

As antibiotics, herbs and phytogenic products could control and limit the growth and colonization of a variety of pathogenic and nonpathogenic species of bacteria in chicks'gut. This may lead to a greater efficiency in the utilization of feed, resulting in increased growth and improved feed efficiency [20].

The caecum is one of the areas of greatest microbial activities in the gastrointestinal tract of chickens [21]. Paired caeca are situated at the junction of the small and large intestine and they normally contain a stable population of many different bacterial species [22, 23]. The results in the present study have no clear effects observed on the caecal microflora populations, which may have been due to an insufficient degree of replication.

Among the different blood constituents measured, Cholesterol and glucose concentration, were not significantly affected at the end of the experiment in broilers receiving plant extracts. While, triglyceride and protein concentrations were increased by the addition of 2g and 4g of aqueous extract to basal diet, respectively. Also, supplementation of two levels of *Abies numidica* extracts to diet has no harmful effect on liver, heart and spleen of broilers.

## CONCLUSION

The application of phytobiotics to chicken nutrition is at an early stage of implementation and will require further studies input to minimize the negative effects of them on the other nutritional or feed ingredients, improper combination, excessive use and inappropriate dosage.

Based on the obtained results of body weight gain, 4g/kg diet of hydroalcoholic extract of *Abies numidica* might be the appropriate supplementation level to broiler diets and more detailed studies are needed to determine the optimal dietary inclusion level in regard to effects on microbial numbers and performance.

More investigation of phytobiotics could lead to the development of feeding strategies for chickens to improve bird health and on-farm food safety that will reduce the use of antibiotics as growth promoters.

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