

# Plant of the *Juglandaceae* family as alternative to antibiotic growth promoters in broiler production

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Two experiments were conducted in order to evaluate the effect of the dietary addition of a plant belonging to the *Juglandaceae* family and strongly inhibiting *in vitro* growth of *Clostridium perfringens*, on the production results of male broilers (240 birds, Ross 308). From d11 to d28, the birds received a diet with a high content of fishmeal and animal fat, which was formulated to increase the intestinal growth of *Clostridium perfringens*. The diet was fed either not supplemented or supplemented with 1% freeze-dried plant material (green leaves) or salinomycin (60 mg/kg feed). At 28 d, the dietary inclusion of the plant material increased the body weight (1338 g vs. 1165g,  $P < 0.01$ ) and improved the FCR (kg feed/kg body weight, 1.38 vs. 1.52,  $P < 0.001$ ). Further, in one of the two experiments the intestinal *Clostridium perfringens* counts were numerically reduced in the range of 1.2-1.5 log units. The effect of salinomycin with respect to the improvement of the production results and intestinal growth inhibition of *Clostridium perfringens* was superior to that of the plant material. However, it is concluded that this plant of the *Juglandaceae* family has a potential as an alternative to antibiotic growth promoters.

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**Keywords:** *Juglandaceae*; broilers; growth promotion; *Clostridium perfringens*

## Introduction

After the ban of antibiotic growth promoters in the EU, the use of ionophore coccidiostats as feed additives is presently discussed and will probably be forbidden in 2012. Beyond their action as anticoccidials, ionophores exert antibiotic action against certain Gram-positive bacteria, e.g. *Clostridium perfringens* (*C. perfringens*) causing necrotic enteritis; a serious production disease connected with production losses due to increased mortality and growth depression (Immenseel et al., 2004). It is very likely that a future ban of ionophores will result in increased production problems related to an increased prevalence of *C. perfringens* which necessitates the search for appropriate alternative feed additives. This issue is currently addressed by the EU project REPLACE under the sixth framework programme. The aim of this project is to examine the possible use of plants and their extracts as natural alternatives to antimicrobials in feeds ([www.replace-eu.com](http://www.replace-eu.com)).

In the present study, a plant belonging to the *Juglandaceae* family was examined with respect to its efficacy to inhibit growth of *C. perfringens* and to promote broiler growth.

## Material and methods

### *In vitro* experiment

The effect of the plant of the *Juglandaceae* family on survival and growth of *C. perfringens* was examined in an *in vitro* test system. The analysis involved the use of a growth medium, which was supplemented with 10% extract of broiler small intestinal contents in order to simulate intestinal conditions. The medium was inoculated with  $10^7$  cells per ml of a *C. perfringens* field strain (strain

6963206 or PFGE type 37 according to Nauerby *et al.* 2003) isolated from a necrotic enteritis outbreak. The plant material was added in concentrations of 0%, 0.1%, 1% and 5% and incubations were performed over 4 hours. Samples were taken at 0, 2, and 4 hours of incubation and *C. perfringens* was quantified after 24 hours anaerobic incubation on tryptose–sulfite–cycloserine (TSC) agar plates.

### Production experiment

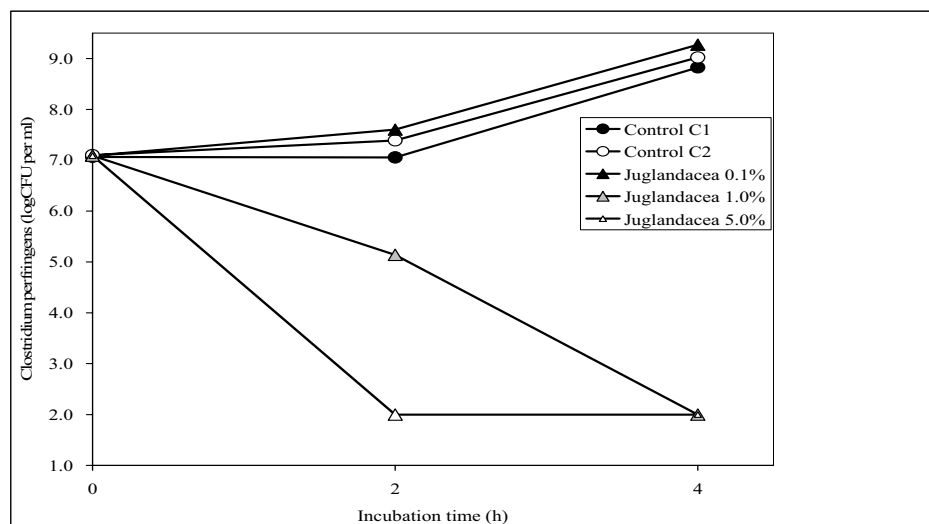
Two experiments were carried out with a total of 240 day-old male broiler chickens (Ross 308) over an experimental period of 28 days. In both experiments the birds were randomly divided into 3 experimental groups and housed in 12 floor pens (4 replicates). In the starter period (1-10 days), the birds received a diet of good quality. From day 11-28, the birds were offered a basal grower diet containing approximately 10% fishmeal and 10% animal fat (lard: tallow, 1:1), formulated to increase the intestinal growth of *C. perfringens*. Both starter and grower diet were offered without any supplementation (None), or supplemented with either 1% freeze-dried leaves from the *Juglandaceae* family plant (*Juglandaceae*) or with salinomycin (60 mg/kg feed).

At days 1, 10, 14, 21 and 28, the birds were weighed individually, and the feed intake per pen was registered. At the end of the experiment, 4 birds from each pen were killed by cervical dislocation and samples from the small intestine (ileum) and caeca were taken. The number of *C. perfringens* in intestinal samples was determined using TSC agar plates as described above.

The statistical calculation of results was performed using the GLM procedure of the SAS taking the effect of dietary treatment (None, *Juglandaceae*, Salinomycin), the effect of experiment (Exp. 1 and 2) and the interaction between experiment and treatment into consideration. Results are given as least-square means with a pooled standard error (SE).

## Results and discussion

As shown in *Figure 1*, the addition of the *Juglandaceae* plant material in concentration of 5% to the ileum extract medium strongly inhibited the growth of *C. perfringens in vitro*. After 2 hours of incubation, the bacterial numbers were below the detection limit (log 2 CFU/ml). The addition of 1% reduced *C. perfringens* with approximately 2 log units after 2 hours of incubation and after 4 hours, the counts were below the detection limit. Following addition of 0.1% of the plant material, no growth inhibiting effect on *C. perfringens* was registered. The bacterial counts increased up to log 9 CFU/ml over the incubation period, which was similar to the numbers obtained in the non-supplemented control incubations (C1, C2).



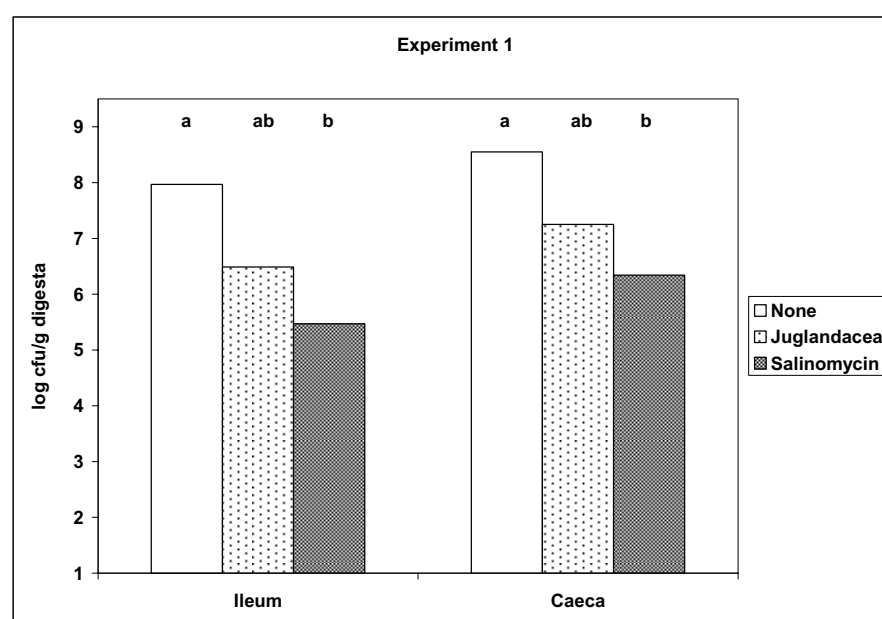
**Figure 1** *In vitro* growth of *C. perfringens* strain 6963206 in ileum extract medium after addition of different concentrations of a plant belonging to the *Juglandaceae* family

Throughout the *in vivo* experiment, broilers supplemented with material of the *Juglandaceae* family plant had higher body weights than those receiving the non-supplemented diet (Table 1). After 28 days, the birds receiving the plant material weighed approximately 170 g more than the broilers without dietary supplementation. However, the addition of salinomycin was still significantly superior to that of the *Juglandaceae* addition. The feed intake of birds tended to increase following dietary supplementation with the plant material and with salinomycin ( $P > 0.05$ ). The highest feed intake was registered in the group receiving the diet containing salinomycin (results not shown). The feed conversion ratio (FCR) was significantly improved ( $P < 0.05$ ) by the supplementation of the plant material as well as salinomycin (Table 1). There was no significant difference between salinomycin and the *Juglandaceae* with respect to their effect on the FCR. However, salinomycin seemed to improve the FCR to a higher extent than the plant material (Table 1).

**Table 1 Production results of broilers fed diets either not supplemented or supplemented with 1% freeze dried *Juglandaceae* leaves or salinomycin (60 mg/kg feed)**

	None	Juglandaceae	Salinomycin	SE	P-value
Body weight, g					
10 d	168 <sup>b</sup>	191 <sup>a</sup>	208 <sup>a</sup>	6.4	0.001
14 d	299 <sup>b</sup>	340 <sup>ab</sup>	380 <sup>a</sup>	14.3	0.003
21 d	649 <sup>b</sup>	735 <sup>b</sup>	843 <sup>a</sup>	31.4	0.002
28 d	1165 <sup>c</sup>	1338 <sup>b</sup>	1488 <sup>a</sup>	43.9	< 0.001
FCR, g feed /g body weight					
10 d	1.44 <sup>a</sup>	1.26 <sup>b</sup>	1.25 <sup>b</sup>	0.039	0.005
14 d	1.46 <sup>a</sup>	1.32 <sup>b</sup>	1.27 <sup>b</sup>	0.035	0.004
21 d	1.42 <sup>a</sup>	1.29 <sup>b</sup>	1.19 <sup>b</sup>	0.039	0.003
28 d	1.53 <sup>a</sup>	1.38 <sup>b</sup>	1.29 <sup>b</sup>	0.035	< 0.001

With respect to *C. perfringens* growth, a significant interaction was observed between experiment and the dietary treatment. In the first trial (Figure 2), the plant addition numerically reduced the numbers of *C. perfringens* by 1.5 and 1.2 log units in ileal and caecal content, respectively, whereas no effect of the plant addition was found in experiment 2 (Figure 3). In both experiments, the supplementation with salinomycin resulted in a reduction of the *C. perfringens* ( $P < 0.05$ ) in the range of 3 log units in intestinal samples (Figure 2, and Figure 3).



**Figure 2 Counts of *Clostridium perfringens* in intestinal content of broilers fed diets either not supplemented or supplemented with 1% freeze dried *Juglandaceae* leaves or salinomycin (60 mg/kg feed)**

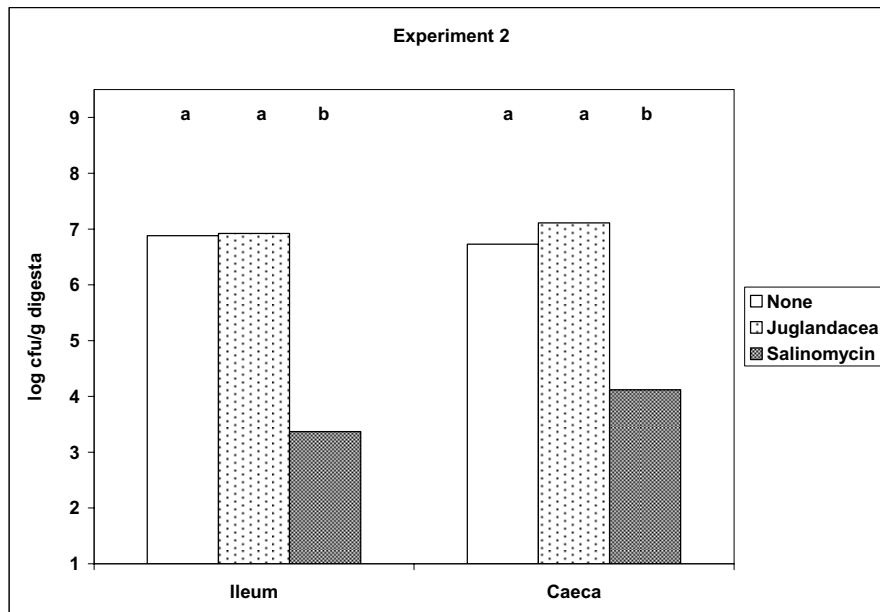


Figure 3 Counts of *Clostridium perfringens* in intestinal content of broilers fed diets either not supplemented or supplemented with 1% freeze dried *Juglandacea* leaves or salinomycin (60 mg/kg feed)

The occurrence of high intestinal *C. perfringens* numbers has been shown to be associated with poor broiler growth (Lovland and Kaldhusdal, 2001), which is partly explained by the ability of *C. perfringens* to de-conjugate bile acid, thus reducing fat digestion (Knarreborg *et al.*, 2004). It is therefore surprising, that the addition of the plant in both broiler experiments consistently improved growth, whereas suppression of *C. perfringens* was less consistent. But, the potential active compound or compounds of the *Juglandacea* plant, responsible for the antimicrobial and growth promoting effect, respectively, are so far unknown and elucidating their mode of action will be the matter of future research.

It is concluded from the present results that this plant of the *Juglandaceae* family has a potential as an alternative to antibiotic growth promoters.

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