Effect of a dietary supplementation combining a probiotic and a natural anticoccidial in broiler chickens

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In order to improve the growth performance of broilers and prevent coccidiosis in our farms, two groups of broiler chicks (Hubbard F15) were bred under the same conditions for a period of 52 days. "Experimental group" received an aliment added with a natural anticoccidial based on herbal extracts and a probiotic but water free of antibiotics while "Control group" received the same food without probiotic and natural anticoccidial, but added with a chemical anti-coccidial and a water containing antibiotics. The obtained results showed, in support of the subjects belonging to the "experimental group", a difference in weight without bearing statistically significant difference, higher consumption rates accompanied by a low mortality rate and a length of upper intestines. The enumeration of oocyst excretion showed a marked increase, characterized by three peaks corresponding to three episodes of coccidiosis in the "control group" and a much smaller increase without clinical expression in the "experimental group". The autopsy of the animals sacrificed in the "experimental group" showed the total absence of clinical coccidiosis lesions unlike those performed to the subjects in the "control group" who presented a final average lesion score of 3.5 in D22, 3.8 and 3.2 respectively, on D30 and D45, confirming the recurrence of coccidiosis. The weight of the plucked and eviscerated carcasses and of the edible offal of the chickens who consumed food supplemented with probiotics and natural anticoccidial based on herbal extracts are superior to the weight of the subjects belonging to the "control group".

Key words: Pediococcus acidilactici, Yucca schidigera, Trigonella foenum-graecum, supplementation, broiler, feeding, zootechnical performance.

INTRODUCTION

The antibiotics stand among the most common additives used to improve feed efficiency, growth rate and consequently increase the productivity and profitability of poultry farms. However, they favored the emergence of antibiotic residues in the food chain, a large number of resistant animal bacterial strains (Ungemach et al., 2006) and allergic reactions of the consumers, as well as failures of the antibiotic treatment on humans (Corpet,
Five hundred and twenty, 1 day-old chicks of Gallus gallus domesticus belonging to the Hubbard F15 strain, mixed sexes, homogeneous weight, coming from the same hatchery, were divided into two groups (n=260) with five repetitions of 52 subjects each. They were put in place in January 2012 to be raised under the same breeding conditions for a period of 52 days, in a traditional building, partitioned as to provide ten areas of life, 6 m² each, undergoing the same environmental conditions.

The flouro-type food used, based on sweet corn, soybean cake, wheat bran, di-calcium phosphate, calcium and vitamin-mineral concentrates was produced especially for our experiment, based on a formula taking into account the three breeding phases [starting (D1-D28), growing (D29-D42) and finish (D43-D52)].

The animals in the “experimental group” received a drinking water free of additives and an aliment supplemented with the anticoccidial “Yuquina XO™” (NOR- FEED, South France) based on the herbal extracts of “Y. schidigera and T. foenum graecum” at a rate of 0.5 kg/t and lyophilized P. acidilactici CNCM MA18/5M strain (Bactocell MA, France) at a rate of 10⁷ CFU/g, while those subjects belonging to the “control group”, were administered the same dietary aliment, free of probiotic and herbal extracts, but supplemented with a chemical anticoccidial (Robenidine, Cystostat) and water rich in antibiotics, the treatments most frequently administered on the Algerian field throughout the breeding period.

The subjects of the two groups were vaccinated on D6 against Newcastle disease (UNICLEVA®), the vaccination being repeated on D15 and D19 against Gumboro disease (IBD L CEVA®).

Zootechnical parameters and intestinal morphometry

The average live weight was calculated by weighing 200 subjects (starting phase) and 100 subjects (growing and finish phases). Feeds distributed for control and experimental groups were weighted at the end of each breeding phase (D28, D42 and D52 of age) to calculate feed conversion ratio.

Death cases were recorded daily and mortality rates were determined at the end of each breeding phase (D28, D42 and D52). We have not recorded death cases suffered during the first three days due to transport stress.

The intestinal morphometry was performed on 10 subjects from each group, on D28, D42 and D52. After anesthetic treatment, the animals were sacrificed by bleeding and the length of the intestine as a whole (from the duodenum gizzard-junction up to the distal end of the colon) was measured.

Oocysts excretion and intestinal lesion scores

A 20 g sample of fresh droppings, issued on litter, is collected daily from the five areas from each batch during the period (D13 to D52). The oocysts, contained in 5 g of droppings of each sample were analyzed by the method of Mc Master, according to concentration, by flotation in a dense saturated solution of magnesium sulfate (density: 1.3) (EUZEBY 1981, 1987). The average number of oocysts is expressed per gram of droppings (o.p.g).

The lesion scores were determined based on the autopsy of five subjects taken from different locations in the building and sacrificed, at the first suspicious signs of coccidiosis (diarrhea and death), according to the method of Johnson and Reid (1970) as amended by Bouheller (2005).

The research of the lesions was carried out systematically at the autopsy of all fresh mortalities.

Carcass yield

To evaluate carcass yield and perform the intestinal morphometry(*) at the end of the breeding cycle (D52), 10 subjects were
randomly selected from each group, previously fasted (for 12 h), were individually weighed, sacrificed by bleeding and plucked. After cold storage (+8°C) for a period of 12 h, the carcasses - head and legs were first cleared and then reweighed. After evisceration, the edible organs (gizzard, heart and liver), the abdominal fat and carcass were systematically collected and weighed separately.

Statistical analysis

The statistical analysis was performed based on the test of homogeneity applied on two means of two populations ("experimental" and "control" groups). We used the hypotheses test \( H_0 \) and \( H_1 \) based on the calculation of the critical ratio (CR) on the sample database which is compared to the value of the table of the normal distribution with threshold value \( \alpha = 5\% \).

Formulation of hypothesis: \( H_0: \mu_1 = \mu_2 \) and \( H_1: \mu_1 \neq \mu_2 \)

Sampling distribution is a Student distribution because standard deviations are unknown. There are estimated from the samples data. The Student's distribution is approximated by the Gauss distribution because size of sampling is greater than 30; then \( t (\alpha/2, n_1 - 1 + n_2 - 1) = z_{\alpha/2} \).

By default, the significance's level \( \alpha = 5\% \) brings us to compare the statistically calculated (Zcal) to the tabulate value \( z_{\alpha/2} = 1.96. \)

If Zcal < \( z_{\alpha/2} \), then hypothesis \( H_0 \) is accepted: the two population means are homogeneous.

RESULTS AND DISCUSSION

Zootechnical parameters

The results of the zootechnical and morphometric parameters obtained at the end of each breeding phase are reported in Table 1. The results showed a difference in weight between the subjects of the control and experimental groups (CG and EG) at the end of breeding (2678 g vs 2791 g, respectively), but no statistically significant difference (\( \alpha=5\% \)). The positive effect of this probiotic on growth has been demonstrated in fattening pigs, broilers and laying hens by Awaad et al. (2003), Vittorio et al. (2005), Chevaux et al. (2006), Di Giancamillo et al. (2008), Alkhalf et al. (2010) and Abd-El-Rahman et al. (2012).

The best feed conversion ratio, achieved by the subjects of the "experimental group" versus the ones achieved by the subjects in the "control group", counting for the three phases of breeding (1.32 vs 1.48: starting, 1.57 vs 1.73: growing and 2.39 vs 2.83: finish) could find an explanation based on the positive effect of lactic acid bacteria on feed efficiency, which was reported by Jin et al. (1998) and Simon et al. (2001).

We recorded a high rate of mortality in the "control group" as compared to the "experimental group" (14.7% vs. 6.5%). The situation on the high mortality observed in the "control group" appears to be consistent with the development of coccidiosis on \( D_{18} \) (10.1%: starting). It could be a result of the low level of the anticoccidial in the food. As for the low mortality recorded by the "experimental group", it appears to be the result of anticooccidial based on herbal extracts, introduced preventively.

The average length of the intestines of chickens supplemented with probiotics is significantly higher than those having received the food without additives at the end of the three phases of breeding. This increase reaches 10% (\( p<0.001 \)) on \( D_{28} \), approximately 7% (\( p<0.001 \)) on \( D_{42} \) and 15% (\( p<0.05 \)) on \( D_{52} \). According to Samli et al. (2007), Enterococcus faecium NCIMB 10415 increases the weight gain, the conversion rate and the size of the villi in the ileum.

Oocyst excretion and lesion scores of the coccidiosis

**Enumeration of oocysts**

Average value of o.p.g. for each day and group during \( D_{13} - D_{52} \) period is graphically represented in Figure 1. The obtained results show a pronounced increase and statistically significant oocyst excretion in the "control group" characterized by three peaks on \( D_{19-24}, D_{30} \) and \( D_{45} \) corresponding to three episodes of coccidiosis. This ascertainment is confirmed by the appearance of blood in faeces (Photo 1) strengthening our hypothesis regarding the anticooccidial under-dosage used, or the potential resistance of coccidia. In the experimental group, the excretion is much smaller and appears with a slight delay (\( D_{25} \) and \( D_{37} \)).

However, it should be noted that the sharp decreases in oocyst excretion observed in the control group are consistent with the administration, on \( D_{22} \) and \( D_{30} \) of sulfonamides (Coccidiopan®) and on \( D_{45} \), of a chemical anticooccidial (Toltrazuril, Baycox®). These treatments may have reduced the gap between the zootechnical performance in experimental group and control group.

**Lesion scores of coccidiosis**

Upon observation of mortality cases (\( D_{22}, D_{30} \) and \( D_{45} \) with onset of diarrhea (Photo 1) on the litter of the control group, the autopsy of the animals sacrificed from the two groups revealed the average final lesion indexes reported in Table 2.

The autopsy of the animals sacrificed in the experimental group showed no clinical coccidiosis lesion (Photo 2a) during the entire breeding period (scores below 2). On the contrary, the subjects in the control group showed pathognomonic signs of coccidiosis (Photo 2b). The mean lesion score of 3.5 obtained on
Table 1. Zootechnical and morphometric parameters.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>End of the three phases of breeding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>( D_{28} )</td>
</tr>
<tr>
<td>&quot;Control&quot;</td>
<td>Average live weight by subject (g)</td>
<td>996 ± 23</td>
</tr>
<tr>
<td></td>
<td>Feed ratio</td>
<td>1.48</td>
</tr>
<tr>
<td></td>
<td>Mortality rate (%)</td>
<td>10.1</td>
</tr>
<tr>
<td></td>
<td>Average length of the intestines (cm)</td>
<td>191 ± 13</td>
</tr>
<tr>
<td>&quot;Experimental&quot;</td>
<td>Average live weight by subject (g)</td>
<td>1011 ± 27</td>
</tr>
<tr>
<td></td>
<td>Feed ratio</td>
<td>1.32</td>
</tr>
<tr>
<td></td>
<td>Mortality rate (%)</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>Average length of the intestines (cm)</td>
<td>212 ± 17</td>
</tr>
</tbody>
</table>

Figure 1. Kinetics of oocyst excretion in Control and Experimental Groups.

Photo 1. Presence of bloody droppings on litter of "control group" at (a) \( D_{22} \) and (b) \( D_{30} \).
Table 2. Average final lesion indexes obtained for the two groups at the end of each breeding phases.

<table>
<thead>
<tr>
<th>Groups</th>
<th>End of each breeding phases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D_{22}</td>
</tr>
<tr>
<td>Control</td>
<td>3.5</td>
</tr>
<tr>
<td>Experimental</td>
<td>1.5</td>
</tr>
</tbody>
</table>

D_{22} revealed the first episode of clinical coccidiosis, those of 3.8 and 3.2 obtained on D_{30} and D_{45} respectively and confirmed the recurrence of coccidiosis.

**Autopsy of fresh cadavers**

The autopsy of the fresh cadavers from the “experimental group” revealed the presence of punctate congestions scarcely scattered in the duodenum in two sporadic cases on D_{24} and tracheitis in a sporadic case on D_{35} (Photo 3a and b). However, the autopsy of the subjects of the Control group revealed the presence of pericarditis associated with a pericarditis translating into a colibacillosis complication in two sporadic cases on D_{22} and the presence of macerated blood at the level of the intestines and caeca marking the clinical episodes of coccidiosis on D_{22}, D_{30} and D_{45} (Photo 3c and d).

**Carcass yield**

The average weights and yields of carcasses obtained at the end of breeding (D_{52}) are reported in Table 3. We clearly see that the weight of the plucked and eviscerated carcasses is higher in the chickens that have been administered a diet supplemented with probiotics and herbal extracts. The weight of the edible offal (gizzard, heart and liver) of the experimental group is higher than those of the control group; as to the abdominal fat, the weight difference between the two groups was not significant.

It could be mentioned that the association “probiotics and herbal extracts” does not induce excess abdominal fat as compared to the Control group which is likely to endorse the weight of the eviscerated carcass (the abdominal fat being removed at the slaughterhouse). Indeed, anatomical differences were highlighted between the animals (broilers) fed either with an aliment made of wheat (D+) or with food made from corn-soybean (D-). The pro-ventricle and gizzard are more developed in D+, by way of contrast, the small intestine is more developed in D- (Peron et al., 2006; Garcia et al., 2007; Rougiere et al., 2009; Rougiere and Carré, 2010). Rougiere and Carré (2010) were also able to highlight the food retention time in the gizzard and significantly longer pro-ventricles in D+ relative to D-, whereas at intestinal level, no difference was visible.
Photo 3. Lesions at autopsy of fresh cadavers from the two groups, viz: Experimental group (a) Punctate congestions of the serous (D24) (b) Tracheitis (D35); Control group (c) Pericarditis and perihepatitis (D27) and (d) Presence of blood in the ceaca (D45).

Table 3. Average weights and carcass yield.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Weight (g) of ( \bar{X} \pm SEM )</th>
<th>Carcass yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Plucked carcasses (*)</td>
</tr>
<tr>
<td>Control</td>
<td>2678 ± 29</td>
<td>2182 ± 153</td>
</tr>
<tr>
<td>Experimental</td>
<td>2791 ± 27</td>
<td>2423 ± 137</td>
</tr>
</tbody>
</table>

(*): carcasses to which have been removed the head and legs.

**Conclusion**

The probiotic *P. acidilactici*, used alone or in combination in poultry feed, increases the gain of live weight, meat, edible offal and size of the intestines without causing an excess of abdominal fat. It improves the dietary efficiency by acting favorably upon the balance of the intestinal flora of chickens.

The low oocyst excretion and the absence of clinical signs of coccidiosis observed in the subjects belonging to the experimental group could be the result of the effectiveness of anticoccidial based on herbal extracts (*Y. schidigera* and *T. foenum graecum*).

Faced with the alarming situation of excessive use of...
anticoccidial (antibiotics and other chemicals) in poultry breeding, this biological product based on herbal extracts, requiring no waiting time, could stand as a real alternative product.

The combination of these biological products would, in addition, allow us maintain a satisfactory level of production, address the issues related to other antibiotics resistance and anticoccidials, preserve the quality of chicken meat (drug residues) and consequently, consumers’ health.

Conflict of Interest

The author(s) have not declared any conflict of interest.

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