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Impact of Dietary Supplementation with *Pediococcus acidilactici* on Zootechnical and Sanitary Performances of Broilers in Algeria

[1]Djezzar Redha, [2]Benamirouche Karima, [2]Baazize-Ammi Djamila, [2]Khoubei Amine,
[2]Merrouki Aicha, [3]Maghni Euthmane and [2]Guetarni Djamel
[1]Higher National Veterinary School of Algiers, Ageria Algiers, El Harrach, Algeria
[2]Faculty of Agro-Veterinary and Biological Sciences,
[3]Faculty of Economics and Management Sciences, Saad Dahlab University, Blida, Algeria

Abstract: Two groups of 1763 broiler chicks of (Hubbard F15) strain and of mixed sexes were bred under the same breeding conditions for a period of 58 days. The experimental group received a food supplemented with probiotics *Pediococcus acidilactici* in a quantity of 10^9 cfu kg^{-1} of food and water free of antibiotics. The control group received the same food but without probiotic supplement whereas the drinking water was treated according to the protocol closest to those currently used in aviary breeding on the Algerian territory. The results related to the zootechnical performance emphasized that the addition of probiotic has significantly improved body weight gain during the growing phase, thing translated into a better feed conversion ratio and a mortality rate significantly improved after the 3rd week. The lesions observed at the autopsy of the corpses from the control group do not reflect any specific pathology. This situation could be explained by the overuse of antibiotics which triggered, relative to the animals belonging to this group, an effective coverage against various microbial attacks while those belonging to the experimental group are being indicatives of Colibacillary complications and coccidiosis episodes treated on D_{30} and D_{43} with an anticoccidial drug (Toltrazuril, Baycox®) requiring a minimum waiting period of 12 days. The monitoring of the evolution of the floras made it possible to highlight the stimulating effect of *Pediococcus acidilactici* on the growth of lactic bacteria on the one hand; the fluctuating loads in Enterobacteriaceae observed during D_7-D_{19} which could be explained by the traditional breeding conditions and the immune status of the broiler that is yet to become mature as well as their regression (starting from D_{19}) accompanied by the stabilization of the lactic flora at relatively large thresholds. Therefore, researchers can assert that there is a close relation between the barrier effect and the improvement of the zootechnical performance.

Key words: *Pediococcus acidilactici*, probiotic supplementation, broilers, feeding, zootechnical performance, Algeria

INTRODUCTION

The use of antibiotics in the poultry farms is a widespread practice. Facing the prohibition of their use several alternative research paths have been explored in view of the substitution thereof with the use of probiotics (Lactobacilli). These living microorganisms, once ingested, exert a beneficial effect on the health of the host thus and so improving its intestinal balance (Fuller, 1991).

The claimed zootechnical effectiveness of the probiotics would trigger an improvement of the zootechnical performance and sanitary conditions or even the animals wellbeing (Temim *et al*., 2009). Various researchers have reported during the use of *Pediococcus acidilactici*, positive effects on the balance of the intestinal flora, thing translating into an improved zootechnical and sanitation performance of the chickens (Jin *et al*., 1998; Vittorio *et al*., 2005). The disparity between the results of the different tests would relate to the fact that these products act specifically by modulating the flora of the host which varies, itself according to the breeding conditions (Ahmad, 2006).

The present research focusing on the value of the dietary supplementation with *Pediococcus acidilactici* during a complete breeding cycle of the broilers under the local breeding conditions, aims at equally assessing the impact of this probiotic on the growth, mortality and evolution of the digestive flora.

Corresponding Author: Guetarni Djamel, Faculty of Agro-Veterinary and Biological Sciences, Saad Dahlab University, Route de Soumaa Ouled Yaich, BP 270 Blida, Algeria

MATERIALS AND METHODS

Animals and feed: Three thousand five hundred twenty six, 1 day old chicks of Hubbard strain F15, mixed-sex, coming from the same hatchery were weighed and divided into two, groups (n = 1763) of homogeneous weight (48.3 g). They were introduced during late January, 2010 for a period of 58 days in a traditional style building located in Boufarik (governorate of Blida), partitioned so as to provide two living areas of 180 m^2 each and undergoing the same environment conditions.

A starchy food type was distributed *ad libitum* during the three phases of breeding: starting (D_1-J_{10}), growing (D_{11}-D_{42}) and finishing (D_{43}-D_{58}). The animals of the experimental group received food supplemented with Bactocell® (*Pediococcus acidilactici* MA18/5M, Lallemand SAS, France in a quantity of 10^9cfu kg^{-1} from day 1 (D_1) and water free of additives, namely, free of antibiotics. Those animals belonging to the control group received the same food free of probiotics but their water was supplemented with antibiotics, treatments most frequently administered in the field.

The subjects in both groupes were vaccinated against Newcastle disease UNI L CEVA® on D_6 and boosted with NEW L CEVA® on D_{19} and also against the Gumboro disease IBD L CEVA® on D_{15}.

MATERIALS AND METHODS

Zootechnical parameters: The living weight, feed conversion ratio and mortality rate are being determined at the end of each breeding phase (D_{28}, D_{42} and D_{58}). Researchers have not considered the deaths cases registered during the first 3 days due to the stress caused by the transport conditions.

Lesion parameters: The death cases were systematically subjected to autopsy.

Monitoring of the digestive flora evolution: Researchers assessed the action of probiotics on the intestinal flora by seeking and counting the Lactobacillus and Enterobacteriaceae flora within the intestinal mass of the sacrificed animals.

Sampling plan: The samplings taken out of the intestinal mass were performed aseptically after sacrificing the animals that have been earlier subjected to a 4 h starvation on a weekly basis in view of monitoring the evolution of the floras (periods D_7-D_{13} and D_{25}-D_{55}) and every 2 days as to highlight the settlement time for a sufficient lactic flora (D_{13} and D_{25}).

Sample preparation: For a sampling exceeding 25 g, researchers have aseptically mixed the intestinal masses of 3 subjects sacrificed for the purpose of sampling during the period D_7-D_{13} with only 2 subjects sacrificed during the period D_{15}-D_{55}.

About 25 g of intestinal mass are being introduced aseptically into a sterile bag type Stomatcher 400 preloaded with 225 mL of Tryptone Salt Broth (TSE, Ideal Labo®). The content is being homogenized by using a Stomacher® device for 8 min.

Starting from the mother solution obtained herein, a series of decimal dilutions (10^{-1} up to 10^{-8}) is carried out in tubes containing 9 mL of TSE. These dilutions will serve to the research of the lactic flora and Enterobacteriaceae.

Detection and counting of the lactobacilli flora: The counting of intestinal lactobacilli flora is being performed based on the conventional method of counting the colonies in MRS Agar (Bourgeois *et al*., 1991). About 0.1 mL of each dilution was aseptically placed on the surface of a Petri dish box containing a MRS Agar and then carefully spread. After 48 h of incubation at 37°C under anaerobic conditions, the boxes containing a number of typical colonies ranging between 30 and 300 are being selected there of for further counting of lactic acid bacteria. The counting is being performed according to the following Eq. 1:

$$N = \frac{\Sigma c}{1{,}1 \times d} \times 0{,}1 \quad (1)$$

Where:

N = Concentration of viable cells (cfu/mL)
Σc = Sum of colonies counted on the two successive boxes
d = The dilution ratio corresponds to the first dilution
0, 1 = Volume of the suspension seeded (mL)

Detection and counting of Enterobacteriaceae: Abot 1 mL of each dilution was aseptically placed in a sterile Petri dish then were added about 15 mL of VRBG super cooled agar culture medium (40-45°C). Homogenize the seeded Petri dishes by slow horizontal rotation movements. After solidification of the agar, the dishes are being incubated. After 24 h of incubation at 37°C, under anaerobic conditions, the dishes containing a number of typical colonies ranging between 30 and 300 are being selected there of for further counting of lactic acid bacteria. The counting is being performed according to the following Eq. 2:

$$N = \frac{\Sigma c}{1.1 \times d} \quad (2)$$

N = Concentration of viable cells (cfu/mL)
Σc = Sum of the colonies counted on the two successive dishes
d = Dilution rate corresponding to the first dilution

Statistical analysis: The statistical analysis was performed based on the test of homogeneity applied on two means of two populations (experimental and control groups). Researchers used the hypotheses test (H0 and H1) 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 $\alpha = 5\%$.

RESULTS AND DISCUSSION

Zootechnical parameters: The results of the zootechnical parameters obtained at the end of each breeding phase are shown in Table 1. The results of the zootechnical parameters obtained at the end of the breeding process emphasized a difference in weight between the subjects of the Control and Experimental groups (2788 vs. 2701 g, respectively) however from the statistical point of view, the difference beared little significance ($\alpha = 5\%$). Nevertheless, the statistical treatment of data by period (starting, growing, finishing) gave a CR of 0.016; 4.16 and 1.71, respectively. Only the weight difference recorded between the two groups (experimental and control) and corresponding to the growing period is significant that is to say that the populations are heterogeneous.

The best feed conversion ratios-recorded by the experimental group subjects as compared to those belonging to the control group during the three breeding phases (starting: 1.35 vs 1.50; growing: 1.51 vs. 1.68; finishing: 2.41 vs. 2.86) could be explained based on the positive effect of lactic bacteria on the feed efficiency which was a fact reported by Jin *et al.* (1998) and Simon *et al.* (2001).

The increased mortality rate recorded relative to the experimental group as compared to the control group (8.08 vs. 4.1%, respectively) is the result of the two pathological episodes of coccidiosis occurred during which researchers have counted more than half of the total mortality cases (74/142 subjects). The low mortality rate identified in the control group seems to be the consequence of effective drug coverage. The animal performance achieved by the subjects of the experimental group also proves to be convincing, better than those achieved by the subjects of the control group.

Lesion parameters: The autopsy performed on the Control corpses revealed an inceptive inflammation of the pectoral muscle (D_{37}) probably the result of an overweight condition of the animal predisposed to recurrent rash due to the sternum friction against the bedding (Fig. 1a) on the one hand and a rather hypertrophic liver (D_{37}) but heavily congested, describing caecal content in slurry (Fig. 1b), on the other hand. The encountered lesions not reflecting any specific pathology could be explained by the overuse of antibiotics that triggered an effective coverage against

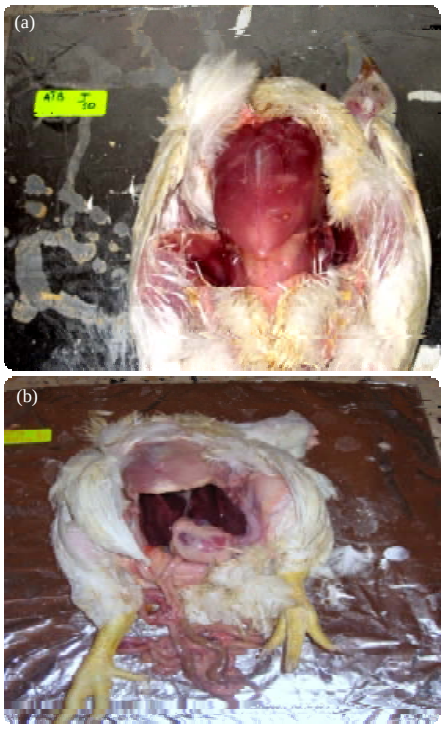

Fig. 1: The lesions observed at the autopsy of the dead corpses from the control group; a) Inflammation of the breast muscle (D_{37}); b) Highly hypertrophic and heavily congested liver and caecal content in slurry (D_{37})

Table 1: Zootechnical parameters

Groups	Parameters	D_{28}	D_{42}	D_{58}
Experimental	Average weight living subject (g)	1007±11.20	1771±9.80	2701±7.40
	Feed conversion ratio	1.35	1.51	2.41
	Mortality rate (%)	2.90	5.70	8.05
Control	Average weight of the subject (g)	1006±13.80	1872±11.70	2788±7.90
	Feed conversion ratio	1.50	1.68	2.86
	Mortality rate (%)	1.87	2.20	4.10

various microbial attacks for the animals belonging to this group. The autopsies performed on the experimental dead bodies revealed:

- Pericarditis and Perihepatitis resulting into a colibacillary complication in two sporadic cases (D_{23}) (Fig. 2a)
- Swollen and congested intestines encountered at a sporadic death case in D_{30} (Fig. 2b)
- Caeca distended with blood marking a first episode of caecal coccidiosis (D_{36}) (Fig. 2c)
- The presence of blood soaked into the intestines and particularly caeca, marking a second episode of caecal coccidiosis (D_{43}-D_{48}) (Fig. 2d)

The observed lesions are indicative of the colibacillus-related complications and coccidiosis episodes. Indeed, coccidiosis, major and recurrent pathology in the Algerian farms is present in the experiment.

Facing this severe symptomatology, researchers were forced to introduce during D_{30} and D_{43}, a specific treatment of coccidiosis, nevertheless compatible with the lactic flora; the treatment was based on Totrazuril (Baycox®) equivalent to 1 mL L^{-1} of water over 48 h.

Evaluation of the probiotics action on the digestive flora: The result of the Lactic and Enterobacteriaceae flora counts are being shown in Table 2 and graphically shown in Fig. 3.

Lactic flora: The results of the lactic flora count revealed its presence within the intestinal mass of the birds sacrificed ever since the first sampling (D_7). Starting from D_{15}, it begins to evolve positively, actually installing in D_{19}. This situation could be explained by the small amount of food ingested that is closely related to the subject's weight that increases at 44 g (D_1), 124 g (D_7), 317 g (D_{14}), hitting a double value 624 g (D_{21}) and reaching the weight of 1007 g at the end of the growing phase. The addition of *P. acidilactici* in chicken feed ever since the first breeding day would favor the growth and installation of lactobacilli. However, Mountzouris *et al.* (2007, 2010) showed that the mixture of *P. acidilactici* with other lactic bacteria such as Lactobacillus, Bifidobacterium and Enterococcus was responsible for the increase in the population of Lactobacillus in the ileum and caeca. This stimulating effect of *Pediococcus acidilactici* on the growth of lactic bacteria can be explained by the positive interaction between the two which can be caused by either reducing the gastrointestinal pH or the production of metabolites.

The period describing D_{21}-D_{42} is being characterized by a stable flora, except for D_{25} for which researchers have noted a slight drop which could be explained by the incidence of the first episode of coccidiosis, a major aviary parasitose which is well known in the farms, confirmed by necropsy findings on the dead

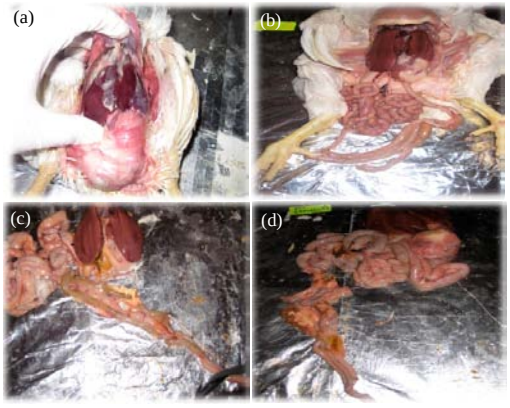

Fig. 2: The lesions observed at the autopsy of the corpses from the experimental group; a) Fibrinous pericarditis and perihepatitis (D_{23}); b) Swollen and congested intestines (D_{30}); c): Caeca distended with blood (D_{36}); d) The presence of blood soaked into the intestines and caeca (D_{43}-D_{48})

Table 2: Results of the lactic and Enterobacteriaceae flora counts starting from the intestinal mass of the sacrificed birds belonging to the experimental group during the breeding period

	Lactic flora		Enterobacteriaceae	
Sampling plan	cfu mL^{-1}	Log cfu mL^{-1}	cfu mL^{-1}	Log cfu mL^{-1}
D_7	17.9×10^3	4.25	34.5×10^3	4.55
D_{13}	1.9×10^3	3.28	10^3	3.00
D_{15}	0.06×10^3	1.77	74.5×10^3	4.87
D_{17}	1.6×10^3	3.20	545	2.74
D_{19}	216.4×10^6	8.33	195.5×10^3	5.29
D_{21}	229.1×10^6	8.36	3.5×10^3	3.54
D_{23}	105×10^6	8.02	136×10^3	2.13
D_{25}	9.9×10^6	7.00	145	2.16
D_{34}	130.9×10^6	8.11	1	0.00
D_{41}	727.3×10^6	8.86	1	0.00
D_{48}	16×10^3	5.80	51.8×10^3	4.71
D_{55}	0.75×10^3	2.87	145	2.16

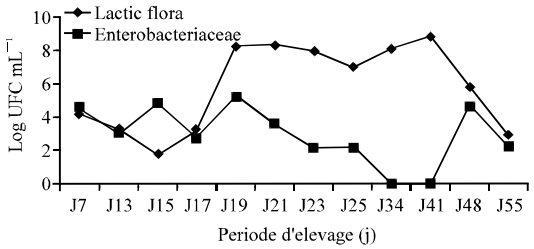

Fig. 3: Evolution curve of the lactic and Enterobacteriaceae flora counts starting from the intestinal mass of the sacrificed birds belonging to the experimental group during the breeding period

subjects during the period D_{30}-D_{36} (Fig. 2b and c). The use of the anticoccidial Toltrazuril (Baycox®) since, the incidence of coccidiosis in D_{30} did not inhibit the growth of the lactic flora. Researchers can therefore strengthen the idea that Toltrazuril is compatible with the lactic flora. The negative evolution of the lactic flora observed between D_{42} and the end of the breeding period (D_{58}) is due to the voluntary cessation of the diet supplementation with probiotics.

Enterobacteriaceae: The Enterobacteriaceae count results shown in Table 2 have revealed their presence during the period D_7-D_{19} also describing some loads fluctuation that could be explained by the traditional-type breeding conditions and immunity status of the chicks that are yet to become mature.

Their gradual Decline (D_{19}), accompanied by the stabilization of the Lactic flora at relatively high thresholds is probably due to the antagonistic effect of the Lactic bacteria explained by several mechanisms, namely, the production of metabolites of which the bacteriocins and lactic acid (pH drop) trigger the intestinal environment favorable to the growth of lactic bacteria and however unfavorable to the growth of harmful bacteria and pathogens (Patterson and Burkholder, 2003). The results are similar to those reported by Idoui *et al.* (2009) showing a decrease of the Enterobacteriaceae in chickens receiving the probiotic *Lactobacillus plantarum.*

Their disappearance between D_{34} and D_{41} appears to be the result of the anticoccidial treatment (Toltrazuril) acknowledged being compatible with the lactic flora. The reappearance of this population on D_{41} and the constant increase thereof up to D_{48} was then followed by a decrease up to the end of the breeding. Indeed, the voluntary cut-off on D_{40} of the chickens dietary supplementation with probiotic *Pediococcus acidilactici* could be the cause of the imbalance of the intestinal flora (decrease of the lactic bacteria and augmentation of the Enterobacteriaceae). According to Ahmad (2006) and Kabir (2009), the probiotic ingested (exogenous bacteria) plays a role in maintaining the balance of the endogenous flora, stimulates the growth of Lactobacilli and on the other hand, reduces the proliferation of the pathogens, probably by the phenomenon of competitive exclusion.

The increase of the Enterobacteriaceae that took place between (D_{41}-D_{48}) seems to be related to the emergence of enteritis lesions of unknown origin that caused the deaths of 12 subjects during the period D_{44}-D_{48}. The decrease in the Enterobacteriaceae manifested as of D_{48} up to the end of the breeding could be explained by the administration of Toltrazuril following the 2nd episode of coccidiosis.

The analysis of the overall results enables us to say that a barrier effect exerted by the lactic flora against the Enterobacteriaceae is shown in D_{19} the starting point for the stabilization of the lactic flora at a relatively important level on the one hand and the decrease in Enterobacteriaceae on the other hand. However, it should be noted that the weight gain and the improved feed conversion ratio of the broilers were positively correlated with the appearance of this barrier effect as of D_{19}. Better zootechnical parameters were observed in chickens treated with *Pediococcus acidilactici* on D_{14} by Vittorio *et al.* (2005) and on D_{23} by Djezzar *et al.* (2012). The researches of Jin *et al.* (1998) showed that the use of probiotics based on *Lactobacillus* sp. induced a significant increase of the lactic flora starting from D_{30}.

Therefore, researchers can assert that there is a close relation between the barrier effect and the improvement of the zootechnical performance.

CONCLUSION

The results obtained in this study showed that the use of probiotics can improve weight performance and trigger a better feed conversion ratio. However, the lesions observed at the autopsy of the death cases have been indicatives of the Colibacilli-related complications and of the two coccidiosis episodes. The latter, present in the experiment is the major and recurrent pathology of the breeding farms in Algeria. The barrier effect due to the installation of a large lactobacilli flora induced a competitive inhibition of the pathogenic flora based on the synthesis of the metabolites, lactic acid and a lower pH but is ineffective against the development of coccidiosis. The failure to observe the waiting times for the administered treatments especially in late breeding, puts the consumer health into a real risk.

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