EFFECTS OF *PEDIOCOCCUS ACIDILACTICI* ON GROWTH PERFORMANCE AND INTESTINAL MORPHOMETRY IN BROILER CHICKENS

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Abstract: This study was carried out to evaluate the effect of dietary supplementation with *Pediococcus acidilactici* on zootechnical performances and intestinal morphometry in broiler chickens. A total of 600 one-day-old chicks are divided into 2 experimental groups (5 repetitions of 60 chicks per treatment) and are fed, for 49 days, with a basic feed supplemented (Probiotic batch) or not (Control batch) with *Pediococcus acidilactici*. Under our conditions, the addition of the probiotic did not significantly modify the growth of the chickens but it reduced the mortality rate (2.33% vs.4.01%). In addition, this treatment significantly reduced feed conversion ratio. These results indicate a better assimilation of nutrients, which could be explained by a larger intestinal absorption surface as indicated by the significant increase in total intestinal length ($p<0.05$) found in the supplemented chickens compared to the controls.

Keywords: Broiler chickens, Intestinal morphometry, Probiotic, Supplementation, Zootechnical performances.

INTRODUCTION

Antibiotics have been widely used as growth promoters (AGPs) in the field of animal production since 1940s (Al-Khalaifah, 2018). Their zootechnical and economic efficiency is at the origin of their systematic use (Donoghue, 2003), Motl et al. (2005). However, with the emergence of bacteria acquiring resistance to antibiotics and their residue in end-products, the European Commission has decided, since January 1, 2006 (EC regulation No. 1831/2003), to ban the marketing and use of antimicrobial as growth promoters in animal feed. In Algeria, their incorporation in animal feed was prohibited since 2007 (ministerial decision of December 24, 2006). This suppression caused a deterioration of the sanitary state of the animals, an increase in the mortality rate, a decrease in body weights, an increase in consumption index and consequently a decrease in the economic profitability of poultry farms (Dibner and Richards, 2005).

A variety of different supplements, have been explored to maintain growth performance of broilers. Particular attention has been paid to the use of probiotics Menconi et al. (2011), (Park and Kim, 2014), prebiotics Pourabedin et al. (2016), Rajani et al., (2016), enzymes, natural plant extracts (Dorman and Deans, 2000) and organic acids Fernandez-Rubio et al. (2009), Saleem et al. (2016), as AGPs alternatives in poultry feed.

In the present study, our objective is to evaluate the effect of the use of the probiotic *Pediococcus acidilactici*, added to the feed, on growth and health performances, and on the intestinal morphometry of broiler chickens.

MATERIAL AND METHODS

Animals and feed: Six hundred (600) one day-old chicks of strain ISA15 (mixed sexes), and coming from the same hatchery were weighed and were allocated in two experimental groups (n=300) of homogeneous weight (≈ 41.0 g), each comprising five replicates of 60 subjects (density of 11 chickens/m^2). The first group, Control (C), was fed with a standard feed adapted to each rearing phase: a starter feed distributed from D0 to D10 (Metabolisable Energy: 2800 kcal/kg; Crude Protein: 21%), a grower feed from D10 to D42 (Metabolisable Energy: 2900 kcal/kg; Crude Protein: 19%) and a finisher feed from D42 to D49 (Metabolisable Energy: 2930 kcal/kg; Crude Protein: 17%). The second group, Probiotic (P), was fed the same basic feed (starter, grower and finishing) supplemented with 109 CFU/kg of *Pediococcus acidilactici* MA 18/5M (BACTOCELL® France).

Throughout the trial, the chickens were fed and watered ad libitum and were raised in the same building to ensure similar rearing conditions.

Parameters recorded:

Growth performance: Body weight, body weight gain, feed intake, feed conversion ratio, and mortality rate were determined at the end of each rearing phase (at D10, D42, and D49).

Intestinal morphometry: A histometric study of the intestine was performed at 10, 28 and 49 days of age on 10 control chickens and 10 probiotic-supplemented chickens (2 subjects per flock with a weight representative of its batch). These chickens were weighed and then sacrificed by bleeding. The entire intestine (from the junction with the gizzard to the colon) and the 2 caeca (detached at the ileocaecal junction) were juxtaposed and the total length was measured. Histological sections, stained by the Hemalun-eosin technique (Martoja and Martoja-Pierson, 1967), were taken from the proximal and distal portions of the duodenum and ileum to determine the volume of the intestinal villi as previously described Uni et al. (1998). For each specimen, histological slides were photographed (at x10 magnification) using a microscope equipped with an image capture process (Motic Co., Ltd), then 10 villi per specimen were measured using image acquisition and analysis software (Motic Images Plus 2.0 ML, Motic Co., Ltd).

Statistical analysis: The data collected were entered into an Excel 2010 database. All data were presented as means with standard deviation, were then subjected to one-way analysis of variance (ANOVA), to determine the effect of probiotic supplementation on the parameters considered. Statistical significance was declared at $P < 0.05$. Analyses were performed using the Statview program (Abacus Concepts, 1996, Inc, Berkeley, CA94704-1014, USA).

RESULTS

Growth performance: The growth performances (body weight, body weight gain, feed intake and feed conversion ratio) measured during the trial are presented in table 1.

Our results show that the addition of *Pediococcus acidilactici* to the feed did not modify the weight of the chickens: no significant variations between the two batches at D10, D42 and D49. At D49, the average weights were almost identical in both groups. Similarly, the differences in weight gains recorded between chickens in batch P and C are not statistically different ($p > 0.05$).

The feed intake of the probiotic supplemented chickens in the start-up phase was comparable to that of the control chickens. However, consumption is reduced by about 3% in the growth phase, and, it is significantly reduced by 7% in the finishing phase ($P<0.05$). The overall feed intake (from D0 to D49) was significantly reduced by 4%, by the addition of the probiotic ($P<0.01$).

Table 1

Growth performances of chickens receiving a standard feed (Control batch) or supplemented with *Pediococcus acidilactici* (Probiotic batch) (values expressed as means ± standard deviation; n=5, *: $P<0.05$, **: $P<0.01$).

Body weight (g)			
D0	40.87±0.51	40.93±0.64	0.69
D10	210±4,74	212±2.72	0.98
D42	2107±120.19	2086±86.6	0.70
D49	2494±138.25	2472±76.25	0.31
Body weight gain (g)			
Start-up (D0-D10)	170±5.13	171±2.86	0.99
Growth (D10-D42)	1897±122.18	1874±89.01	0.67
Finishing (D42-D49)	387±54.19	386±13.59	0.98
Cumulus (D0-D49)	2454±138.02	2431±76.33	0.24
Feed intake(g)			
Start-up (D0-D10)	302±5.24	302±7.68	0.98
Growth(D10-D42)	3032±173.58	2951±140.95	0.22
Finishing (D42-D49)	1339±69.06	1240±166.58	*
Cumulus (D0-D49)	4673±209.61	4493±156.32	**
Feed conversion ratio (g/g)			
Start-up (D0-D10)	1.78±0.03	1.77±0.05	0.78
Growth (D10-D42)	1.60±0.04	1.57±0.05	0.23
Finishing (D42-D49)	3.50±0.36	3.20±0.37	**
Cumulus (D0-D49)	1.91±0.04	1.85±0.07	*

Concerning the feed conversion ratio, our results show that it is significantly improved at the end of rearing in batch P, difference of 3% from batch C ($P<0.05$).

Mortality: Mortality rates recorded in chickens supplemented with *P. acidilactici* are lower than those observed in control chickens (table 2). The probiotic intake reduced the cumulative mortality rate by 42% compared to the control batch.

Intestinal morphometry: The histological study carried out on the intestine reveals, in chickens supplemented with probiotics, very significant increases in the length of the intestine and the volume of the villi in the proximal and distal portions of the duodenum and ileum, whatever the age of the chickens (table 3).

Table 2

Mortality rate in chickens fed a standard diet (Control batch) or a diet supplemented with *Pediococcus acidilactici* (Probiotic batch) (values expressed as means ± standard deviation; n=5).

Mortality rate		
Stat-up (D0-D10)	2.67±0.90	1.66±1.66
Growth (D10-D42)	1.03±0.94	0.68±0.93
Finishing (D42-D49)	0.34±0.77	0.00
Cumulus (D0-D49)	4.01±1.49	2.33±2.23

Table 3

Intestinal morphometry measured at 10, 28 and 49 days of age in chickens fed a standard diet (Control batch) or a diet supplemented with *Pediococcus acidilactici* (Probiotic batch) (values expressed as means ± standard deviation; n=10, *: $P<0.05$, **: $P<0.01$, ***: $P<0.001$).

Total length of the intestine (cm)			
D10	105.2±7.67	114.3±4.78	***
D28	169.1±11.23	195.7±6.54	***
D49	223.4±13.14	241.6±9.32	*
Volume of intestinal villi ($mm^{3)}$			
Proximal duodenum			
D10	0.009±0.001	0.029±0.002	***
D28	0.016±0.001	0.066±0.005	***
D49	0.030±0.004	0.082±0.009	***
Distal duodenum			
D10	0.010±0.001	0.023±0.002	***
D28	0.020±0.002	0.126±0.010	**
D49	0.031±0.003	0.099±0.004	***
Proximal ileum			
D10	0.005±0.001	0.014±0.001	***
D28	0.013±0.001	0.052±0.006	***
D49	0.048±0.005	0.070±0.008	**
Distal ileum			
D10	0.008±0.001	0.020±0.001	***
D28	0.011±0.001	0.039±0.004	***
D49	0.047±0.006	0.067±0.007	**

DISCUSSION

In the present study, dietary supplementation with *P. acidilactici* did not significantly modify the growth of the chickens, as reported by several studies, demonstrating that probiotic supplemented to the birds improve the performances of

growth Mountzouris et al. (2007), Jazi et al.(2018). However, it improved significantly the feed conversion ratio, by reducing the feed intake, as reported by Jin et al. (1998).

According to Mountzouris et al. (2007), it is difficult to directly assess different studies using probiotics, because the efficacy of a probiotic application depends on many factors, such as species composition and viability, administration level, application method (e.g., spraying, feed, or water), frequency of application (e.g., once, intermittent, or continuous), overall diet, bird age, overall farm hygiene, and environmental stress factors (e.g., temperature, stocking density).

The positive effect of the probiotic on the feed conversion ratio reflects an increase in the efficiency of food processing which is consistent with the significant increase in the size and volume of the intestinal villi and reveals a better digestive valorization of the food Awad et al. (2009). Indeed, the addition of *P. acidilactici* in the feed induced an increase in the total length of the intestine associated with a marked increase in the dimensions of the villi measured in the different intestinal compartments and at the different phases of rearing. This finding is in agreement with the histomorphological data reported by Awad et al. (2009).

The mortality rate recorded in chickens supplemented with probiotic was reduced compared to controls; these findings are in agreement with those of Alkhalf et al. (2010) who observed a lower mortality rate in birds fed the probiotic. According to Vitorrio et al. (2005), the favorable role on health status of the host induced by the addition of lactic acid bacteria in the food would be due to competitive exclusion, the synthesis of lactic acid leading to a drop in pH or the stimulation of local or systemic immunity.

CONCLUSIONS

The results obtained in the present study indicate that the addition of the probiotic *P. acidilactici* in broiler feed has reduced mortality and improved feed conversion ratio. This better digestive use of the nutrients is consistent with the increase in absorption surface indicated by the increase in total intestinal length and the volume of the villi. Probiotics therefore seem to constitute, an interesting alternative to antibiotics used as growth promoters now banned.

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