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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 D_{22}, 3.8 and 3.2 respectively, on D_{30} and D_{45}, 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,

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1996; Mathlouthi et al., 2009). The desire to maintain a satisfactory level of production requires the research of non-therapeutic alternatives to substitute antibiotics as growth factors that should be equally effective from the zootechnical, health and economical point of view. Among the proposed additives we include the enzymes, the organic acids, the natural plant extracts, the probiotics and prebiotics (Dorman and Deans, 2000).

Probiotics are able to control the carrying and dissemination of pathogens and zoonotic agents and can also contribute to potentiate the food and consequently the profitability of farming (Trufanov et al., 2008; Niderkorn et al., 2009). Several studies have shown, in addition to the zootechnical efficiency (Simon et al., 2001; Vittorio et al., 2005), their beneficial effects on the health of poultry (Awaad et al., 2005; Vandeplas et al., 2009; Higgins et al., 2010). The improvement of the zootechnical performance by orientation of the flora has been reported by many authors (Cabuk et al., 2004; Alfaro et al., 2007); nevertheless, the results regarding their efficacy are not consistent (Zhang et al., 2005). Our recent works (Djezzar et al., 2012) have shown that the use of *Pediococcus acidilactici* (Bactocell MA 18/5M strain) certainly allowed some improvement of the zootechnical performance, but its effectiveness remains limited due to the problem of coccidiosis, a major and recurrent pathology in broilers breeding.

Coccidiosis, the most common parasitic disease to be found in poultry farming, causes considerable economic losses due, in part, to the massive mortality rate in their acute and very dangerous form, as hidden in their chronic or subclinical and weaken the health status of animals, predisposing them to other bacterial and viral diseases (Naciri et al., 2005). The endogenous development of parasites may be limited by the use of food additives or by stimulating immune defenses by making use of anticoccidial vaccines. The coccidiostats (synthetic products or ionophores) are facing increasing resistance of coccidia (Reperant et al., 2012). The herbal extracts rich in steroidal sapogenins, especially *Yucca schidigera*, have various biological activities acting preemptively against the coccidial risk. In Algeria, the means to fight against coccidiosis confine to the use of chemical anticoccidials in food and drinking water. Vaccination remains absent due to its high cost (Alloui and Barberis, 2012).

The purpose of this study is to test the effectiveness of a dietary supplementation combining a probiotic (*P. acidilactici*) and an anticoccidial based on herbal extracts (*Y. schidigera and Trigonella foenum graecum*) in our local breeding conditions

MATERIALS AND METHODS

Animals, feed and treatments

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 m^2 each, undergoing the same environmental conditions.

The floury-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 (D_1-D_{28}), growing (D_{29}-D_{42}) and finish (D_{43}-D_{52})].

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 (Bactocell ®, France) at a rate of 100 ppm (concentration of 10^9 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, Cycostat) 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 D_6 against Newcastle disease (UNI L CEVA ®), the vaccination being repeated on D_{15} and D_{19} 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 (D_{28}, D_{42} and D_{52} of age) to calculate feed conversion ratio.

Death cases were recorded daily and mortality rates were determined at the end of each breeding phase (D_{28}, D_{42} and D_{52}). 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 D_{28}, D_{42} and D_{52}. 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 (D_{13} to D_{48}). 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 Bouhelier (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 (D_{52}), 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 α = 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 standards 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 $t(\alpha/2, n_{1-1} + n_{2-1}) \approx z\alpha/2$.

By default, the signification's level α = 5% brings us to compare the statistically calculated (Zcal) to the tabulate value zα/2=1.96.

$$Z_{cal} = \frac{|\bar{x}_1 - \bar{x}_2|}{\sigma_{\bar{x}_1 + \bar{x}_2}} \text{ with } \sigma_{\bar{x}_1 + \bar{x}_2} = \sqrt{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}}$$

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 (α=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 anticoccidial 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 $D_{19\text{-}24}$, 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 anticoccidial 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 anticoccidial (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.

Groups	Parameters	End of the three phases of breeding		
		D_{28}	D_{42}	D_{52}
"Control"	Average live weight by subject (g)	996 ± 23	1802 ± 31	2678 ± 29
	Feed ratio	1.48	1.73	2.83
	Mortality rate (%)	10.1	13.4	14.7
	Average length of the intestines (cm)	191 ± 13	245 ± 15	295 ± 14
"Experimental"	Average live weight by subject (g)	1011 ± 27	1778 ± 25	2791 ± 27
	Feed ratio	1.32	1.57	2.39
	Mortality rate (%)	2.5	3.7	6.5
	Average length of the intestines (cm)	212 ± 17	262 ± 15	345 ± 14

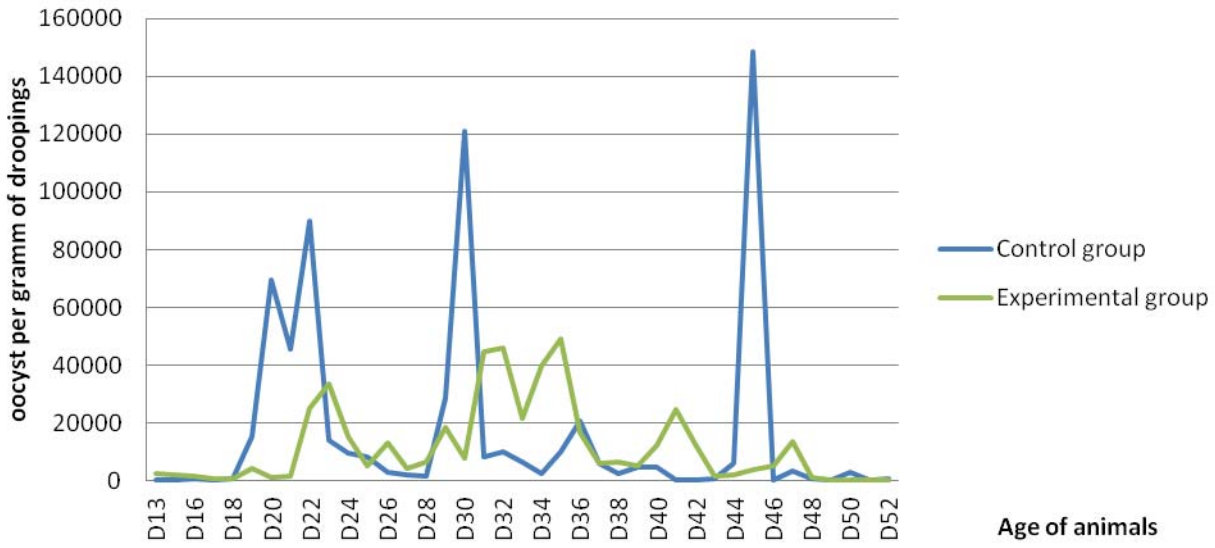
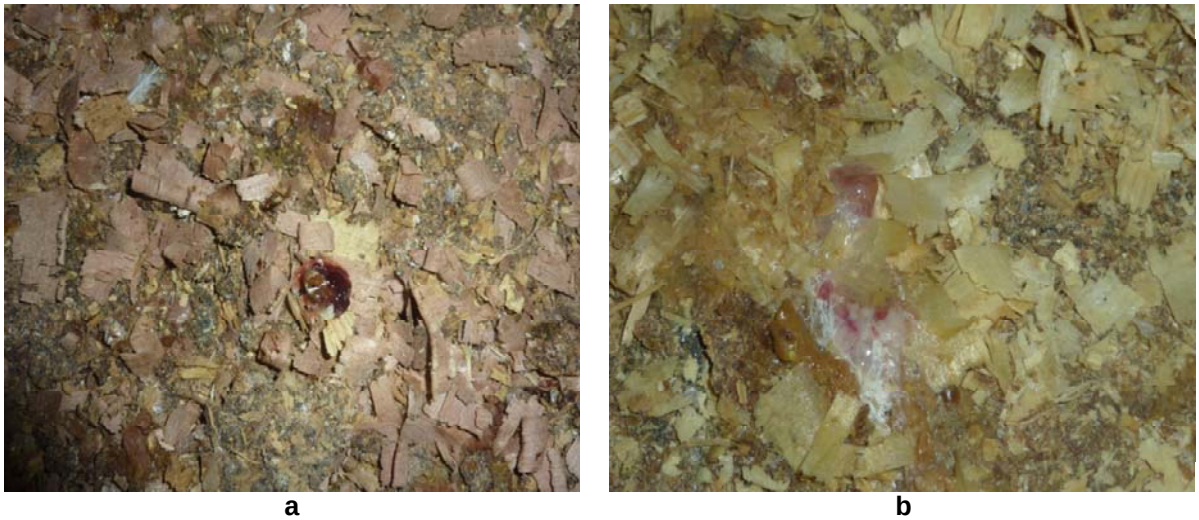


Figure 1. Kinetics of oocyst excretion in Control and Experimental Groups.



a **b**

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.

Groups	End of each breeding phases		
	D_{22}	D_{30}	D_{45}
Control	3.5	3.8	3.2
Experimental	1.5	1.7	1.1

(**a**) (**b**)

Photo 2. Lesions observed at the autopsy of sacrificed subjects, for Experimental group (a) Punctate congestion duodenum (D_{24}) and Control group (b) Caeca hemorrhagic (D_{30}).

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 perihepatitis 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.

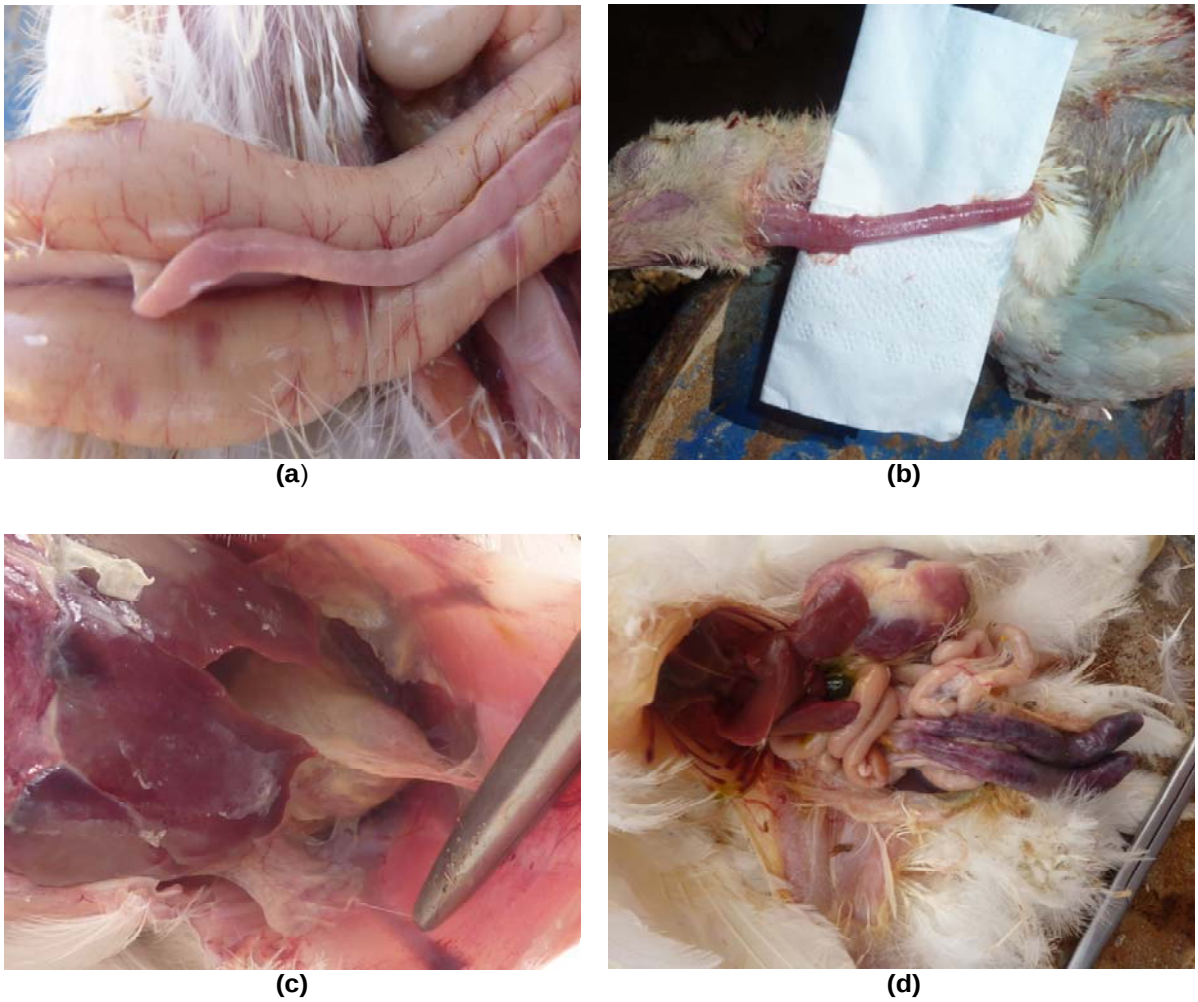


Photo 3. Lesions at autopsy of fresh cadavers from the two groups, viz: Experimental group (a) Punctate congestions of the serous (D_{24}) (b) Tracheitis (D_{35}); Control group (c) Pericarditis and perihepatitis (D_{27}) and (d) Presence of blood in the ceaca (D_{45}).

Table 3. Average weights and carcass yield.

Groups	Weight (g) of ($\bar{x} \pm SEM$)						Carcass yield (%)
	Liver	Plucked carcasses (*)	Eviscerated carcasses	Grease	Edible offal	Intestines	
Control	2678 ± 29	2182 ± 153	1784 ± 131	42 ± 11	77 ± 20	279 ± 27	66.6
Experimental	2791 ± 27	2423 ± 137	1930 ± 127	37 ± 9	105 ± 11	351 ± 36	69.2

(*): 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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