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Research Article
Effect of Mixing ACIDAL® with Drinking Water for Laying Hens on Production Performance

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Abstract

Background: The ban of antibiotics use as growth factors since 2006 affects animal performance and economical viability of farms. Several alternatives including incorporation of organic acids in feed or drinking water in order to improve productivity were studied. **Objective:** The objective of this study is to mix ACIDAL with drinking water of hens in order to improve productivity. **Methodology:** The experiment was carried out with 360 ISA Brown hens (22-44 weeks of age), allocated to 3 groups (control, Aci 1 and Aci 2) of 120 birds each. The three groups received, respectively in drinking water 0, 1 and 2 mL of ACIDAL L^{-1}. Prior to start, every 4 weeks and at the end of the treatments, samples of chicken droppings according to each group were collected and used to determine total *Streptococcus* and *Escherichia coli* and to check the presence of *Salmonella*. During treatments, amount of water consumption, feed intake, body weight, egg weight and egg component weights were recorded weekly. **Results:** Eggs produced were collected daily and every 2 weeks, the litter quality was assessed. Mixing of ACIDAL with drinking water of laying hens reduced significantly the number of total bacteria, eliminated completely *Salmonella* in the droppings, decreased feed intake and improved egg weights and body weight compared to control group witch litter was significantly wetter and more tendentiously crusty compared to those of treated groups. **Conclusion:** In opposite, there is no effect on water consumption, mortality rate, egg laying rate and ratios of albumen, yolk and shell.

Key words: Antibiotics, growth promoters, alternatives, organic acids, ACIDAL ML

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In intensive production systems of poultry meats and eggs, antibiotics and antibiotic products are widely used for therapeutic and prophylactic purposes and as growth promoters. Antibiotics as growth factors are among the most widely used additives to improve feed efficiency and growth rate and consequently increase the productivity and profitability for the farmers. However, frequently use of antibiotics leads to the selection of resistant bacteria and therefore, the increase of incidence of infectious diseases in poultry, mortality with negative impacts on production parameters. Face to this situation, global forums for human and animal health suggested to ban the use of antibiotics as growth promoters in poultry diets. As result, the European Union banned systematically the use of antibiotics as growth promoters in animal feed since 2006. This prohibition of antibiotics use as growth factors affects animal performance and economical viability of farms. Hence, non therapeutic alternatives are needed to maintain high production performance. Several alternatives including incorporation of organic acids, essential oils, probiotics and prebiotics[1] in feed or drinking water in order to improve productivity were studied. Acids are products of normal metabolism of anaerobic intestinal flora[2]. Organic acids and their salts may have excellent antibacterial ability. They can be also involved in regulation of digestive flora and enhance enzymatic digestion. It was reported that incorporation of prebiotics in animal feed may induce organic acids production in the intestinal tract. However, mixing of organic acids directly into feed or drinking water may be more beneficial. But reports are conflicting about the effects of the use of organic acids on *Salmonella* bacteria. Indeed, Izat *et al.*[3] reported no effect of single organic acid, while Thompson and Hinton[4] reported reducing of excreted *Salmonella* by incorporating a mixture of propionic and formic acids in the feed. It is for this purpose that ACIDAL, a combination of formic, fumaric, acetic, propionic and lactic acids was developed as alternative to replace antibiotics. Thus, the objective of this study is to mix ACIDAL with drinking water of laying hens in order to control *Salmonella*. In this line, the effect of ACIDAL on presence of *Salmonella* and amount of total germs in hen droppings were studied. Also, the effects of different levels of ACIDAL in drinking water on production performance were investigated.

MATERIALS AND METHODS

Experimental design: The experiment was designed to study the effects of ACIDAL (Impextraco nv, Belgium) on production performance of laying hens of 22-44 weeks of age at Laboratory of Poultry Science, University of Lomé. Three hundred and sixty ISA Brown pullets of 22 weeks of age were used as starting material for this experiment. The ISA Brown day-old chicks were raised at AYODELE Poultry Farm (Badja, Togo) up to 22 weeks. At that age, the pullets were transferred to poultry house of Laboratory of Poultry Science. The chickens were divided into three groups of 120 pullets each. These groups were (1) Control group (cont), (2) Group that received 1 mL of ACIDAL L^{-1} of water (Aci 1) and (3) Group that received 2 mL of ACIDAL L^{-1} of water (Aci 2). For each group, the chickens were divided into three replicates of 40 pullets each. The replicates were randomly distributed over the poultry house. Because of transfer from AYODELE Poultry Farm to Laboratory of Poultry Science, all the pullets were provided simple water *ad libitum* during 2 weeks in order to be adapted to the new environment. Then, from 24 weeks of age, ACIDAL treatment started for 20 weeks. Prior to start, every 4 weeks and at the end of the treatments, samples of chicken droppings according to each group were collected and used to determine total *Streptococcus* and *Escherichia coli* and to check the presence of *Salmonella*. During treatments, amount of water consumption was measured daily and drinking water pH was recorded before and after incorporation of ACIDAL every week. Also, feed consumption, body weight, egg and egg component weights were recorded weekly. So, 18 eggs per group were weighed and broken to collect meticulously shell, albumen and yolk. Number of egg produced was recorded daily according to the pen and treatment. Every 2 weeks, the litter quality was assessed visually on the basis of the crust aspect with a scale from 1 (dry and crumbly litter) to 5 (totally caked litter or wet) as shown in Table 1.

Microbiological analysis: The microbiological procedures used to analyze vegetable were those recommended in the standardized routine methods adopted in the UEMOA countries (West African Economic and Monetary Union). These analyses related the following germs enumeration: Total aerobic flora, *Escherichia coli*, faecal streptococci and *Salmonella* spp.

For microbiological purposes all media were purchased from Biomerieux (France). Microbial enumeration was

Table 1: Score of assessment of litter quality

Score	Litter aspect
1	Dry and friable litter
2	Friable and slightly wet
3	Friable but crusty in some places
4	Crusty at surface but friable by digging
5	Totally caked litter or wet

performed as follows: 10 g of each sample were crushed in 90 mL tryptone salt in aseptic conditions. Decimal dilutions up to 10^{-1} to 10^{-5} were prepared from these suspensions. One millilitre of each dilution was used for cell enumeration. Total aerobic bacteria were determined with plate count agar after 24 h incubation at 30°C. *Escherichia coli* were enumerated on violet red bile lactose after 24 h incubation at 44°C and faecal streptococci were determined with Slanetz and Bartley agar after 24 h incubation at 37°C. For *Salmonella* spp., buffered peptone water was used for pre-enrichment at 37°C for 24 h. Afterwards enrichment at 37°C for 24 h was made with rappaport vassiliadis soya broth prior for isolation and counting on Hektoen and SS agar at 37°C (24 h). Characteristics bacteria were identified with Api 20E system (Apparatus and Identification Procedures La Balme-les-Grottes Cedex 2 France).

European regulation (CE 1774/2002) and French association of normalization limits for effluent and organic waste of breeding were used to appreciate the conformity of the analyzed samples: Total aerobic bacteria (30°C) 5×10^3 CFU g^{-1}, *Escherichia coli* 5×10^3 CFU g^{-1}, faecal streptococci 5×10^3 CFU g^{-1} and *Salmonella* spp., 0 CFU/25 g.

Statistical analysis: The data were processed with the statistical software package SAS version 9.2 (SAS Institute Inc., Cary, NC). Generalized linear regression procedure was used to analyze layer, egg and egg components or feed weights. When the means of the general model were statistically different, then the means were further compared using Tukey's test. In addition, to quantify replication variability, the CV for every parameter was calculated.

RESULTS

***Salmonella* and total bacteria:** Table 2 shows the incidence of *Salmonella* according to the treatments and experiment stage. Overall, all the groups were natural infected with *Salmonella* at the beginning. But, 4 weeks after incorporation of ACIDAL in drinking water until the end of treatment, *Salmonella* was completely absent in treated layers with ACIDAL, while the control group still showed presence of *Salmonella*.

The amount of total bacteria in the different groups of layers were not similar as shown in Fig. 1 indicating some effects of ACIDAL on them. The lowest number of total germs per gram of droppings was obtained in the group of the layers subjected to 1 mL of ACIDAL in drinking water, while the highest level was obtained in the control group ($p<0.01$). The group of Aci 2 was intermediary but statistically comparable to control group. As for total *Streptococcus* and *Escherichia coli* all groups that received ACIDAL as well as control groups did not show any presence of these germs in droppings.

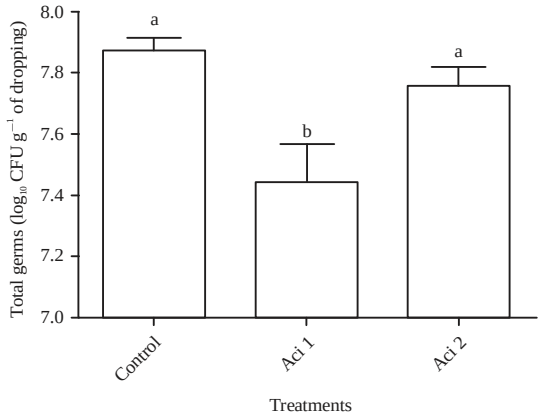

Fig. 1: Level of total germs (log_{10} CFU g^{-1} of droppings) according to treatment, data sharing no common letter are different ($p<0.05$), CFU: Colony forming units (CFU g^{-1} sample)

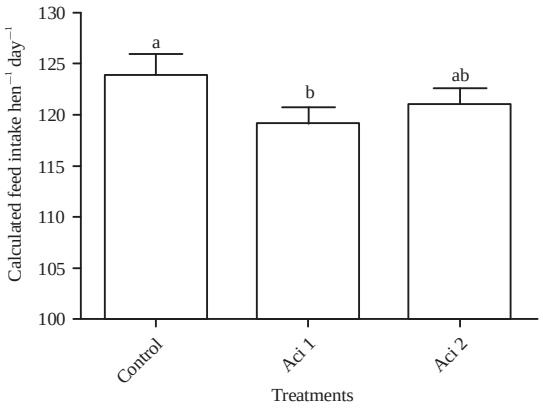

Fig. 2: Average daily feed consumption according to treatments. Data sharing no common letter are different ($p<0.05$)

Table 2: Incidence of *Salmonella* in droppings according to treatments and experimental stage

Stage of experiments	Control	Aci 1	Aci 2
Prior to treatment	+	+	+
4 weeks after treatment	+	-	-
8 weeks after treatment	+	-	-
12 weeks after treatment	+	-	-
20 weeks after treatment	+	-	-

-: Absence of *Salmonella*, +: Presence of *Salmonella*

Feed and water consumption: Average daily feed consumption according to the treatment during the trial period is shown in Fig. 2. Overall, daily feed intake ranged

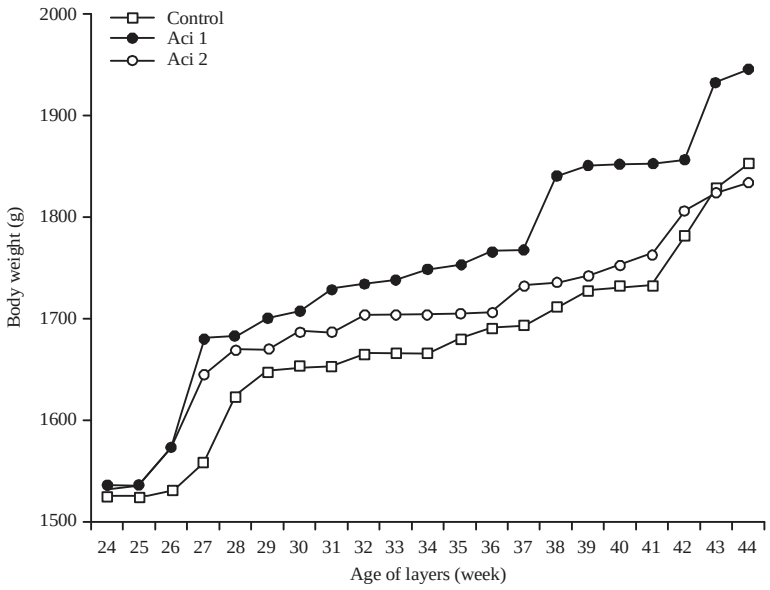

Fig. 3: Weekly body weight according to treatment and age of layers

from 115-130 g irrespective of treatments. However, feed intake was not similar between the groups. The lowest daily feed consumption was obtained in the group of Aci 1 and the highest in the control group ($p<0.05$). Daily feed consumption of layers of Aci 2 group was comparable to both control and Aci 1 groups.

Water consumption was not affected by ACIDAL treatment although the group of layers that received 2 mL of ACIDAL L^{-1} drinking water drank slightly more than the other groups.

Layer body weight: Overall, weekly body weight increased from 24-44 weeks as shown in Fig. 3. With regard to treatments, body weights were similar for all groups during the first 2 weeks of experiment. At week three of treatment, layers that received ACIDAL treatment were heavier than the control group ($p<0.05$). But, from week 4 onward, layers treated with 1 mL ACIDAL L^{-1} of drinking water grew better than all other groups ($p<0.05$). During the same time, growth rate of layers of the group Aci 2 decreased gradually compared to control and Aci 1 groups. Consequently, body weights of layers of Aci 2 group become similar and lower ($p<0.05$) to that of control at 43 (week 19 of treatment) and 44 (week 20 of treatment) weeks of age, respectively.

Egg production rate: Figure 4 shows laying rate according to treatment and age of layers. Egg production level of all groups followed the same trend. Between 35 and 39 weeks of age, there was a very pronounced drop in egg production for unknown reason but it has to be mentioned that the layers were not subjected to any antibiotical treatment during the experiment. There was no clear effect of ACIDAL on egg production rate. However, during the last 3 weeks of the experiment, egg production in the group of layers that received 1 mL of ACIDAL drinking water was slightly higher than that of control and Aci 2 groups.

Table 3: Mean ratios of egg components weights to egg weight according to treatments over the entire experimental period

Treatments	Albumen ratio (%)	Shell ratio (%)	Yolk ratio (%)
Control	62.3±0.395	12.9±0.164	23.5±0.281
Acidal 1	62.1±0.287	12.9±0.201	23.8±0.369
Acidal 2	61.7±0.331	13.2±0.199	23.3±0.460

Egg weight and egg components: In average, egg weights were in the following order: Aci 1>Aci 2>control, indicating that ACIDAL treatment improved egg weight. Figure 5 indicates that egg weight increased with the age of layers. Egg weights were similar for all groups at the beginning of the experiment (24 weeks of age). But from 25 weeks of age onward, egg weights of Aci 1 group were higher than those of control group ($p<0.05$). Egg weights of layers that received 2 mL of ACIDAL L^{-1} of drinking water were lower ($p<0.05$) than those of Aci 1 group between 25-31 weeks of age but comparable to those of control group between 25-30 weeks of age. From 31-44 weeks of age, egg weights of Aci 2 group were higher ($p<0.05$) than those of control group but comparable to those of Aci 1 group.

Table 3 indicates proportions of eggshell, albumen and yolk according to egg weight, respectively. Although eggshell

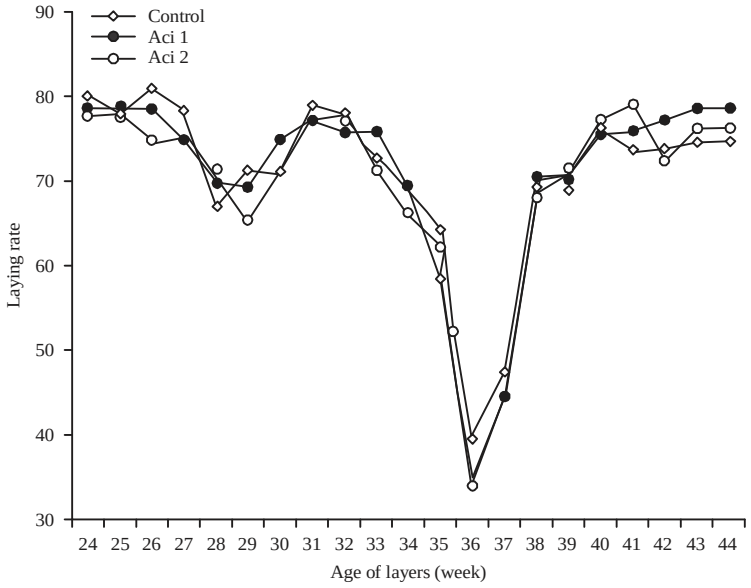

Fig. 4: Laying percentage according to treatment and age of layers

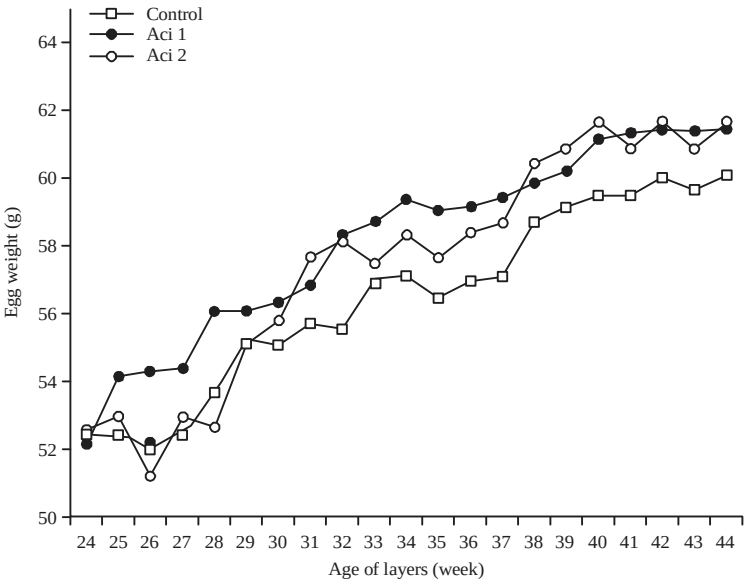

Fig. 5: Egg weight according to treatment and age of layers

proportion increased and albumen ratio decreased with dose of ACIDAL in drinking water, there was statically no significant effect of ACIDAL on these parameters. Ratios of yolk weight/egg weight were similar between all groups. However, eggshell percentage changes were not significantly different among treatments but usage of ACIDAL numerically increased it with the age of the layers (Table 4). Then, at 44 weeks of age, eggshell proportion of layers that received 2 mL of ACIDAL L^{-1} of drinking water is higher ($p<0.05$) than those of control group.

Table 4: Ratios of eggshell weights to egg weight according to the age of layers

Age (week)	Control	Aci 1	Aci 2
28	12.9±0.766	12.8±0.523	12.8±0.679
32	13.3±0.142	12.7±0.199	13.1±0.235
36	12.8±0.174	12.6±0.424	12.8±0.344
40	12.6±0.340	13.5±0.778	13.7±0.648
44	12.8±0.109[b]	13.1±0.014[b]	13.4±0.106[a]

Data sharing no common letter are different ($p<0.05$)

Feed conversion ratio: Feed conversion ratio according to the treatment is shown in Fig. 6. The lowest feed conversion ratio was obtained in the group of Aci 1 and the highest in the

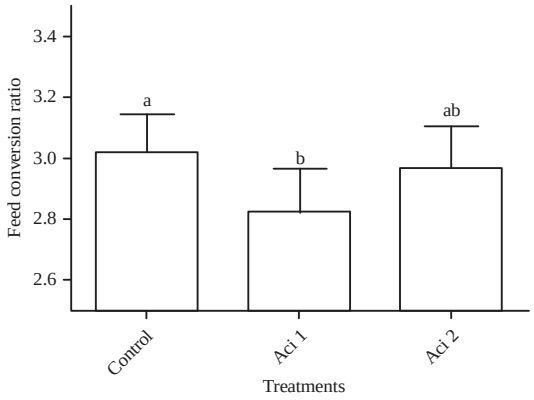

Fig. 6: Feed conversion ratio according to treatment

control group ($p<0.05$). Feed efficiency of layers of Aci 2 group was comparable to both control and Aci 1 groups.

Effects of acidal on litter quality: Litter of control group was significantly wetter and more tendentiously crusty ($p<0.05$) compared to treated batches with ACIDAL where the litter is dry and crumbly (lot C) or slightly wet (lot B). The mean scores of the different groups were 3.00 ± 0.11, 2.13 ± 0.08 and 1.87 ± 0.08, respectively for control, Aci 1 and Aci 2.

DISCUSSION

Mixing of ACIDAL with drinking water of laying hens reduced significantly the number of total bacteria and eliminated completely *Salmonella* germs in the droppings. Similar results were obtained by Hassan *et al.*[5] who obtained a reduction of levels of *E. coli* and *Salmonella* bacteria in the intestines of chicks that were fed from 14th-49th day of age with a diet supplemented with 0.06% of galliacid, a mixture of organic acids (acid fumaric, calcium formate, calcium propionate, potassium sorbate). In addition, Cengiz *et al.*[6] showed that incorporation of organic acids into broiler diets improved beneficial effects of microbial activity in the intestine. Hence, effects of ACIDAL in this study on bacteria levels in the droppings may be due to ability of organic acids, in their non-dissociated form to enter into the bacteria and break the physiological balance by decreasing their internal pH. This decrease of internal pH is incompatible with certain types of bacteria which do not cope with high transmembrane pH gradient[7,8]. But, Fuller[9] pointed out that very high levels of acidification of feed induced the development of acidophilic bacteria in intestine flora. This could explain the higher number of total bacteria obtained in Aci 2 group compared to the group of layers that received 1 mL of ACIDAL L^{-1} in drinking water. Also, Akyurek *et al.*[10] reported an increase in the number of *Lactobacillus* by adding organic acids in ileal digesta. The absence of faecal streptococci bacteria and *Escherichia coli* found to be related to the relatively high level of operating hygiene.

Mixing of ACIDAL with drinking water decreased feed intake but had no effect on water consumption. High feed intake in the control group compared to treated layers may be due negative effect of high number of bacterial germs. This negative effect may be explained by the fact that microorganisms divert carbohydrate and protein intake for their needs hence depriving the host[11]. This detrimental effect leads to high metabolic needs and then high feed consumption[12]. Similar results were obtained by Mohamed *et al.*[13] when they fed the chicks between 14 and 36 days of age with a diet supplemented with 0.06% of a mixture galliacid organic acid. Also, Leeson *et al.*[14] reported that the use of butyric acid at 0.4% in chicken feed has improved feed conversion up to 8%. It can be hypothesized that improvement in feed conversion ratio may be due to improved ileal digestibility of nutrients[15,16].

The ACIDAL treatment improved egg weights compared to control group. This result is consistent with that of Wong and Zahari[17] and Langhout and Sus[18] who reported that incorporation of a mixture of organic acids in the diet of layer chickens improved egg weights.

Surprisely, ACIDAL treatment had no effects on mortality rate, egg laying rate and the ratios of albumen, yolk or shell weights to egg weights. This may be explained by the age of the layers. Indeed, 20-45 weeks of age is the stage of ascending of egg production. Similar results were reported by Vogt and Matthes[19] and Skinner *et al.*[20] incorporating organic acids in layer chicken feeds. In contrast, Rahman *et al.*[21], Soltan[22] and Gama *et al.*[23] reported a significant improvement of egg laying rate between 2 and 9% by incorporation organic acid in the feed of laying hens of 67-74 weeks of age. These positive effects suggest that, with regard to laying rate, mixing of organic acids to drinking water or feed may be more beneficial for older hens probably during the descending phase of egg production (from 45 weeks old onward). Egg component ratios as a mean over the entire experimental period were not significantly affected by ACIDAL treatment. Nevertheless small numerically increased shell ratio percentages were observed in both ACIDAL groups in a dose-dependent way. The increased egg weight in the ACIDAL groups (Fig. 5) rather should decrease percentage of shell as a result of the surface/volume ratio, decreasing with increasing weight. However, the reverse is observed with increasing treatment time with even statistically higher shell ratio in the Aci 2 group at the end of

the experimental period. This clearly indicates an improved shell quality with ongoing ACIDAL treatment. The results of some studies carried out on rats, broiler chickens and pigs have indicated that organic acids may improve the utilization of minerals in monogastric animals[22]. So, the differences in egg shell percentage may be a consequence of the increased mineral absorption[22]. The phenomenon of increased absorption is reflected in the increased calcium deposits of the shell and contributes to improving shell weight.

The results of this study also show that the litter of control group was significantly wetter and more tendentiously crusty compared to treated batches with ACIDAL. This result may be explained by the beneficial effect of ACIDAL on bacterial populations of the digestive flora. Indeed, the presence of pathogenic or non-useful microorganisms leads to digestive disorders followed by diarrhoea[24]. Thus, watery droppings may increase the humidity of the litter which would be eventually crusty. In addition, low pH of feed mixture in the stomach and the intestine may promote the activation of proteolytic enzymes and increased mineral and amino acid absorption[25] and therefore reducing the amount of droppings.

CONCLUSION

It is concluded that mixing of 1‰ ACIDAL in layer chickens drinking water improves health state and therefore, feed intake, egg weights but had no effect on laying rate probably due to the young age of the layers used in this study. This product should be recommended to poultry farmers especially in hot and wet climatic zones where environmental conditions favour bacteria proliferation.

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