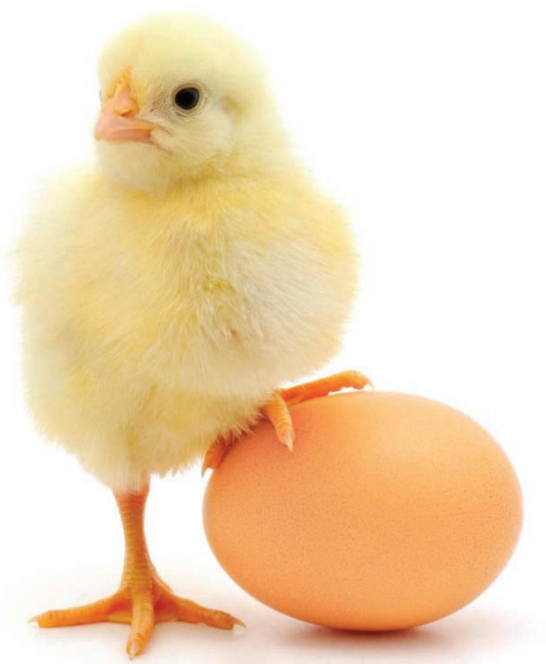
ISSN 1682-8356
ansinet.com/ijps



INTERNATIONAL JOURNAL OF POULTRY SCIENCE

OPEN ACCESS International Journal of Poultry Science

ISSN 1682-8356
DOI: 10.3923/ijps.2020.577.585

Research Article
Effect of Whey and ACIDAL®ML Mixed in Drinking Water on Hen's Growth Performance, Haematochemical and Serum Immunological Parameters

[1]T. Bouassi, [1]D. Libanio, [2]M.D. Mesa, [2]A. Gil, [3]K. Tona and [4]Y. Ameyapoh

[1]Centre d'Excellence Régional sur les Sciences Aviaires, University of Lomé. B.P: 1515, Lomé, Togo
[2]Department of Biochemistry and Molecular Biology II, Institute of Nutrition and Food Technology (Biomedical Research Center, CIBM), University of Granada, Avenida del Conocimiento S/N, 18100 Armilla, Granada, Spain
[3]Department of Animal and Veterinary Sciences, School of Agriculture, University of Lomé, BP: 1515, Lomé, Togo
[4]Laboratoire de Microbiologie et de contrôle de qualité des Denrées Alimenaires, Ecole Supérieure des Techniques Biologiques et Alimentaires, University of Lomé. B.P: 1515, Lomé, Togo

Abstract
Objective: The objective of this study was to evaluate the growth performance, some haematological and serum immunological and biochemical parameters of laying hens supplemented with whey (LW) and ACIDAL®ML mixed in drinking water. **Materials and Methods:** Seven hundred and fifty Isa Brown hens' chicks were randomly assigned to five treatments and five replicates of 150 birds each. The birds were reared for 36 weeks. The treatments were administered in the drinking water at dosages: 250 mL L^{-1} of LW (Lacto 25) and 500 mL L^{-1} of LW (Lacto 50) and 1 mL L^{-1} of ACIDAL®ML (Aci). A positive control group (T+) was treated with 500 mg L^{-1} of Tétracolivit (antibiotic). The negative control group (T-) was offered drinking water only. During experimental period, feed intake, body weight, average daily gain, feed conversion ratio and mortality were recorded. At weeks 12, 24 and 36, blood samples were collected from 10 birds of each treatment group for determination of total proteins, albumin, triglycerides, cholesterol, immunoglobulin (IgA and IgG) concentration and haematological parameters. **Results:** Supplementation of ACIDAL®ML and LW decreased feed intake and mortality leading to increased body weight gains and improved feed conversion ratio (FCR). Administration of LW and ACIDAL®ML increased red blood cells, haemoglobin, haematocrit, serum albumin and IgA values but decreased white blood cells (WBC), lymphocyte and serum IgG. **Conclusion:** LW and ACIDAL®ML supplementation in hens' drinking water, improved growth performance and reduced mortality. Furthermore, LW and ACIDAL®ML improved hen's immune status by decreasing serum IgG, WBC and lymphocytes while increasing serum IgA and albumin content.

Key words: Antibiotic, growth promoter, liquid whey, ACIDAL®ML, laying hens

Citation: T. Bouassi, D. Libanio, M.D. Mesa, A. Gil, K. Tona and Y. Ameyapoh, 2020. Effect of whey and ACIDAL®ML mixed in drinking water on hen's growth performance, haematochemical and serum immunological parameters. Int. J. Poult. Sci., 19: 577-585.

Corresponding Author: Bouassi Tchilabalou, Centre d'Excellence Régional sur les Sciences Aviaires, University of Lomé. B.P: 1515, Lomé, Togo
Tel: (+ 228) 91 80 26 61

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

Health is one of the key factors which strongly influence the production performance of laying hens[1]. Antibiotics are thus used in livestock production for therapeutic purposes and sometimes, to improve performance. As the use of these drugs in laying hens is increasingly minimized to avoid potential egg residue and possible antimicrobial resistance issues, poultry farmers rely on nutritional factors that tend to improve health status and growth performance[2,3]. Therefore, the use of organic acids, prebiotics, probiotics and symbiotics in the diet of poultry is considered as a suitable alternative to improve their growth performance and health status. However, these productsare often synthetic, relatively expensive and in accessible to farmers, especially in the rural areas. Thus, there is an on-going search to find natural alternatives to antibiotics that are available at lower cost.

Many by-products are rejected and discarded by the dairy industry and these can be recovered and used as additives in livestock production according to their nutritional values. One of such by-products is whey: a by-product of the cheese industry rich in nutrients, mineral salts and vitamins[4]. Whey has been considered as a highly nutritive by-product that can be used to feed farm animals in liquid, condensed or dried form, or as dried whey products[5,6]. For many years, whey has been used in feeding monogastrics[7,8]. Supplementation of whey powder has been shown to improve weight gain and nitrogen retention in turkeys[8] and in broiler chickens[9,10]. Lactose is a major component of whey and it can be used as a prebiotic-like compound because it is non-digested by poultry but may promote the growth of beneficial intestinal bacteria. To be metabolized, lactose requires the hydrolysis by the lactase-brush border enzyme in the intestine[11]. However, lactase activity in the proximal intestine of birds is negligible[12], entering to the large intestine at higher concentrations than other sugars[13]. Thus, undigested lactose is fermented by large intestinal microbiota[14], thus affecting the intestinal microorganisms' composition and their fermentation products[15-17]. It is known that inadequate high amounts of lactose might result in formation of gas causing intestinal discomfort, bloating and osmotic diarrhoea[13,18]. Nevertheless, microbial lactose fermentation is considered beneficial to the poultry host if side effects are avoided by supplementing appropriate amounts to the diet[17]. Furthermore, lactose-induced effects such as the maintenance of the immune system and the improvement on ileum morphology[19] have also been attributed to microbial lactose fermentation. Few studies have investigated the use of liquid whey in poultry diets. The few studies carried out in poultry have clearly indicated that, taking into the account of lactose intolerance in poultry, the best way to enhance whey utilization in its liquid form is to mixit in drinking water[10,18,20]. However, these studies have mostly been carried out on broilers or laying hens during the first egg production phase (for very short periods). Consequently, the aim of this study was to investigate the effect of whey and ACIDAL on growth performance, some haematological, immunological and biochemical parameters of laying hens from day-old to 36 weeks of age.

MATERIALS AND METHODS

Experimental animals and experimental design: Due to the lack of an animal welfare committee at the University of Lomé at the time of this research, the research was conducted in accordance to the guidelines of the Canadian Council on Animal Care[21].

A total of 750 Isa Brown hens' chicks at 1 week of age were purchased from the Laboratory of Poultry Production Techniques Hatchery of the University of Lomé. The birds were reared in an open-sided poultry house. Birds were kept in a deep litter system divided into pens measuring 3.30×2.50 m with 2.60 m height. Wood shavings were used as litter materials for the birds. Thirty birds were allocated in each pen and were randomly assigned to the experimental treatments as follows:

Chicks that did not receive any supplement of whey, ACIDAL or antibiotic in the drinking water [Negative control group (T-)], chicks that received 1 g of Tétracolivit (antibiotic) in per litre of drinking water [Positive control (T+)], 1 mL of ACIDAL®ML in per litre of drinking water [ACIDAL group (Aci)], 250 mL of liquid whey in per litre of drinking water (25%) [Lacto 25 group (Lacto 25)], 500 mL of liquid whey in per litre of drinking water (50%) [Lacto 50 group (Lacto 50)]. Each treatment had 5 replicates of 30 birds.

Origin of whey and ACIDAL®ML: Whey was daily obtained from traditional cheese "wagashi" producers from Badja, Todomé and Vodomé in Avé prefecture (south of Togo). Cow milk from these three different farms was obtained to produce the whey. An aqueous extract of the stems of *Calotropis procera* were added as coagulant. The extract was prepared by pounding the washed stems in a mortar. Then, the residue material was mixed with a small amount of water and filtered with a sieve. Caseins were precipitated by the addition of the *Calotropis procera* aqueous extracts to the milk. After a gentle heating coagulation occurred and the curd formed was separated from the whey by dripping. The whey was prepared and used every day and kept in sterilized cans until use. ACIDAL®ML (organic acids) used in this study, was provided by Impextraco, nv, Belgium. The dosage rate 1 mL L^{-1} of water used in the current study was based on manufacturer's recommendation and our previous study[22].

Supplement administration: Whey was mixed with the water at two different concentrations: 50 and 25%. The water was prepared by mixing 50% of water and 50% of whey for one experimental group and by mixing 75% of water and 25% of whey for the second experimental group. On the other hand, ACIDAL®ML supplement was prepared by mixing 1 mL in 1 L of water, while Tétracolivit supplement was prepared by mixing 1 g in 1 L of water. The amount of the supplement ingested per day was calculated by multiplying the daily water consumption for each treatment by each supplement concentration. Then the average individual daily supplement intake was: 130 and 65 mL day^{-1}, for 50 and 25% whey treatments, respectively; 0.26 mL day^{-1} for the ACIDAL®ML treatment (over all experimental time) and 260 mg day^{-1} for the Tétracolivit treatment (during 5 consecutive days per month).

Feeding and management: All birds were fed the same standard diet, which was formulated to meets their nutritional needs in terms of energy and protein and according to the age: starter diet for chicks under 8 weeks of age; grower diet, for pullet between 9 and 20 weeks of age; and layer diet for hens over 20 weeks of age. The nutritional composition of the experimental diets is presented in Table 1. Feed and water were provided *ad libitum*.

Data collection

Growth performance: Feed intake and body weights of the birds were recorded weekly in each replicate. During the laying period, total eggs weights produced per pen were recorded daily. The average daily feed intake (ADFI), daily water consumption, average daily gain (ADG) and feed conversion ratio (FCR) were calculated. From 0-20 weeks of age, FCR of the birds was calculated by dividing daily feed intake (g) by daily gain (g) and from 21-36 weeks of age, FCR was calculated by dividing daily feed intake (g) by the weights of the eggs laid daily. Mortality was recorded daily and ADG, ADFI and FCR were corrected for mortality.

Immunological and Biochemical characteristics determination: At 12, 24 and 36 weeks of age, two 12 h fasted hens from each replicate were randomly selected (ten hens per treatment) and blood samples were collected from each hen through the wing vein using sterilized needles and syringes into non anticoagulant bottles. Serum were separated by centrifugation (3000×g for15 min) at room temperature and stored at -20°C until analysis. Biochemical

Table 1: Ingredients and nutritional composition of the experimental diets

	Composition (%)		
Ingredients	Starter diets	Grower diets	Layer diets
Maize	58.00	56.00	58.00
Wheat bran	7.00	18.00	8.00
Spent grain	0.00	0.00	0.00
Soybean meal	10.00	6.00	0.00
Roasted soybeans	18.00	8.00	15.00
Fish meal	0.00	3.50	9.00
oyster shell	2.00	2.00	6.25
Concentrate	4.00	6.00	3.00
L-Lysine	0.50	0.20	0.20
DL-Methionine	0.50	0.30	0.30
NaCl	0.00	0.00	0.25
Total	100.00	100.00	100.00
Calculated and determined composition[1]			
ME (kcal kg^{-1})	2907.29	2719.66	2832.77
Crude protein	20.78	17.59	17.63
Lysine	1.36	1.06	1.11
Methionine	0.89	0.73	0.71
Meth+cystine	1.11	0.92	0.92
Calcium	1.17	1.45	2.55
Phosphorus	0.41	0.54	0.71
Crude fat	6.37	4.90	6.35
Crude fibre	4.70	5.03	5.19
Ash	1.29	1.41	2.40
Moisture	1.37	2.57	1.78

[1]Data are based on analysed values of main feed ingredients (i.e., maize, soybean, fishmeal, wheat bran)

parameters (total proteins, Albumin, triglycerides and cholesterol concentration) of blood serum were determined using commercial diagnosing kits (SPINREACT, S.A./S.A.U. Ctra. Santa Coloma, 7 E-17176 Sant Esteve de Bas Spain). Serum immunoglobulin (IgG and IgA) were determined using a sandwich enzyme immunoassay kit (ABYNTEK BIOPHARMA S.L, Spain; ABK1-E7717 and ABK1-E804).

Haematological studies: Blood samples were collected from 2 birds selected randomly from each replicate at 12, 24 and 36 weeks of age. Blood samples were collected from the wing vein into anticoagulant (heparin) bottles and analysed for red blood cells, haemoglobin, packed cell volume, white blood cells, lymphocyte and Neutrophil using a Haematological Auto Analyzer (Mindray Automated Haematology Analyzer, BA-88A).

Statistical analyses: All results are expressed as Means±standard error of the mean (SEMs). The normality of variables was assessed using Q-Q graphs. One-way ANOVA test and a general lineal model were used to determine differences among the three times (12, 24 and 36 weeks) and among the five treatments (T-, T+, Aci, Lacto 25 and Lacto 50). Multiple comparison post-hoc analysis adjusted by Tukey were done to identify the differences between groups. In all cases, more than 95% of the data were analysed. A p value <0.05 was considered significant. All statistical analyses were performed using the Statistical Package for Social Science (SPSS) version 20.0 for windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Growth parameters and mortality: The effect of LW and ACIDAL®ML treatment on growth performance of laying hens is presented in Table 2. At the end of the first 8 weeks of age, no differences were observed in the ADFI (p = 0.970) and ADG (p = 0.243) of the birds. But, birds in T-group had higher FCR values when compared with the Aci and Lacto 50 groups (p = 0.016). Average mortality values in T-group was higher (p<0.0001) than those of others groups. From 9-20th weeks of age, ADFI values remained unaffected by treatments (p = 0.526), while ADG was lower and FCR was higher in negative control group (p<0.001) when compared with others groups. Mortality was higher (p = 0.035) in the birds of T- group than those of Aci group. From 21st week of age onward, birds in T-group had higher ADFI and FCR values when compared with the others groups (p<0.001). The mortality values were higher in T-group compared with Aci group (p = 0.031).

For the entire experimental period, birds treated with antibiotic, LW and ACIDAL®ML had higher ADG (p<0.001), lower FCR and mortality (p<0.001) than those in negative control group. But there were no differences in the ADG, FCR and mortality of the birds offered antibiotic, LW and ACIDAL®ML. A decrease in ADFI was observed in Aci and Lacto 50 groups compared with the negative control group (p = 0.014), while no difference was found between others treatments.

Blood Indices: The effect of different levels of LW and organic acid in drinking water on some haematological parameters of the laying hens at different age is presented in Table 3. During the experimental period, the treatments had no statistically significant difference (p>0.05) on red blood cell number (RBC), haematocrit and haemoglobin values. However, at 12 weeks of age, the number of RBC in Lacto 25 and Aci Group was numerically higher than those of other groups. Also

Table 2: Daily feed consumption, daily body weight gain, feed conversion ratio and mortality according to treatments and hens Age period

		Treatments[1]						
Age period (weeks)	Parameters[2]	T-	T+	Aci	Lacto 25	Lacto 50	SEM[3]	p-value
0-8	ADFI	35.30	33.80	32.10	33.00	33.20	4.610	0.9700
	ADG	8.48	9.52	9.67	9.57	9.94	0.670	0.2430
	FCR	4.02[a]	3.37[a]	3.13[b]	3.26[ab]	3.13[b]	0.290	0.0160
	Mortality (%)	23.30[a]	2.33[b]	5.33[bc]	6.66[bc]	7.00[c]	1.550	<0.0001
9-20	ADFI	52.20	53.40	51.30	52.10	51.80	1.240	0.5260
	ADG	12.20[b]	13.60[a]	13.80[a]	13.50[a]	13.60[a]	0.300	<0.0001
	FCR	4.21[b]	3.55[a]	3.60[a]	3.76[a]	3.44[a]	0.150	<0.0001
	Mortality (%)	5.33[a]	2.66[ab]	0.00[b]	2.66[ab]	4.00[ab]	1.600	0.0350
21-36	ADFI	118.00[a]	113.00[b]	111.00[b]	112.00[b]	111.00[b]	1.620	<0.0001
	FCR	3.31[a]	2.79[b]	2.55[b]	2.58[b]	2.75[b]	0.100	<0.0001
	Mortality (%)	9.16[a]	4.66[ab]	2.00[b]	4.33[ab]	4.00[ab]	2.096	0.0310

[1]T-: Drinking water without antibiotic (Tétracolivit), LW and ACIDAL®ML; T+: Drinking water +500 mg L^{-1} of antibiotic 5 consecutive days per month, Aci: Drinking water + 1 mL of ACIDAL®ML per liter; Lacto 25: Drinking water +250 mL of LW per liter; Lacto 50: Drinking water +500 mL of LW per liter. [2]From 0-20 weeks of hen's age, Feed conversion ratio (FCR) was calculated by dividing daily feed consumption (ADFI) by daily body weight gain (ADG) and from 21-36 weeks of hen's age, FCR was calculated by dividing ADFI by daily produced eggs mass. [a,b,c]Within row, values sharing no common letter were significant different according to the treatment (p<0.05). [3]Standard error of the mean

Table 3: Effect of liquid whey and organic acid on some haematological parameters of the laying hens at different periods

		Treatments[1]						
Parameters	Age (weeks)	T-	T+	Aci	Lacto 25	Lacto 50	SEM[2]	p-value
White blood cells $\times 10^3$ µL^{-1}	12	186.00	185.00	178.00	181.00	175.00	4.070	0.0630
	24	175.00^a	161.00^{ab}	159.00^b	155.00^b	172.00^a	5.490	0.0040
	36	175.00^a	164.00^{ab}	156.00^b	162.00^{ab}	168.00^{ab}	4.280	0.0030
Lymphocytes (%)	12	13.60^a	12.70^a	9.80^b	11.20^{ab}	10.20^b	0.930	0.0010
	24	12.20^a	10.90^{ab}	9.43^{bc}	9.20^{bc}	8.01^c	0.730	<0.0001
	36	11.20^a	6.75^b	7.46^b	7.90^b	7.73^b	1.060	0.0030
Neutrophils (%)	12	57.20	54.60	54.40	52.10	54.50	1.980	0.2020
	24	58.30	55.60	57.20	54.10	55.20	3.800	0.8150
	36	60.20	60.10	56.10	59.40	56.40	1.790	0.0630
Red blood cells $\times 10^6$ µL^{-1}	12	2.48	2.64	2.63	2.61	2.54	0.080	0.3180
	24	2.46	2.36	2.45	2.45	2.56	0.130	0.6990
	36	2.45	2.39	2.42	2.66	2.40	0.110	0.1420
Haemoglobin (g dL^{-1})	12	11.80	12.80	12.00	13.40	12.00	0.720	0.1480
	24	10.90	11.30	11.20	11.00	11.70	0.334	0.1450
	36	10.80	10.80	10.40	11.50	11.10	0.410	0.1150
Haematocrit (%)	12	32.30	34.90	33.50	34.90	33.60	1.560	0.4330
	24	30.40	30.90	31.50	31.40	33.30	1.550	0.4170
	36	31.80	31.30	31.00	34.10	32.20	1.240	0.1340

[1]T-: Drinking water without antibiotic (Tétracolivit), LW and ACIDAL®ML, T+: Drinking water +500 mg L^{-1} of antibiotic 5 consecutive days per month, Aci: Drinking water +1 mL of ACIDAL®ML per liter; Lacto 25: Drinking water +250 mL of LW per liter, Lacto 50: Drinking water +500 mL of LW per liter. [a,b,c]Mean values with different superscripts in same row are significantly different (p<0.05). [2]Standard error of the mean

Table 4: Effect of liquid whey and organic acid on some serum biochemical indices

		Treatments[1]						
Parameters	Age (weeks)	T-	T+	Aci	Lacto 25	Lacto 50	SEM[2]	p-value
Albumin (g dL^{-1})	12	2.40	2.38	2.33	2.59	2.46	0.04	0.342
	24	1.64^b	2.17^a	1.84^a	1.74^{ab}	1.53^b	0.07	0.028
	36	1.88	1.99	1.89	1.98	1.72	0.06	0.635
Total protein (g L^{-1})	12	27.30	27.80	25.40	25.80	20.40	1.05	0.159
	24	41.70	34.80	43.40	32.80	41.20	1.57	0.117
	36	45.90	41.50	44.80	39.90	42.00	1.30	0.633
Cholesterol (mg dL^{-1})	12	77.10	75.20	70.80	80.40	76.70	3.29	0.930
	24	76.70	60.70	87.60	87.60	71.80	3.89	0.154
	36	68.60	68.40	57.20	70.90	56.40	3.01	0.379
Triglycerides (mg dL^{-1})	12	44.50	36.40	43.00	41.20	37.00	2.04	0.689
	24	179.00	181.00	150.00	154.00	149.00	10.83	0.816
	36	76.20	148.00	67.50	77.40	108.00	13.29	0.379

[1]T-: Drinking water without antibiotic (Tétracolivit), LW and ACIDAL®ML, T+: Drinking water +500 mg L^{-1} of antibiotic 5 consecutive days per month, Aci: Drinking water +1 mL of ACIDAL®ML per liter, Lacto 25: Drinking water +250 mL of LW per liter, Lacto 50: Drinking water +500 mL of LW per liter. [a, b]Mean values with different superscripts in same row are significantly different (p<0.05). [2]Standard error of the mean

numerically increased RBC number was observed at 36 weeks of age in Lacto 25 Group (2.66$\times$106 µL^{-1}) as compared to T- (2.45$\times$106 µL^{-1}), T+(2.39 x 106 µL^{-1}), Aci (2.42$\times$106 µL^{-1}) and Lacto 50 (2.40$\times$106 µL^{-1}) groups. Overall, a slight increase was observed in haemoglobin and haematocrit values in the groups that received whey and organic acid in drinking water. However, this increase was not significant statistically (p>0.05) in comparison to the control groups.

With respect to the age of the hens, white blood cell (WBC) number and lymphocytes percentage decreased in all treatments from 12-36th weeks of age. WBC number and lymphocytes percentage were reduced (p<0.05) in whey and organic acid group in comparison with the negative control group. In addition, WBC number was increased with whey level in drinking water. As for the heterophils, their proportion remained comparable across the treatments during the experimental period.

Serum immunological and biochemical indices: Table 4 shows the effect of LW and organic acid in drinking water on some serum biochemical parameters of laying hens. Serum total protein level increased (p<0.0001) with hens' age in all treatment groups but ,was not affected by the treatments. At 12 weeks of age, serum albumin concentration was comparable across the treatments. At 24 weeks of age serum albumin levels decreased in all groups and with regard to the

Table 5: Effects of liquid whey and organic acid on serum IgA and IgG

		Treatments[1]						
Parameters	Age (weeks)	T-	T+	Aci	Lacto 25	Lacto 50	SEM[2]	p-value
IgA (mg mL^{-1})	12	1.01	1.00	1.14	1.02	1.07	0.02	0.087
	24	0.98	1.08	1.04	1.1	1.03	0.03	0.705
	36	0.99	1.01	1.26	1.08	1.04	0.04	0.323
IgG (mg mL^{-1})	12	8.88	8.76	9.77	9.87	8.71	0.36	0.749
	24	10.04^{a}	8.66^{ab}	7.26^{ab}	6.26^{b}	7.11^{ab}	0.43	0.033
	36	4.46	8.03	4.79	5.73	4.99	0.38	0.061

[1]T-: Drinking water without antibiotic (Tétracolivit), LW and ACIDAL®ML, T+: Drinking water +500 mg L^{-1} of antibiotic 5 consecutive days per month, Aci: Drinking water +1 mL of ACIDAL®ML per liter, Lacto 25: Drinking water +250 mL of LW per liter, Lacto 50: Drinking water +500 mL of LW per liter. [a, b,c]Mean values with different superscripts in same row are significantly different (p<0.05). [2]Standard error of the mean

treatment, albumin value in T+, was higher (p = 0.028) than those in T- and Lacto 50 groups but was comparable to those in Aci and Lacto 25 groups. At 36 weeks of age, albumin levels slightly increased in all treatments but there were no significant differences (p = 0.635) among the treatments. Total serum triglyceride and cholesterol were not affected by treatments in the current study. But, with respect to hen's age, total serum triglyceride increased (p<0.0001) in all treatments during the laying period. Total serum cholesterol decreased (p = 0.049) across the treatment groups at 36 weeks of age. In general, the highest values in triglyceride were obtained at 24th week of age.

As shown in Table 5, there was no effect of treatments on the serum IgA content during the experimental period. But a numerically increase (p = 0.078) in serum IgA contents was observed in ACIDAL®ML group compared to the negative control group. On the other hand, serum IgG contents (Table 5) at 12 and 36th weeks of age was not affected by the treatments but at 24 week of age the use of liquid whey at 25% in drinking water, reduced the content of serum IgG (p = 0.033) compared with the negative control. In general, the use of organic acid and LW in drinking water, reduced the content of serum IgG (p = 0.010) compared with the negative control. According to hen's age, serum IgG contents decreased in all treatments group during the laying period. In this study, serum IgA and IgG content were not affected by LW levels in drinking water of laying hens.

DISCUSSION

Supplementation of organic acid and whey in poultry drinking water or diets have been reported to modify gut microflora population[10,23] and stimulate the digestive system[24], ultimately improve growth and health of birds. Organic acids and prebiotics supplementations have the capacity to stimulate growth performance[19], nutrient utilization[25,26] and health of the birds[27]. The current study showed that, supplementation of ACIDAL®ML and liquid whey (LW) through water, decreased feed consumption (from week 21-36) and mortality (throughout the experimental period), leading to increased body weight gains (from week 9-36) and improved feed conversion ratio (FCR). The reduction in feed intake could be attributed to the improved feed retention in the gastrointestinal tract (GIT) and increased nutrient utilization[28,29] as a result of a better GIT milieu enabled by beneficial microorganisms[26,30]. Zarei *et al*.[31] observed a similar trend when dried whey was fed alone or in combination with probiotic to broilers. Moreover, similar results were also found by Wang *et al*.[32] when encapsulated essential oils and organic acids were fed to hens during the early laying period. The improvement in the average daily gain (ADG) was also found by Majewska *et al*.[33] who reported that the inclusion of fresh acid whey or lactic acid at the rate of 4 mL L^{-1} of water, twice a week for 4 h sustained overall production results and contributed in particular to a decrease in mortality. It is well known that organic acids improved nutrient digestion and availability[34,35]. It is therefore expected that ACIDAL®ML supplementation should supply more nutrient for hen's metabolism and lead to a better growth performance. Also, the lactose contained in LW is not absorbed in the hen's intestine but it is fermented to short chain fatty acids (SCFAs)[19]. High energy metabolites such as SCFAs would supply more energy for poultry metabolism[30,36] and enhance growth performance. Similar results were reported by Marlina *et al*.[37] in a study where, Dairy Wastewater Sludge was offered to broiler chickens and resulted in a significant higher body weight gain and suppressed the growth of pathogenic bacteria. The records of mortality in this study were found to be higher in the negative control group. One function of prebiotic and organic acid is to improve gut health by enhancing beneficial microorganisms associated with health and well-being[35,38]. The better GIT environment of the birds administered with ACIDAL®ML and LW would have enhanced health status and reduced mortality rate. Besides that, in the present study, the number of RBC in Lacto 25 and Aci Group was numerically higher than those of others groups and a slight increase was observed in haemoglobin (Hb) and haematocrit values in the groups offered LW and ACIDAL®ML. These findings are in agreement with the observations of Nyamagonda *et al.*[39] who found that the mean total erythrocyte count in Group supplemented with prebiotic, probiotic or symbiotic were numerically higher than that of the control group. This improvement in RBC could be attributed to improved health status and physiological well-being of the birds administered with ACIDAL®ML and LW at 25%. Better health status has thus resulted in mortality reduction in these groups. The slight increase in Hb and haematocrit concentration in the hens supplemented with ACIDAL®ML and LW may be due to the better GIT environment enabled by ACIDAL®ML and LW. The improved GIT environment might have enhanced better iron salt absorption in the small intestine and consequently enhanced blood-forming processes. Also, in the present study, there was a significant decrease in WBC counts and lymphocyte in ACIDAL®ML and LW supplemented groups. The lower white blood cells and lymphocyte counts in organic acid and LW supplemented birds may be due to the antimicrobial activity of ACIDAL®ML and the effect of LW on the GIT harmful bacteria[35]. It is well known that high WBC and lymphocytes count is associated with bacterial[40] infection, or the presence of foreign body or antigen in the circulating system. The increased WBC and lymphocytes count observed in the negative control hens, in the present study, may thus indicate a greater phagocytic and humoral immune activity in these hens due to the presence of harmful bacteria[41]. This might have caused the poor performance of the birds observed in this group[42]. It is known that activation of immune cells is an energy-consuming process[43]. Khiaosa-Ard and Zebeli[44] reported that the activation of the immune response diverted energy away from production. Furthermore, the nutrient transport function of the gastrointestinal tract (GIT) decreased when the intestine is under prolonged inflammatory or metabolic stress[45]. The use of ACIDAL®ML and LW in drinking water tended to increase serum IgA content and decreased IgG content. Elevated serum IgA antibody titers, may indicates beneficial effects[46] of supplemental organic acid and LW on birds' immune system. The presence of IgA is one of the most important factors in intestinal mucosal immunity because IgA protects the host against various pathogenic invaders[47]. Supplementation of drinking water with organic acid and LW significantly reduced the content of serum IgG, indicating that organic acid and LW could alleviate the immune stress. Indeed, higher pathogenic exposure is often associated with higher immune response. Ahiwe *et al.*[42] reported an increase in serum IgG on day 24, when broiler chickens were challenged with salmonella lipopolysaccharide (LPS). Repeated exposure to endotoxin may result in immunosuppression and low resistance against secondary infections. In the current study, increased IgA production in treated groups would supply boosted mucosal immunity, which reduced immune system activation and IgG production[46]. Currently, information on the effect of whey on immune function in laying hens is scarce but many studies have reported that organic acids and prebiotics could alleviate the inflammatory response induced by endotoxin. The results of the present study agree with the earlier findings[48]. Furthermore, present study showed that the lower concentration of serum IgG in hens during laying period, may be due to the transportation of circulating IgG into the yolk of the ovum[49]. The lower serum albumin in T- and Lacto 50 groups could be explained by the increase of serum IgG content in T-group and serum IgA content in Lacto 50 group which could have balanced the serum total proteins level which was similar in all groups. Indeed, according to Tennant[50], the level of albumin can decrease as a result of certain infections without affecting the value of the total protein because of the increase in gamma globulin. In the present study, there was no significant change in the triglyceride and cholesterol of the birds due to the treatments but with respect to the age of hens. Triglyceride increased during the laying period. The high level of triglyceride in circulating blood during egg production may be attributed to the fact that hepatic lipid metabolism increases considerably in laying hens during egg production period[51].

CONCLUSION

Addition of LW and ACIDAL®ML in drinking water improved growth performance and reduced mortality rate of hens. Furthermore, addition of LW and ACIDAL®ML improved hens' immune status by decreasing serum IgG and increasing serum IgA and albumin.

SIGNIFICANCE STATEMENT

The study revealed that the liquid whey and ACIDAL®ML (organic acid) improved the growth performance and feed conversion ratio of hens which indicates the possibility of lower cost of poultry production. This study has also shown the prebiotic effect of liquid whey on hen's growth performance and immune status.

ACKNOWLEDGMENTS

The authors would like to thank Centre d'Excellence Régional sur les Sciences Aviaires (CERSA)" of University of Lomé (Togo) for funding this research through World Bank grant International Development Association (IDA) 5424. This work was partially achieved during the first visit of the first author to the Department of Biochemistry and Molecular Biology II, Institute of Nutrition and Food Technology (Biomedical Research Center, CIBM) of the University of Granada in the framework of the Erasmus+International Dimension programme and also with the financial support of the Coimbra Group Short Stay Scholarship Programme for young researchers from Sub-Saharan Africa.

REFERENCES

1. Ahmadi, F. and F. Rahimi, 2011. Factors affecting quality and quantity of egg production in laying hens: A review. World Applied Sci. J., 12: 372-384.
2. Yörük, M.A., M. Gül, A. Hayirli and E. Laçin 2004. Laying performance and egg quality of hens supplemented with sodium bicarbonate during the late laying period. J. Applied Anim. Res., 26: 17-21.
3. Lensing, M., J.D. van der Klis, I. Yoon and D.T. Moore, 2012. Efficacy of *Saccharomyces cerevisiae* fermentation product on intestinal health and productivity of coccidian-challenged laying hens. Poult. Sci., 91: 1590-1597.
4. Brunner, J.R., 1981. Cow milk proteins: Twenty-five years of progress. J. Dairy Sci., 64: 1038-1054.
5. Schingoethe, D.J., 1976. Whey utilization in animal feeding: A summary and evaluation. J. Dairy Sci., 59: 556-570.
6. Siso, M.I.G., 1996. The biotechnological utilization of cheese whey: A review. Bioresour. Technol., 57: 1-11.
7. Damron, B.L., D.P. Eberst and R.H. Harms, 1971. The influence of partially delactosed whey, fish meal and supplemental biotin in broiler diets. Poult. Sci., 50: 1768-1771.
8. Balloun, S.L. and J.K. Khajarern, 1974. The effects of whey and yeast on digestibility of nutrients in feather meal. Poult. Sci., 53: 1084-1095.
9. Al-Ubaidi, Y.Y. and H.R. Bird, 1964. Assay for the unidentified growth factor in dried whey. Poult. Sci., 43: 1484-1488.
10. Kermanshahi, H. and H. Rostami, 2006. Influence of supplemental dried whey on broiler performance and cecal flora. Int. J. Poult. Sci., 5: 538-543.
11. Deng, Y., B. Misselwitz, N. Dai and M. Fox, 2015. Lactose intolerance in adults: Biological mechanism and dietary management. Nutrients, 7: 8020-8035.
12. Denbow, D.M., 2000. Gastrointestinal Anatomy and Physiology. In: Sturkie's Avian Physiology, Whittow, G.C. (Ed.). 5th Edn., Chapter 12, Academic Press, San Diego, CA., USA., ISBN: 978-0-12-747605-6, pp: 299-325.
13. Morishita, Y., R. Fuller and M.E. Coates, 1982. Influence of dietary lactose on the gut flora of chicks. Br. Poult. Sci., 23: 349-359.
14. Siddons, R.C., 2005. Effect of diet on disaccharidase activity in the chick. Br. J. Nutr., 27: 343-352.
15. Atkinson, R.L., F.H. Kratzer and G.F. Stewart, 1957. Lactose in animal and human feeding: A review. J. Dairy Sci., 40: 1114-1132.
16. Van der Wielen, P.W.J.J., L.J.A. Lipman, F. Van Knapen and S. Biesterveld, 2002. Competitive exclusion of *Salmonella* enterica serovar Enteritidis by *Lactobacillus crispatus* and *Clostridium lactatifermentans* in a sequencing fed-batch culture. Applied Environ. Microb., 68: 555-559.
17. Venema, K., 2011. Intestinal fermentation of lactose and prebiotic lactose derivatives, including human milk oligosaccharides. Int. Dairy J., 22: 123-140.
18. Shariatmadari, F. and J.M. Forbes, 2005. Performance of broiler chickens given whey in the food and/or drinking water. Br. Poult. Sci., 46: 498-505.
19. Gülşen, N., B. Coşkun, H.D. Umucalilar, F. İnal and M. Boydak, 2003. Effect of lactose and dried whey supplementation on growth performance and histology of the immune system in broilers. Arch. Tierernaehrung, 56: 131-139.
20. Al-Dabbas, F.M., Z.H.M. Abu-Dieyeh and S.Y.A. Al-Dalain, 2007. Effect of drinking labaneh whey on growth performance of broilers. Int. J. Poult. Sci., 6: 842-845.
21. Tennessen, T., L. Connor, A.M. de Passille, K. Stanford, M. von Keyerserlingk and G. Griffin, 2009. CCAC guidelines on: The care and use of farm animals in research, teaching and testing. Canadian Agri-Food Research Council, Ottawa ON, Canada.
22. Bouassi, T., Y. Ameyapoh, V. Van Hamme, K. Anani and Y. Adjrah *et al.*, 2016. Effect of mixing ACIDAL®with drinking water for laying hens on production performance. Int. J. Poult. Sci., 15: 365-372.
23. Adil, S., M.T. Banday, G.A. Bhat, S.D. Qureshi and S.A. Wani, 2011. Effect of supplemental organic acids on growth performance and gut microbial population of broiler chicken. Livest. Res. Rural Dev., Vol. 23.
24. Szczurek, W., M.N. Alloui and D. Józefiak, 2018. The effects of dietary whey lactose and lactobacillus agilis bacteria on the growth performance, physicochemical conditions of the digestive tract and the caecal microbial ecology of broiler chickens. Ann. Anim. Sci., 18: 483-500.
25. Biggs, P. and C.M. Parsons, 2008. The effects of several organic acids on growth performance, nutrient digestibilities and cecal microbial populations in young chicks. Poult. Sci., 87: 2581-2589.
26. Olukosi, O.A. and N.D. Dono, 2014. Modification of digesta pH and intestinal morphology with the use of benzoic acid or phytobiotics and the effects on broiler chicken growth performance and energy and nutrient utilization. J. Anim. Sci., 92: 3945-3953.

27. Canibe, N., S.H. Steien, M. Overland and B.B. Jensen, 2016. Effect of K-diformate in starter diets on acidity, microbiota and the amount of organic acids in the digestive tract of piglets and on gastric alterations. J. Anim. Sci., 79: 2123-2133.
28. Bonsu, F.R.K., A. Donkoh, S.A. Osei, D.B. Okai and J. Baah, 2014. Direct-fed microbial on the health status, productive performance and internal egg characteristics of layer chickens under hot humid environmental conditions. Afr. J. Agric. Res., 9: 14-20.
29. Dono, N.D., N.H. Sparks and O.A. Olukosi, 2013. Association between digesta ph, body weight and nutrient utilization in chickens of different body weights and at different ages. J. Poult. Sci., 51: 180-184.
30. Pineda-Quiroga, C., R. Atxaerandio, I. Zubiria, I. Gonzalez-Pozuelo, A. Hurtado, R. Ruiz and A. Garcia-Rodriguez 2016. Productive performance and cecal microbial counts of floor housed laying hens supplemented with dry whey powder alone or combined with Pediococcus acidilactici in the late phase of production. Livestock Sci., 195: 9-12.
31. Zarei, A., A. Lavvaf and M.M. Motlagh, 2017. Effects of probiotic and whey powder supplementation on growth performance, microflora population and ileum morphology in broilers. J. Applied Anim. Res. 46: 840-844.
32. Wang, H., S. Liang, X. Li, X. Yang, F. Long and X. Yang, 2019. Effects of encapsulated essential oils and organic acids on laying performance, egg quality, intestinal morphology, barrier function and microflora count of hens during the early laying period. Poult. Sci., 98: 6751-6760.
33. Majewska, T., K. Pudyszak, K. Kozlowski, K. Bohdziewicz and P. Matusevicius, 2009. Whey and lactic acid in broiler chickens nutrition. Vet. Med. Zootechnol., 47: 56-59.
34. Rafacz-Livingston, K.A., C.M. Parsons and R.A. Jungk, 2005. The effects of various organic acids on phytate phosphorus utilization in chicks. Poult. Sci., 84: 1356-1362.
35. Yadav, S. and R. Jha, 2019. Strategies to modulate the intestinal microbiota and their effects on nutrient utilization, performance and health of poultry. J. Anim. Sci. Biotechnol., Vol. 10, No. 2, 10.1186/s40104-018-0310-9
36. Jozefiak, D., A. Rutkowski and S.A. Martin, 2004. Carbohydrate fermentation in the avian ceca: A review. Anim. Feed Sci. Technol., 113: 1-15.
37. Marlina, E.T., R.L.L. Balia and D.W. Lukman, 2020. The effect of addition fermented dairy-waste water sludge by *Aspergillus niger* in ration on growth performance and the caecal microbial of broiler. Anim. Prod., 21: 43-48.
38. Huyghebaert, G., R. Ducatelle and F. van Immerseel, 2011. An update on alternatives to antimicrobial growth promoters for broilers. Vet. J., 187: 182-188.
39. Nyamagonda, H., M.N. Swamy, T. Veena, H.D.N. Swamy and K. Jayakumar, 2009. Effect of prebiotic, probiotic and G-probiotic SPL®on certain haematological parameters in broiler chickens. Vet. World, 2: 344-346.
40. Ahamefule, F.O., B.E. Obua, I.A. Ukweni, M.A. Oguike and R.A. Amaka, 2008. Haematological and biochemical profile of weaner rabbits fed raw or processed pigeon pea seed based diets. Afr. J. Agric. Res., 3: 315-319.
41. Morales-Lopez, R. and J. Brufau, 2013. Immune-modulatory effects of dietary *Saccharomyces cerevisiae* cell wall in broiler chickens inoculated with *Escherichia coli* lipopolysaccharide. Br. Poult. Sci., 54: 247-251.
42. Ahiwe, E.U., M.E. Abdallh, E.P. Chang'a, M. Al-Qahtani, A.A. Omede, H. Graham and P.A. Iji, 2019. Influence of autolyzed whole yeast and yeast components on broiler chickens challenged with salmonella lipopolysaccharide. Poult. Sci., 98: 7129-7138.
43. Ghareeb, K., W.A. Awad, J. Böhm and Q. Zebeli, 2016. Impact of luminal and systemic endotoxin exposure on gut function, immune response and performance of chickens. World's Poult. Sci. J., 72: 367-380.
44. Khiaosa-ard, R. and Q. Zebeli, 2014. Cattle's variation in rumen ecology and metabolism and its contributions to feed efficiency. Livestock Sci., 162: 66-75.
45. Oviedo-Rondón, E.O., 2019. Holistic view of intestinal health in poultry. Anim. Feed Sci. Technol., 250: 1-8.
46. Liu, Y.J., L.H. Zhao, R. Mosenthin, J.Y. Zhang, C. Ji and Q.G. Ma, 2019. Protective effect of vitamin E on laying performance, antioxidant capacity and immunity in laying hens challenged with *Salmonella* enteritidis. Poult. Sci., 98: 5847-5854.
47. Sauer, N., M. Eklund, E. Bauer, M.G. Gänzle, C.J. Field, R.T. Zijlstra and R. Mosenthin, 2012. The effects of pure nucleotides on performance, humoral immunity, gut structure and numbers of intestinal bacteria of newly weaned pigs. J. Anim. Sci., 90: 3126-3134.
48. Shang, Y., A. Regassa, J.H. Kim and W.K. Kim, 2015. The effect of dietary fructooligosaccharide supplementation on growth performance, intestinal morphology and immune responses in broiler chickens challenged with *Salmonella* enteritidis lipopolysaccharides. Poult. Sci., 94: 2887-2897.
49. Cetin, N., B.K. Guclu and E. Cetin, 2005. The effects of probiotic and mannanoligosaccharide on some haematological and immunological parameters in turkeys. J. Vet. Med. Ser. A, 52: 263-267.
50. Tennant, B.C., 1997. Hepatic Function. In: Clinical Biochemistry of Domestic Animals, Kaneko, J.J., J.W. Harvey and M.L. Bruss (Eds.). Academic Press, Cambridge, Massachusetts, USA., ISBN-13:978-0-12-396305-5, pp: 327-352.
51. Lorenz, F.W., C. Entenman and I.L. Chaikoff, 1938. The influence of age, sex and ovarian activity on the blood lipids of the domestic fowl. J. Biol. Chem., 122: 619-633.