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Control of Acute Thermal Stress in Broilers Ross 308 Line by Inclusion of Betaine in Drinking Water and its Economic Analysis in El Quinche Parish, Ecuador

Control del Estrés Térmico Agudo en Pollos de Engorde Línea Ross 308 mediante la Inclusión de Betaína en Agua de Bebida y su Análisis Económico en la Parroquia El Quinche, Ecuador

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Abstract

This study evaluated different concentrations of inclusion of betaine supplied in the drinking water for the control of acute heat stress in broiler chickens Ross 308 Line and its effect on the productive parameters and economic benefit in two production cycles. In two poultry houses, the experiment began with 2672 and 2304 broiler chickens that were distributed in the third week in 16 quadrants with four pseudo-replicates of 167 and 144 chickens each quadrant. Three treatments of betaine (1.5, 2 and 2.5 g/l) and one control treatment (without betaine) distributed at random were supplied in the drinking water during the last week of breeding (seven week). The supply of 1.5 g/l (T2) of betaine level showed the highest average weight (2441 ± 52.2 g) and lowest mortality (2.96%) during the first cycle, whereas T4 exhibited highest average weight (2925 ± 60.2 g) and lowest mortality (3.43%) during the second cycle. All treatments revealed acute thermal stress with no significant differences in body temperature. In the economic analysis T2 and T4 showed the highest net income with about 60.44% and 67.36% with reasonable cost-benefit ratio (1.42 and 1.93) during first and second cycle, respectively. This study suggests the supply of betaine between 1.5-2.5 g/l in the water during the last week of rearing period in Mediterranean areas along with good management practice to mitigate the acute thermal stress in commercial chicken broilers Line Ross 308.

Keywords: Betaine, broiler, economic analyses, productive parameters, acute thermal stress.

Resumen

Este estudio evaluó diferentes concentraciones de inclusión de betaína suministrada en el agua de bebida para el control del estrés térmico agudo en pollos de engorde de la línea Ross 308 y su efecto sobre los parámetros productivos y el beneficio económico en dos ciclos de producción. El experimento se inició en dos galpones con 2672 y 2304 aves que fueron distribuidas en la tercera semana en 16 cuadrantes con cuatro pseudo-réplicas de 167 y 144 aves en cada cuadrante. Tres tratamientos de betaína (1,5; 2 y 2,5 g/l) y un tratamiento control (sin betaína) distribuidos al azar se suministraron en el agua de bebida durante la última semana de crianza (séptima semana). El suministro de 1,5 g/l (T2) de betaína mostró un mayor peso promedio (2441 ± 52,2 g) y menor mortalidad (2,96 %) durante el primer ciclo, mientras que el T4 (2,5g/l) mostró el mayor peso promedio (2925 ± 60,2 g) y menor mortalidad (3,43 %) durante el segundo ciclo. Todos los tratamientos revelaron un estrés térmico agudo sin diferencias significativas en la temperatura corporal. En el análisis económico, los T2 y T4 mostraron los ingresos netos más altos de alrededor del 60,44 % y 67,36 %, con una relación costo-beneficio de 1,42 y 1,93 durante el primer y segundo ciclo, respectivamente. Este estudio sugiere el suministro de betaína entre 1,5-2,5 g/l en el agua de bebida durante la última semana de crianza en zonas mediterráneas junto con buenas prácticas de manejo para mitigar el estrés térmico agudo en los pollos de engorde Línea Ross 308.

Palabras clave: Betaína, broiler, análisis económico, parámetros productivos, estrés térmico agudo.

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1 Introduction

Broiler chickens are homeothermic animals because they have the ability to regulate and maintain their own internal body temperature (Araújo et al., 2015; Mascarenhas et al., 2020). This mechanism is only efficient when its internal temperature is within the thermo-neutral zone, (27.5-37.7 °C) (Ajakaiye et al., 2011; Mutibvu et al., 2017; Saeed et al., 2019). Several factors may influence the thermo-neutral zone in broilers as body weight, amount of plumage, acclimatization, and state of dehydration (Pereira and Nääs, 2008; Araújo et al., 2015; Bhadauria et al., 2017).

The resulting heat is a product of metabolism (glycolysis, Krebs cycle, phosphate derivation pathway) and muscle activity produced within the body of the animal (Zahoor et al., 2016; Lu et al., 2017; Tickle et al., 2018; Zaboli et al., 2019; Barzegar et al., 2020). Different factors can affect the amount and production of heat as physical activity, ambient temperature, circadian rhythms, among others (Syafwan et al., 2011; Lara and Rostagno, 2013; Fisinin and Kavtarashvili, 2015; Baracho et al., 2019). Therefore, to maintain body temperature within the thermo-neutral zone, broilers dissipate excess heat to the surrounding environment through cell conduction and vascular convection (Baracho et al., 2011; Da Silva et al., 2015; Nilsson et al., 2016; Nascimento et al., 2017).

Poultry farming mainly depends on climatic conditions such as temperature and humidity (Nawab et al., 2018). The internal body temperature of an adult chicken is around 40.5°C. This temperature increases as the ambient temperature rises or falls above or below the thermo-neutral zone (Aengwanich, 2007; Nascimento et al., 2011). When the core body temperature reaches its critical point (47°C), called the upper lethal temperature point, chickens may die from heat prostration (Scanes, 2016). When the balance between heat production and heat loss in the chicken's body is disturbed, heat loss is decreased while heat production is increased, resulting in the onset of heat stress (Dayyani and Bakhtiari, 2013; Lara and Rostagno, 2013; Saeed et al., 2019).

Stress can be defined as a non-specific response of the animal organism to adverse environmental conditions that produces physiological and metabolic adjustments to maintain homeostasis, generates effects on the central nervous system, the neuroendocrine system and the immune system (Lin et al., 2006; Martin et al., 2011). Heat stress negatively affects not only productive and reproductive performance but also the profitability of the poultry farmer and the welfare of chickens (Pawar et al., 2016; Alagawany et al., 2017; Ranjan et al., 2019). The increase in the cost of maintenance energy triggers heat stress in poultry (Syafwan et al., 2011; Rath et al., 2015; Shlomo, 2015; Zhang et al., 2016). On a biochemical level, an increase of up to 20 times in the breathing rate of chickens can cause greater loss of CO_2 through the lungs (Knížatová et al., 2010; Nascimento et al., 2017). This loss results, in an increase in the blood pH causing an acceleration of the acid-base balance (Olanrewaju et al., 2006; Borges et al., 2007) which ends up affecting the health, welfare and performance of the chickens.

Heat stress also causes lipid peroxidation due to excessive generation of reactive oxygen species (Akbarian et al., 2016). Antioxidant supplementation has the ability to reduce oxidative instability of proteins and lipids, and this may be associated with increased activity of cellular antioxidant enzymes (Delles et al., 2014).

Betaine can be found in different plants and animal species as a natural substance (Nudiens et al., 2001). Betaine acts in the metabolism of chickens as a donor of methyl groups for protein synthesis, nucleic acids and choline (McDevitt et al., 2000). Is a methyl derivative of glycine and a metabolite of choline degradation that acts further as an osmolite helping to maintain cellular water, ion balance, methionine conservation, and fat distribution (Eklund et al., 2005; Ratriyanto et al., 2009; Ahmed et al., 2018; Shakeri et al., 2018). Among the benefits of betaine is that can enhance water retention due to the osmotic effect increasing cell volume and hence anabolic activity, cell membrane integrity and overall performance of the chicken (Shakeri et al., 2018; Liu et al., 2019). The osmolytic property of betaine permits cellular adaptation to adverse osmotic environments noticed in hot and humid climates. As a donor of the methyl group, betaine can also replace up to 20% of the dietary methionine and up to 100% of the choline in the diets of commercial broilers, saving feed costs (Sakomura et al., 2013).

Heat stress is a major challenge to the welfare of the chickens and the profitability of the poultry farm. Therefore, growers must be aware and vigilant in managing and maintaining adequate house internal temperatures in the final weeks of rearing period, especially in areas with a Mediterranean climate during summer. Behavioral events as well as production parameters provide relevant information on the responses of poultry to heat stress conditions due to high ambient temperature and high relative humidity (Lara and Rostagno, 2013; Nyoni et al., 2019).

In Ecuador, there is a lack of scientific studies on betaine administration in drinking water to cope heat stress in chicken, their effect on production parameters and its economic benefit during hot seasons with Mediterranean climates. This study hypothesizes that the higher concentration of betaine in the drinking water, the lower mortality and lower average weight of the flock at the end of the rearing period, given a better hydration of the animals and less food intake in the hottest hours. Therefore, this study, evaluates different concentrations of inclusion of betaine supplied in the drinking water for the control of acute heat stress in chickens of the Ross 308 Line from 38 days of age and its effect on the productive parameters, as well as the economic benefit of the product applied to a Mediterranean climate zone in the Sierra region of Ecuador.

2 Methodology

2.1 Experimental site and microclimatic conditions

The research was carried out in the commune of La Victoria, belonging to the parish of El Quinche, province of Pichincha, 40 km east from the city of Quito. The study area is located at an altitude of 2619 meters above sea level (masl), with a mediterranean climate, registering average temperatures of 18°C with variations of 0.6°C throughout the year. The hottest month is September, with maximum temperatures up to 30°C and a monthly rainfall of 53 mm. The rainiest month is registered in April with 126 mm monthly average.

The experiment was conducted from August to November 2019, during the hottest season of the year. Two poultry houses with an area of 264 m^2 each, housed 2700 male birds during August to September for the first production cycle (FPC), and 2330 male birds during October to November for the second production cycle (SPC). However, the experiment initiated at the last week (seventh week) of the rearing period with 2672 and 2304 animals for the FPC and SCP, respectively, due to the normal mortality registered.

The internal temperature conditions of the houses were checked prior to the reception of the chickens, recording a temperature of 33°C each. The animals were vaccinated against Marek's disease from the hatchery and were fed with fresh, clean, commercial feed and water throughout their cycle.

2.2 Poultry management and betaine supply

At the end of the third week of life (21 days), the chickens were placed in 16 quadrants of 15 m^2 each, and evenly distributed within each poultry house to allocate 167 chickens per quadrant during the FPC and 144 chickens per quadrant during the SPC. The animals were distributed in quadrants in this week since at this age the animals are able to self-regulate their internal body temperature and not depend on external artificial heat (brooders), as well as to facilitate the management of the chickens during the experimental period.

Within each poultry house, the 16 quadrants were classified into four treatments distributed randomly with four pseudo-replications of each treatment. Each experiment consisted of a control treatment (T1: without betaine) and three treatments with different concentrations of betaine (T2: 1.5 g/l; T3: 2 g/l and T4: 2.5 g/l). Betaine was supplied in the drinkers from day 43 and 44 onwards, in the first and second house, respectively, during the hottest hours of the day (12:00 am-15:00 pm). Betaine was suspended in both houses two days prior to slaughter, to avoid the existence of residues inside the animal's body at the time of sale. Only clean water was supplied on the last day of the production cycle. The animals were in the houses until day 48 and 49 during FCP and SCP, respectively.

In broilers, glucocorticoids are produced as corticosterone, and 75% are excreted in the urine (Scanes, 2016). For this reason, a non-invasive technique was used to determine corticosterone levels avoiding generate stress in the chickens by manipulation. Fecal samples (later separation of the liquid portion of the poultry feces) were collected during the experimental period (day 43 to 46 [FCP]; day 44 to 47 [SCP]). This procedure was performed with caution during the first hours of the day (7:00 am-9:00 am) to avoid generating any type of stress (physiological or behavioral) in the animals. The samples collected for each treatment in both cycles were sent to the laboratory for subsequent analysis.

The internal body temperature was registered at day 43, 45 and 47 in both houses in 10% sample of the total chickens by each quadrant, to determine the signs of heat stress using a penetration/immersion probe. The probe was gently introduced 5 to 6 cm (depend on age and size of chickens) at the level of the terminal colon with caution to not generate stress. This procedure was performed in early hours of the morning. Finally, an economic feasibility analysis of the use of betaine in broilers was done to determine the profitability and best cost-benefit (C/B) ratio of betaine supply,

3 Statistical Analysis

Data on average weight, feed intake, weight gain per animal, and body temperature were tested for normality for the two production cycles using the Shapiro-Wilk test. If the data followed normal distribution, the ANOVA one-way test was applied to determine the differences in means between treatments and between cycles. Otherwise, a Mann-Whitney U test was used. In addition, a Multiple Linear Regression Analysis (MLRA) was done to test the hypothesis and analyze the influence of the different levels of betaine on each of the independent variables (final weight, feed consumption, weight gain and body temperature) using a least-squares approach.

Finally, a t-test was done to compare the means of final weights at each production cycle as a proxy to determine statistical differences in the C/B ratio. The most informative model was selected based on the Akaike Information Criterion (AIC). All statistical analyses were performed in version 3.4.1 of the R software (The developmet Core Team, 2017).

4 Economic Feasibility Analysis

The economic analysis was carried out for both cycles considering the final average weight of the animals, the total number of chickens for each treatment at the end of the cycle, and the price per pound in the market at that time. Only the variable costs involved during the period of the experiment (week 7) were considered in the analysis (feed and betaine cost). The net income of the poultry was determined based on the difference between the sale of the chickens (gross income) and the variable costs. Based on the net benefit, the cost-benefit relation was calculated to determine which treatment was the most adequate, with better productive parameters and better profitability.

5 Results and discussion

The results exhibited T2 (1.5 g/l) as the highest average weight during the FCP (2441 $\pm$ 52.2 g) (Table 1) with significant differences between the four treatments (p<0.05; n=97). During SCP, T4 (2.5 g/l) showed the highest average weight (2925 $\pm$ 60.2 g) (Table 2) with significant differences between treatments ($p<0.05$; n=83). There were significant differences between cycles, achieving a higher average weight in the SCP (2735 $\pm$ 193.21 g) than in the FCP (2315 $\pm$ 93.33 g).

During FCP and SCP, T2 showed the highest weight gain / animal / day with 66.1 $\pm$ 8,46 g and 111,3 $\pm$ 9,44 g, respectively (Table 1; Table 2) with significant differences between treatments. Significant differences were also observed between cycles, obtaining a greater weight gain in the SCP (79.7 $\pm$ 21.66 g/animal/day) than in the FCP (47.16 $\pm$ 13.18 g/animal/day). The highest feed intake during the FCP was 185.7 $\pm$ 1.21 g belonging to T4 (Table 1), with differences in all treatments except between T3 and T4 (p=0.353; W=244; n=97). During SCP, the highest feed intake was for T3 with an average of 179.6 $\pm$ 1.72 g / animal / day (Table 2), showing significant differences between treatments ($p<0.05$; n=83). Significant differences were observed between cycles, showing an overall average feed intake of 184.5 $\pm$ 1.76 g/animal day and 173.4 $\pm$ 4.68 g/animal/day, for FCP and SCP, respectively.

Table 1. General performance of the chickens Ross Line 308 at the end of the rearing period with the supplementation of different levels of betaine during the first cycle.

Treatment	Average weight ± SD (g)	Weight gain /animal/ day (g)	Food intake/animal/ day (g)	% Mortality	Body temperature (°C)	Corticosterone level (nmol/L)
T1 (control)	2275 ± 32.8	70.2 ±5.20	184.5 ±1.10	4.29	42.5 ±1.90	113.13 ±11.55
T2 (1.5 g/L)	2441± 52.2	66.1 ±8.46	182.3 ±1.24	2.96	43.0 ±2.62	123.10 ±5.20
T3 (2 g/L)	2213 ± 35.6	35.1 ±4.83	185.5 ±1.06	5.33	42.8 ±2.71	102.61 ±15.13
T4 (2.5 g/L)	2335 ± 48.1	50.7 ±5.96	185.7 ±1.21	4.44	43.2 ±1.89	112.64 ±18.86

Table 2. General performance of the chickens Ross Line 308 at the end of the rearing period with the supplementation of different levels of betaine during the second cycle.

Treatment	Average weight ± SD (g)	Weight gain /animal/ day (g)	Food intake/animal/ day (g)	% Mortality	Body temperature (°C)	Corticosterone level (nmol/L)
T1 (control)	2600 ± 50.4	71.6 ±5.8	174.9 ±1.6	5.2	43.7 ±1.97	116.27 ±14.9
T2 (1.5 g/L)	2903± 50.0	111.3 ±9.4	172.1 ±1.0	3.7	42.7 ±2.3	124.32 ±9.0
T3 (2 g/L)	2498 ± 41.9	55.7 ±5.0	179.6 ±1.72	7.2	43.5 ±2.2	110.56 ±15.5
T4 (2.5 g/L)	2925 ± 60.2	79.0 ±8.4	167.3 ±1.28	3.4	43.0 ±2.2	120.8 ±4.5

In the FCP, the lowest mortality at the end the rearing period was for T2 with 2,96 % (Table 1) whereas during the SCP was for T4 with 3,43 % (Table 2). No differences were observed between cycles. In total, 28 and 26 dead birds were registered in the FCP y SPC, respectively. Finally, the body temperature during the FCP showed no significant differences between treatments ($p>0.05$; $n=97$), recording the highest temperature for T4 with an average of 43.2 ± 1.89°C (Table 1). During the SCP, the highest temperature was for T1 with an average of 43.7 ± 1.97°C (Table 2), without showing significant differences between treatments. No significant differences were observed between cycles. It can be noted that in both cycles, the body temperatures of the chickens were less than the upper lethal temperature point (47°C).

The corticosterone analysis showed that chickens suffered acute heat stress in all treatments during the FCP (Table 1). In the SCP, the average corticosterone levels in T1 and T3 were within normal ranges (80-120 nmol/l) in spite of some animals showed more than 120 nmol/l. The T2 and T4 revealed higher average corticosterone levels with 124.3 nmol/l and 121.75 nmol/l, respectively (Table 2). No correlations between variables were observed in any of the cycles, with no problems of multicollinearity (VIF<10). The results of ARLM are summarized in Table 3 and Table 4 for FCP SCP, respectively. Data for all variables in both production cycles did not follow a normal distribution ($p<0.05$, $n=97$ and $n=83$), thus the Mann-Whitney U Test was applied to determine differences in the means between treatments and cycles.

Table 3. Multiple regression of the betaine supplementation influence in chickens Line Ross 308 at the end of rearing period during the FCP.

		Estimated	Std. Error	t-value	p-value	R^2	R^2 Adjusted	F-statistic	AIC
T1	Intercept (weight)	1243.06	320.74	3.87	0.000874 ***				235.69
	Weight gain	-0.7156	0.24	-2.92	0.008150 **	0.42	0.36	7.73	
T2	Intercept (temp)	44	0.9266	47484	<2e-16 ***				122.01
	Corticosterone (1.5 g/l)	-3.5	1.5366	-2278	0.0345 *	0.28	0.13	1.86	
T3	Intercept (weight)	2280	24.82	91861	< 2e-16 ***				231.54
	Corticosterone level (2 g/l)	-128	35.1	-3647	0.037 **	0.76	0.51	3.03	
T4	Intercept (weight gain)	330.5	226.9	1456	0.1793				
	Costicosterone level (2 g/l)	16.0	5.1	3135	0.0120*	0.82	0.55	3.04	

Hence, in the economic analysis T2, showed the best higher net income ($371.99) with the highest C/B ratio (1.42), follow by the control treatment (T1) (1.28) (Table 5). In this cycle a total of 299.8, 390 and 490.6 g of betaine were utilized for T2, T3 and T4, respectively. For the SCP, In this cycle, T4 showed the best C/B ratio (1.93), presenting a higher net income ($398.81) followed by T2 (Table 6). A total of 256.5, 327.4 and 427.6 g were consumed for T2, T3 and T4, respectively. This study evaluates different concentrations of betaine inclusion in the drinking water for the control of acute heat stress in broilers of the Ross 308 Line from 38 days age onwards and its effect on the productive parameters, and its economic benefit in dry areas of the Inter-Andean region of Ecuador. Overall, the results showed that the final weight of the broilers increased with higher level of betaine in the drinking water compared to the control treatment (T1), particularly with the addition of 1.5 g/l (T2) and 2.5 g/l (T4) at both cycles, respectively.

During the FCP, the results indicated that the weight gain showed a significant effect on the final weight of the broilers in T1 compared to the other variables (food intake, body temperature). In T2, the betaine had no significant effect with any of the variables, except with body temperature. In T3, it was observed that the inclusion of betaine of 2 g/l had a significant effect on the final weight of broilers. The inclusion of this level significantly influenced weight gain but not feed consumption. It should be noted that in the latter, the effect of betaine was obscured by the presence of the other variables in the model. This can be explained the decreased of body weight and gain weight.

Gain weight was also improved significantly with higher levels of inclusion of betaine at both cycles. These results obtained here are similar with those studies that exhibited better weight gain in broilers during heat stress period (Attia et al., 2005; Chen et al., 2018). Many other studies showed no significant effects of betaine in gain weight nor feed intake (Harms and Russell, 2002; Park and Ryu, 2010). Due to its methyl donation property, betaine could be accessible for other key functionalities such as protein synthesis and immune modulation, resulting in enhanced performance of broilers.

Several authors have found that the application of different levels of betaine (0, 0.5, 1.0 and 1.5 g/kg) in the diet have significant effects on body weight, weight gain, feed consumption and feed conversion (Awad et al., 2014). On the other hand, Nofal et al. (2015) showed that betaine levels of 0.1% and 0.2% in the diet improved body weight, weight gain, feed conversion ratio and mortality rate. Other authors have discovered that different levels of betaine in feed improve the feed conversion ratio (Tolba et al., 2007; Honarbakhsh et al., 2007; Zulkifli et al., 2004). Shaojun et al. (2015) found that 0.1%, 0.2%

and 0.4% betaine use exhibited increased in feed consumption, associated with an increased in body weight and lower feed conversion. In contrast, Sakomura et al. (2013) showed that 0.05% and 0.075% betaine supplementation did not have a significant effect on productive parameters such as feed intake and body weight gain. Likewise, El Shinnawy (2015), found that doses of 1.0, 1.5, 2.0 and 2.5 g/kg betaine in the diet indicated a significant increase in body weight and weight gain.

Table 4. Multiple regression of the betaine suministration influence in chickens Line Ross 308 at the end of rearing period during the SCP.

		Estimate	Std. Error	t-value	p-value	R^2	R^2-adjusted	F statistic
T1	Intercept (Weight gain)	1244.2	458.8	2.7	0.0161 *			
	Food Intake	-5.6	2.2	-2.4	0.0247 *	0.38	0.17	1.84
	Intercept (Food Intake)	193.1	4.4	42.9	<2e-16 ***			
	Temperature	-0.37	0.08	-4.6	0.000286 ***			
	Weight gain	-0.05	0.02	-2.4	0.024932 *			
	Corticosterone level (1.5 g/l)	1.5	0.45	3.4	0.003643 **	0.67	0.59	8.47
T2	Intercept (Temperature)	310.7	57.53	5.4	5.89e-05 ***			
	Food Intake	-1.5	0.32	-4.61	0.000286 ***			
	Weight gain	-0.09	0.04	-2.15	0.046975 *			
	Corticosterone level (1.5 g/l)	2.73	0.98	2.78	0.013171 *	0.59	0.49	5.82
T3	Intercept (Food Intake)	154.9	14.6	10.5	3.69e-09 ***			
	Weight gain	-0.009	0.003	-2.617	0.0175 *	0.61	0.57	14.6
T4	Intercept (Food Intake)	169	0.63	264.9	<2e-16 ***			
	Corticosterone level (2.5 g/l)	-2.28	0.76	-2.99	0.00772 **	0.33	0.25	4.49

According to the hypothesis of the present study showed that the higher concentration of betaine in the drinking water, the lower mortality, associated to a higher average weight, with slightly less weight gain and no mark tendency in the food intake at the end of the rearing period compared to the control treatment (T1). Surprisingly, for T4, no significant relationship was observed between the betaine level and any of the explanatory variables (weight, weight gain, feed intake, corporal temperature). Excessive inclusion of betaine in the drinking water can cause energy loss due to their excretion and increasing betaine level may reduce its efficacy.

The final weight of the herd was not compromised at the end of the productive cycle. It was assumed that during the hottest hours, the animals would reduce feed consumption; however, this only happened for SCP in which consumption slightly decreased in a relative -0.4% between the sixth and seventh week, whereas for FCP increased by 13%. There results may be reflected in corticosterone levels where animals in the second cycle showed higher average levels (117.9 nmol/l), close to the limit of the normal range (80-120 nmol/l), which would be associated with a reduction in feed intake. Several factors can explain these differences as the duration and extent of heat stress, species of broilers, growth stages, type of diets, during periods of osmotic disturbances, caused by heat stress in which betaine may protect and improve the morphological characteristics of intestinal epithelia. These results are also partially consistent with several authors (Zulkifli et al., 2004) which reported no significant effect of betaine supplementation on feed intake. However, are consistent with those obtained by Attia et al. (2005), Zhang et al. (2016), and He et al. (2015). The betaine supplementation exhibited improved growth performance with the increasing on final weight and weight gain.

Table 5. Economic feasibility analysis of the suministration of betaine in the drinking water during the first cycle.

Treatment	Average weight (g)	Average weight (pounds)	Price per pound ($)	Number of animals	Raw income ($)	Feed intake (g/animal/day)	Total intake ($)	Quantity of food (20 kg)	Unitary cost (20 kg)	Total cost ($)	Net income ($)	Cost/ benefit ratio
T1	2275	5.00	0.75	162	607.5	184.3	209.0	10.00	25	261.3	346.1	1.32
T2	2441	5.37	0.75	164	660.5	182.9	210.0	10.00	25	261.3	371.9	1.42
T3	2213	4.87	0.75	160	584.4	185.3	207.5	10.37	25	259.4	298.9	1.15
T4	2335	5.13	0.75	161	619.4	185.4	209.0	10.45	25	287.3	332.1	1.15

Table 6. Economic feasibility analysis of the suministration of betaine in the drinking water during the second cycle.

Treatment	Average weight (g)	Average weight (pounds)	Price per pound ($)	Number of animals	Raw income ($)	Feed intake (g/animal/day)	Total intake ($)	Quantity of food (20 kg)	Unitary cost (20 kg)	Total cost ($)	Net income ($)	Cost/ benefit ratio
T1	2600	5.72	0.70	137	548.5	174.9	167.8	8.39	25	209.7	338.7	1.61
T2	2903	6.38	0.70	140	625.8	172.1	168.7	8.43	25	210.9	388.9	1.84
T3	2498	5.49	0.70	134	515.4	179.6	168.4	8.42	25	210.5	278.8	1.32
T4	2925	6.43	0.70	140	630.4	168.0	164.6	8.23	25	205.8	398.8	1.93

In the economic analysis, the best treatments with higher net income and lower percentage of mortality at the end of the rearing period were levels of 1.5 g/l (T2) and 2.5 g/l (T3) of betaine for the FCP and SCP, respectively. T2 showed the best cost-benefit ratio (1.42) and T4 (1.93) during the SCP. The latter can be explained due to higher average final weight of the herd at the end of the rearing period that it reflects in a better C/B ratio. These results suggest that the C/B ratio may be influence by the final weight of the herd as well as the price in the market that will determine the rentability for the farmer. Amer et al. (2018) have found that feed intake improved growth performance with better return in the net income and C/B ratio. They suggest that the improvement in the final weight and weight gain due to betaine supplementation might be attributed to the osmolytic property of betaine that supports intestinal cell growth and enhances cell activity, resulting in improving nutrient digestibility.

6 Conclusions

The results obtained in this study suggests that supplementation of betaine in the drinking water in broiler chickens Line Ross 308, can enhance average final weight particularly with levels of 1.5 g/l that an enhance gain weight and reduce the mortality at the end of the period. Higher levels of betaine (2.5 g/l) can also improve the rentability of the farmer, nevertheless, might be depend on factors such as final weight, market price, appropriate management of the herd and suitable environment conditions of the poultry house. Finally, this study encourages the application of betaine in the drinking water in commercial chickens at small as well as large-scale productions especially in the last days of the rearing period to overcome the acute heat stress in zones with mediterranean climates. Complementary, an appropriate management along the production cycle is essential to minimize the heat stress in the animals. Practices as good ventilation inside the poultry house, adjusting bird density, ensuring availability of fresh, low-salt drinking water, serving feed at cooler times of the day or reducing the effects of excessive temperatures by separating birds by sex can help mitigate its effects.

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