DIETARY ASCORBIC ACID AND CHROMIUM SUPPLEMENTATION FOR BROILERS REARED UNDER THERMONEUTRAL CONDITIONS VS. HIGH HEAT STRESS

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Abstract

The present study evaluates the effect of dietary ascorbic acid and chromium (AACr) on performance and microflora balance of broiler reared under thermoneutral conditions (TN) vs. high heat stress (HS). Two experiments were conducted on 112 Cobb 500 broilers, assigned in two experimental groups (28 chickens/group) and housed in an environmental controlled hall. Two groups (C-TN and AACr-TN) were kept in thermoneutral conditions. In the second experiment, other two groups were kept (C-HS and AACr-HS) in high heat stress (32° C). The structure of diets was the same in both experiments. Compared with the control diet (C), the experimental diet included the addition of a premix with 25 g ascorbic acid / kg premix + 20 mg chromium picolinate/ kg premix (AACr). At the end of the experiment, 8 broilers / group were slaughtered and samples of intestinal and caecal content were collected for bacteriological assessment. Dietary AACr did not affect (p>0.05) body weight, daily feed intake, feed conversion rate neither under TN nor under HS condition. The use of AACr in broiler diet led to a significantly reduction of staphylococci in the caecum. Both in caecum and intestinal content of AACr broilers, irrespective the temperature conditions the number of lactobacilli was significantly higher than in the C broilers. Combination of ascorbic acid and Cr (AACr) has a positive effect in limiting the development of the pathogenic bacteria colonizing the intestine and caecum during the heat stress.

Key words: broiler, ascorbic acid, chromium, microflora balance, heat stress

INTRODUCTION

Heat stress is known to be one of the most detrimental factors in overall poultry production [15]. Although it is clearly elucidated the thermoneutral zone of broilers (18-22° C; [7]), it is not easy to maintain that temperature in a specific area or a period or the combination of both.The issue of controlling stressful conditions and their consequences is becoming a big challenge. Heat stress is a major source of systemic oxidative stress since it causes a redox imbalance between the pro- and anti-oxidants in favor of prooxidants [17]. The gastrointestinal tract is one of the main organs affected by the high heat stress.

In these conditions, the intestinal integrity is impaired, which resulted in increased intestinal permeability to endotoxin and translocation of intestinal pathogens such as *Salmonella spp*. [3]. Vitamins and minerals play a vital role in animal growth and metabolism, as they are essential for many physiological processes [23]. During thermal stress, a reduction in levels of vitamins (vitamin C, E, folic acid) and minerals (Zinc, Chromium) in plasma and tissues was observed, which may be associated with reduced food intake and high water consumption. in chicken [28]; [1]. Thus, supplementation of exogenous vitamins and

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antioxidants scavenge ROS, being beneficial in mitigating oxidative stress in the gastrointestinal tract [2].

Ascorbic acid (vitamin C) is a water soluble and natural antioxidant to protect the animals under heat stressors conditions and also had an activity against oxidation [16]. However, vitamin C can be synthesized regularly in chickens [13]. Many authors have shown that vit. C (40–200 ppm) improved growth parameters of broiler chicks exposed to multiple environmental stressors [4], [5], [20].

Chromium (Cr) is an essential trace element involved in counteracting the oxidative stress associated with insulin resistance [27]. Chromium is used due to its role in nutritional metabolism, such as proteins, carbohydrates, fats, amino acids and nucleic acids [14]. If separately, the vitamins and minerals included in the ratio of chickens can mitigate the negative effects of the thermal stress, the use of their combinations has been tried [26], [10], [22]. There are studies which support synergistic action of vitamin and mineral such as ascorbic acid and chromium (Cr) during stress conditions, by sparing each other resulting in enhanced performance of birds [11]. But, no studies focused on the efffect on broiler microflora were found.

In this context, the present study evaluates the effect of dietary ascorbic acid and chromium (AACr) on performance and microflora balance of broiler reared under thermoneutral conditions (TN) vs. high heat stress (HS).

MATERIAL AND METHOD

The feeding trial was conducted in an experimental hall of the Laboratory of Chemistry and Nutrition Physiology of the National Research Development Institute for Animal Biology and Nutrition (IBNA-Balotesti, Romania) according to an experimental protocol, approved by the Ethics Commission of the Institute. Two experiments were conducted on 112 Cobb 500 broilers, assigned in two experimental groups (28 chickens/group) and housed randomly in digestibility cages (6 chicks/cage), allowing the daily recording of the feed intake and excreta. Two groups (C-TN and AACr-TN) were kept in thermoneutral conditions, according to broiler requirements (Management guide of Cobb 500). In the second experiment, other two groups were kept (C-HS and AACr-HS) in high heat stress (32° C). In both experiments (under thermoneutral condition and under heat stress) the microclimate parameters were controlled. The following parameters were ensured in the experiment under thermoneutral conditions: 27.07 ± 2.75 ° C temperature, 64.8 ± 9.57% humidity, 0.5 ± 0.24% ventilation / broiler, and 686.39 ± 104.38 ppm CO2 emission. In the experiment carried out in heat stress, the microclimate parameters were: 33.44 ° C, humidity 50%, ventilation / broiler 0.48 ± 0.36% and CO2 emission 846.39 ± 122.4 ppm. The light regimen was adequate to broiler age, i.e. 23h light / 1h darkness.

The structure of diets was the same in both experiments, in agreement with the nutritional requirements (NRC, 1994) and the nutritional requirements of the Cobb 500 hybrid (Table 1). The chicks from the control groups (C-TN, C-HS) received a conventional feed (C), based on corn and soybean meal (3039,79 kcal/kg metabolisable energy, 23% crude protein, 5.48% ether extractive) . Compared with the control diets (C-TN; C-HS), the experimental diets (AACr- TN; AACr-HS) included the addition of a premix with 25 g ascorbic acid / kg premix + 20 mg chromium picolinate/ kg premix (AACr). Ascorbic acid supplement (99% purity) was purchased from Shandong Luwei Phramaceutical Co., Ltd. Chromium supplement was used as chromium picolinate (Cr(C6H4NO2)3) purchased from a commercial company (Santa Cruz Biotechnology). None of the groups (C-TN; C-HS, AACr-TN; AACr-HS) had monoensin in the premix.Feed and water were provided for *ad libitum* consumption

Table 1 Diet formulation

Ingredient	Starter (1-14 d)		Grower (15 – 28 d)		Finisher (29-42 d)	
	C	AACr	C	AACr	C	AACr
	%					
Corn	32.73	32.73	36.63	36.63	40.64	40.64
Wheat	20	20	20	20	20	20
Gluten de porumb	2	2	4	4	6	6
Soybean meal	36.17	36.17	30.2	30.2	23.95	23.95
Sunflower oil	3.85	3.85	4.3	4.3	4.72	4.72
Monocalcium phosphate	1.68	1.68	1.52	1.52	1.43	1.43
Calcium carbonate	1.5	1.5	1.38	1.38	1.31	1.31
Salt	0.39	0.39	0.38	0.38	0.33	0.33
Methionine	0.33	0.33	0.25	0.25	0.21	0.21
Lysine	0.3	0.3	0.29	0.29	0.36	0.36
Choline	0.05	0.05	0.05	0.05	0.05	0.05
Vitamin-mineral premix*	1	1**	1	1**	1	1**
TOTAL	**100**	**100**	**100**	**100**	**100**	**100**
Calculated Metabolisable energy, kcal/kg	3039,79		3128,99		3217,72	
Chemical composition- calculated						
Crude protein, %	23.00		21.50		20.00	
Ether extractives, %	5.48		6.01		6.49	
Crude fibre, %	3.77		3.57		3.36	
Calcium, %	0.96		0.87		0.81	
Phosphorus, %	0.77		0.70		0.65	
Available phosphorus, %	0.48		0.43		0.41	
Lysine, %	1.44		1.29		0.16	
Methionine, %	0.69		0.61		0.32	
Triptophan, %	0.25		0.22		1.19	

*1kg premix contains: = 1100000 IU/kg vit. A; 200000 IU/kg vit. D3; 2700 IU/kg vit. E; 300 mg/kg vit. K; 200 mg/kg Vit. B1; 400 mg/kg vit. B2; 1485 mg/kg pantothenic acid; 2700 mg/kg nicotinic acid; 300 mg/kg vit. B6; 4 mg/kg Vit. B7; 100 mg/kg vit. B9; 1.8 mg/kg vit. B12; 2000 mg/kg vit. C; 8000 mg/kg manganese; 8000 mg/kg iron; 500 mg/kg copper; 6000 mg/kg zinc; 37 mg/kg cobalt; 152 mg/kg iodine; 18 mg/kg selenium.
**Vitamin-mineral premix + 25 g ascorbic acid/ kg premix+ 20 mg Cr picolinate/kg premix
Where: C- conventional diet; AACr- conventional diet + 25 g ascorbic acid/ kg premix + 20 mg Cr picolinate/kg premix

Throughout the experimental period (1-42 days, broiler age) the following parameters were monitored: body weight (g); average daily feed intake (g feed/ broiler/day); average daily weight gain (g/broiler/day) and feed conversion ratio (g feed/g gain). The individual body weight was recorded on a weekly basis. The experimental protocol stipulated the recording of possible mortalities throughout the experimental period. Eight birds per treatment with bodyweight within ± 150 g was slaughtered at day 42 by cervical dislocation and immediately bled. Carcasses were eviscerated manually and the gut, from the oesophagus to the cloaca was carefully excised. Intestinal and caecal contents (2 caeca per bird) were collected aseptically in sterilized plastic tubes and preserved at -20 ºC until the bacteriological analyses (Enterobacteriaceae, E. coli, staphylococci, lactobacilli, Salmonella spp). Any digesta remaining were emptied by gentle pressure. The microbiological analyses of Enterobacteriaceae, E. coli, staphylococci, lactobacilli and Salmonella spp. were determined as described previously [9]. The colony counter Scan 300, Interscience (France) was used to determine the colony count of E. coli, staphylococci and lactobacilli. The results were expressed as log base 10 colony-forming units (CFU) per gram of caecal/ intestinal contents.

The complete randomized model was used to analyse the data for growth performance and intestinal and caecal microflora. The effects of treatments were tested by analysis of variance using the GLM procedure of the Minitab software (version 17, Minitab® Statistical Software), with treatment as fixed effect. When overall F-test was significant, differences between means were declared significant at P<0.05 using the test of Tukey. The comparative graph was done using the free software R version 3.5.1.

RESULTS AND DISCUSSION

Both under TN and under HS condition, during the experimental period (1-42 days) there were no significant differences (p> 0.05) in terms of body weight, average daily gain, average daily feed intake and feed conversion ratio (Table 2). It is observed that although exposed to heat stress, the chicks from the group that included in the diet 20 mg Cr picolinate / kg premix and 25 g ascorbic acid / kg premix had a fed conversion ratio (1-14 days), significantly (p<0.05) smaller than those fed a conventional diet.

Table 2 Effects of dietary treatments on broiler performance (1-42 days)

Variable	Days of age	Thermoneutral condition				Heat stress			
		C-TN	AACr-TN	SEM	P-value	C-HS	AACr-HS	SEM	P-value
Body weight (g)	1	39.26	39.26	0.225	>0.9999	46.36	46.36	0.272	>0.9999
	14	427.66	424.27	4.207	4.375	446.10	458.28	3.602	0.3012
	28	1445.83	1383.33	15.024	0.4244	1212.57	1220.43	12.180	0.8844
	42	2605.83	2644.81	29.237	0.4658	1987.97	2014.13	30.589	0.7660
Average daily feed intake (g/day)	1-14	33.13	33.99	3.448	0.557	34.87	34.71	0.415	0.9105
	15-28	106.48	98.70	1.692	0.0253	77.38	78.21	1.856	0.9846
	29-42	150.14	156.75	2.380	0.1345	102.02	100.54	0.370	0.1535
	1-42	96.58	96.48	1.069	0.1866	71.42	71.15	2.284	0.9975
Body weight gain (g/broiler/ day)	1-14	27.74	27.52	0.447	0.6042	28.56	29.42	0.259	0.3066
	15-28	72.72	68.92	1.735	0.4622	54.75	54.44	0.793	0.9839
	29-42	82.85	88.88	1.701	0.2056	55.39	56.69	2.637	0.8497
	1-42	61.11	61.78	0.639	0.3269	46.23	46.85	0.728	0.7660
Feed conversion ratio (g feed/g gain)	1-14	1.19	1.24	0.013	0.4603	1.22a	1.18b	0.007	0.1703
	15-28	1.47	1.42	0.032	0.8517	1.42	1.44	0.006	0.3596
	29-42	1.82	1.74	0.023	0.0307	1.84	1.77	0.036	0.8643
	1-42	1.58	1.56	0.014	0.7980	1.54	1.52	0.010	0.7039

a, b Means in the same column with different superscripts differ significantly (P <0.05). SEM = standard error of the means; C- conventional diet; AACr- conventional diet + 25 g ascorbic acid/ kg premix + 20 mg Cr picolinate/kg premix.

As expected, although they received the same ratios, the chicks reared under heat tress conditions had significantly lower performance (p<0.05) than those reared under thermoneutral conditions. The 42-day body weights of chicks reared under heat stress registered decreases on average by 23% compared to chicks reared under thermonuetral conditions (23.71% C-HS vs. C-TN; 23.84% AACr-HS vs. AACr-TN). The feed conversion ratio (1-42 days) was lower by 2.53% in the C-HS group compared to those from C-TN. The chicks from AACr-HS group had the feed conversion ratio lower by 2.56% than those in the AACr-TN group. Similarly, under heat stress (32˚C), diet supplementation with ascorbic acid (80 mg/kg of diet) did not affect the performance of Cobb 500 broilers (14-35 days) [19]. Also, other authors [21] indicates that either alone or in combination, Cr methionine (1,200 µg Cr^{+3}) and vitamin C (800 mg vit. C/kg of diet) had no significant effect on final body mass, body mass gain, feed intake, and feed conversion ratio of broilers subjected to heat stress. Many studies suggest that Cr under different forms (chromium picolinate, chromium chloride) performs better in terms of weight gain in combination with antioxidants such as ascorbic acid or vitamin E [24], [20], [6], especially in case of stress conditions such as high or low temperature and humidity. Like in our study, using the same dose of vit. C (250 mg/kg diet) but a higher dose of chromium picolinate (2.5 mg/kg diet), other author [8] had shown significantly higher body weight, weight gain and improved feed efficiency of Vencobb 400 broilers reared under heat stress. Also, ascorbic acid at the level of 200 mg/kg in the diet of heat stress broilers increase weight gain, improve the feed efficiency, nutrients retention (CP, CF, EE, ME) and decreased body temperature [12]. Among others, the differences between the results of the studies may be due to the source, level of inclusion, bioavailability of vitamin C and Chromium in the broiler's diet.

Table 3 Effects of the dietary treatments on intestinal bacterial populations (lg10 CFU/ g wet intestinal content

Variable	Thermoneutral conditions				Heat stress			
	C-TN	AACr-TN	SEM	p-value	C-HS	AACr-HS	SEM	p-value
Enterobacteriaceae	7.410[a]	7.368[b]	0.007	<0.0001	7.461[a]	7.445[b]	0.003	<0.0001
E. coli	6.209[a]	6.108[b]	0.054	<0.0001	6.140[a]	6.115[b]	0.004	<0.0001
Stafilococi	5.967[a]	5.915[b]	0.008	<0.0001	5.852[a]	5.777[b]	0.012	<0.0001
Lactobacili	7.145[a]	7.170[b]	0.004	<0.0001	7.368[a]	7.385[b]	0.003	<0.0001
Salmonella spp.	absent	absent	NA	NA	absent	absent	NA	NA

a, b Means in the same column with different superscripts differ significantly (P <0.05). SEM = standard error of the means; NA= non-adequate; n= 6; C- conventional diet; AACr- conventional diet + 25 g ascorbic acid/ kg premix + 20 mg Cr picolinate/kg premix; CFU- colony forming units.

Both in TN and HS conditions, the lowest count (p<0.05) of Enterobacteriaceae, E. coli and staphylococci was recorded in the intestinal content of AACr broilers (Table 3).

On the other hand, dietary AACr had effect on caecal microbiota only in broilers reared under heat stress condition. Table 4 shows that broiler fed AACr-TN diet did not recorded significantly differences (p>0.05) in caecal microbiota (Enterobacteriaceae, E.coli) than those fed C-TN diet. But staphylococci number was significantly (p<0.05) lower in AACr- TN than in the C-TN group. Under heat stress conditions, dietary AACr lowered (P<0.05) the caecal number of Enterobacteriaceae, E. coli and staphylococci. Both in caecum and intestinal content of AACr broilers, irrespective the temperature conditions the number of lactobacilli was significantly higher than in the C broilers (Table 3, 4). It was noticed that diet supplementation with Artemisia and vit. C (80 mg/kg of diet) led to a significantly decrease of the total count of *Enterobacteriaceae* in the intestinal content of experimental groups compared to that of control group exposed to heat stress (32°C) [19]. Researchers studied the effect of vitamin C supplementation (500 g/kg ascorbic acid) in drinking water of Ross 308 broilers reared under thermoneutral conditions [18]. The authors showed an increased Lactobacillus numbers and reduced the total counts of E. coli in the ileal microbiota compared to the un supplemented treatment. The previously results can be explained, as [11] pointed out, by the synergetic action of vit. C and Cr in maintain the proper microflora of broilers reared under heat stress.

Table 4 Effects of the dietary treatments on caecal bacterial populations (lg10 CFU/ g wet caecal content

Variable	Thermoneutral conditions				Heat stress			
	C-TN	AACr-TN	SEM	p-value	C-HS	AACr-HS	SEM	p-value
Enterobacteriaceae	11.379[a]	11.379[a]	0.003	0.9821	11.390[a]	11.358[b]	0.005	<0.0001
E. coli	10.199[a]	10.208[a]	0.005	0.3780	10.159[a]	10.124[b]	0.006	<0.0001
Stafilococi	8.867[a]	8.84b	0.005	0.0808	8.919[a]	8.716[b]	0.031	<0.0001
Lactobacili	11.465[a]	11.471[b]	0.001	00099	10.992[a]	11.102[b]	0.017	<0.0001
Salmonella spp.	absent	absent	NA	NA	absent	absent	NA	NA

a, b Means in the same column with different superscripts differ significantly (P <0.05). SEM = standard error of the means; NA= non-adequate; n= 6; C- conventional diet; t AACr- conventional diet + 25 g ascorbic acid/ kg premix + 20 mg Cr picolinate /kg premix; CFU- colony forming units.

CONCLUSIONS

Dietary AACr did not affect body weight, daily feed intake, feed conversion rate neither under TN nor under HS condition. Combination of ascorbic acid and Cr (AACr) has a positive effect in limiting the development of the pathogenic bacteria colonizing the intestine and caecum during the heat stress. Under thermoneutral condition, dietary AACr did not significantly affect the number of Enterobacteriaceae and E.coli in the broiler'caecum. Dietary AACr can overcome the disorders of the intestinal microflora balance.

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