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Research Article

Influence of Early Heat Conditioning and Betaine or Ammonium Chloride Supplementation on Performance and Physiological Parameters of Quail Chicks in Hot Climate

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Abstract

Objective: This study was designed to determine the effects of early heat conditioning and betaine or ammonium chloride supplementation on growth performance and physiological parameters of quail chicks in hot climate. **Materials and Methods:** A total of 600-one day old quail chicks were divided into two groups, the first served as a control (non-conditioning) and the second exposed to early age heat condition (40$\pm$1°C for 24 h at the 5th day of age). Then each group were divided into three subgroups, the first was fed the basal diet and served as a control, the second was fed the basal diet supplemented with betaine (1.5 g kg^{-1}) and the third was fed the basal diet supplemented with ammonium chloride (0.5 g kg^{-1}). **Results:** The results at 40 day of age showed that early heat conditioning with or without addition significantly ($p<0.05$) improved body weight and feed conversion ratio. The best improvement on performance was recorded for birds exposed to early heat conditioning and fed betaine or ammonium chloride supplemented diet. Addition of either betaine or ammonium chloride to quail diets resulted in a significant ($p<0.05$) improvement of blood measurements. Exposing quail to early heat conditioning resulted in a significant improvement of total plasma proteins, plasma globulin and the level of thyroid hormones. Addition of either betaine or ammonium chloride has improved level of plasma protein fractions and antioxidant status compared to the control. **Conclusion:** It could be concluded that using early heat conditioning coupled with betaine or ammonium chloride supplementation gave more beneficial effects on performance and blood parameters.

Key words: Ammonium chloride, betaine, growth performance, heat conditioning, heat stress, quail chicks

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INTRODUCTION

Birds at high temperature reduce their feed intake to maintain the balance between heat production and loss. Heat production increases while, heat dissipation decreases. The main pathway of heat dissipation for birds under hot climate is respiratory evaporation, especially when ambient temperatures approach the body temperature. High ambient temperature causes negative effects on performance and physiological status of birds[1]. Heat stress may affect the function of intestinal barriers and suppress the immunity which facilitates gut luminal bacteria and endotoxin invasion and also increase the serum levels of corticosterone and inflammatory cytokines in chicks[2]. In poultry production, a number of feed additives have been used to enhance growth, feed efficiency, immune status and antioxidant capacity[3-5]. So, negative effects of hot weather in poultry production can be alleviated through housing design, setting of cooling systems, changes in dietary constituents to account for decreased feed intake and dietary addition of some feed additives[6,7].

Early heat conditioning is a technique that involves exposing young chicks to high environmental temperatures at an early age preferably before the age of 7 days for 24 h. The period before 7 days is most preferred as it takes advantage of the incomplete development of the hypothalamus. Exposing chicks to high environmental temperatures at this age would help to induce thermo-tolerance in the chicks and would allow the birds to tolerate high environmental temperatures at a later growth stage[8]. According to El-Moniary *et al.*[9] early-age heat conditioning of broiler chickens reduces hyperthermia and mortality in older chicks when they are confronted by high environmental temperatures. This situation suggested that, in broilers a single short exposure to heat stress before the birds reach 7 days of age has profound effects in the preparedness of birds for future events.

On the other hand the high ambient temperature may be reducing protein synthesis and negatively affecting poultry production[10]. Betaine is a methyl group donor and plays an important role in the metabolism of protein and energy[11]. Addition of betaine to the feed has been shown to be beneficial in heat stressed broilers. Rao *et al.*[12] using betaine in broiler diets may increase methionine availability for protein synthesis, thus improving performance. In addition, betaine acts as an intestinal osmolyte and affects the movement of water across the duodenal and ileal epithelium. Thus, dietary betaine supplementation to the chicks would improve the ability of their intestinal tissue to resist water loss in hyperosmotic medium. The normal functions of tissues dependent upon the stability of the total osmolarity of intracellular and extracellular fluids. Dietary betaine may protect intestinal cells and intestinal microbes from osmotic variation along the digestive tract[13]. Betaine has been shown to protect cells from osmotic stress and allow them to continue regular metabolic activities in conditions that would normally inactivate the cell[14]. Abd El-Gawad *et al.*[15] and Singh *et al.*[16] reported that betaine supplementation at 1.3 and 2 g kg^{-1} improve growth performance (body weight and feed conversion ratio) of broilers under thermal stress conditions. The best results in regard to the productive performance of chicks were recorded with 1.5 g kg^{-1} added betaine.

Electrolyte concentrations and the maintenance of acid-base balance in body fluids are studied under the term electrolyte balance. The electrolyte balance varies as a function of diet and water balance and it is particularly important in poultry under high temperatures due to panting. In addition, electrolyte balance values in the diet change with animal species, production category and age, as well as with the intensity and duration of heat stress[17], they added that electrolytes are necessary to maintain physiological functions during hot weather. Performance of thermo stressed quail chicks can be improved by supplements such as ammonium chloride that affect favorably the acid-base status during the heat stress[18].

Therefore, the objective of this study was to evaluate the effects of early heat conditioning and supplementation of betaine or ammonium chloride on growth performance and some physiological parameters of quail chicks under heat stress conditions.

MATERIALS AND METHODS

Birds, management and experimental design: An experiment was conducted to study the effect of early heat conditioning and dietary supplementation of betaine or ammonium chloride (NH_4Cl) on growing quail chicks. A total of 600-one day old quail chicks with average body weight being 9$\pm$0.4 g were used. Chicks were divided into two groups of 300 chicks each. The first group served as control (non heat conditioning group) and the second group exposed to early heat conditioning (40$\pm$ 1°C for 24 h at the 5th day of age). Then each group were divided into three subgroups of 100 chicks each (10 chicks$\times$10 replicates). A basal diet was formulated to cover all the nutrient requirements of growing quails (containing 24% crude protein and 2950 kcal of ME kg^{-1}). The first subgroup was fed the basal diet and served as a control group (non heat conditioning and without supplementation), the second was fed the basal diet supplemented with 1.5 g kg^{-1} betaine and the third was fed the basal diet supplemented with 0.5 g kg^{-1} NH_4Cl. Feed and water were offered *ad libitum* and light was provided 23 h daily during the experimental period. Quail chicks were kept under similar hygienic and environmental conditions.

Growth performance and blood collection and analysis: At 21 and 40 days of age, after fasting 12 h, birds of each replicate were weighed and feed consumption was recorded and feed conversion ratio was calculated (feed intake/body weight). At the end of experimental period ten birds from each treatment were randomly taken, weighed and slaughtered. The blood samples were collected from the ten slaughtered birds for each treatment in heparinized tubes. Few drops of fresh blood samples were taken to determine blood pH and blood haemoglobin (Hb). Blood smears were also done, stained with Wright's stain procedure and used to calculate the number of heterophils (H) and lymphocytes (L) in 100 white blood cells then the H/L ratio was calculated. Plasma total proteins were determined according to the method described by Henry[19]. Plasma albumin was determined based on a colorimetric method described by Doumas *et al.*[20]. Globulin was calculated by subtraction of plasma albumin from plasma total protein. Enzyme activity of Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were determined calorimetrically using the commercial kits purchased from Diamond Diagnostics Company. Plasma thyroxin (T_4) and triiodothyronine (T_3) were determined as reported by Akiba *et al.*[21]. Total antioxidant capacity (TAC) level was determined according to Koracevic *et al.*[22]. Catalase (CAT) level was determined according to Aebi[23]. Superoxide dismutase (SOD) level in plasma was determined according to Nishikimi *et al.*[24].

Statistical analysis: All measured parameters were performed on a two way ANOVA test (factorial 2×3) using the general linear model (GLM) produced by the Statistical Analysis Systems Institute[25]. Statistically significant differences among treatments were determined using Duncan's new multiple range at a probability level of $p \leq 0.05$.

RESULTS

Growth performance: The effect of treatments on growth performance of quail chicks at 21 and 40 days of age are shown in Table 1. At 21 days of age the results showed that adding betaine or NH_4Cl to the control group or heat conditioning group significantly ($p<0.05$) increased body weight (BW) compared to the control group (non-conditioning and without addition). The higher values of BW were recorded with betaine supplementation to the control and heat conditioning group followed by heat conditioning group supplemented with NH_4Cl, with no significant differences among them. Feed conversion ratio (FCR) slightly improved ($p>0.05$) with heat conditioning or betaine and NH_4Cl supplementation, while feed intake (FI) did not affect. Regarding the main effect of heat conditioning no significant effects showed on BW, FI or FCR at this period. Supplementation of betaine significantly improved BW compared to the control group while, supplementation of NH_4Cl improved BW with no significant effect compared with the control group.

At 40 days of age the results showed that birds exposed to heat conditioning and supplemented with betaine or NH_4Cl gained significant ($p<0.05$) higher BW followed by groups fed

Table 1: Effect of treatments on growth performance of quail chicks

	At 21 days of age			At 40 days of age		
Items	BW (g)	FI (g)	FCR (g g^{-1})	BW (g)	FI (g)	FCR (g g^{-1})
Main effects						
Heat conditioning (A)						
Non-conditioning	131.20	530.70	4.050	236.15^{b}	1285.70^{b}	5.470^{a}
Heat conditioning	134.49	530.73	3.950	273.82^{a}	1390.21^{a}	5.080^{b}
SEM±	2.04	5.34	0.050	5.64	15.11	0.088
Dietary supplementation (B)						
Without	128.30^{b}	520.12	4.060	234.80^{b}	1292.97^{b}	5.550^{a}
+Betaine	136.28^{a}	539.73	3.960	265.80^{a}	1364.32^{a}	5.150^{b}
+NH_4Cl	133.96^{ab}	532.30	3.970	264.35^{a}	1356.58^{a}	5.130^{b}
SEM±	2.22	5.71	0.064	9.00	26.59	0.113
Interaction (A×B)						
Non-conditioning	123.93^{b}	519.60	4.200	211.79^{c}	1254.40^{c}	5.930^{a}
Non-conditioning+betaine	136.48^{a}	542.20	3.980	246.19^{b}	1311.50^{bc}	5.330^{b}
Non-conditioning+NH_4Cl	133.20^{ab}	530.30	3.980	250.47^{b}	1291.20^{bc}	5.160^{c}
Heat conditioning	132.66^{ab}	520.60	3.930	257.81^{b}	1331.60^{b}	5.170^{c}
Heat conditioning+betaine	136.08^{a}	537.30	3.950	285.41^{a}	1417.10^{a}	4.970^{d}
Heat conditioning+NH_4Cl	134.72^{a}	534.30	3.970	278.23^{a}	1422.00^{a}	5.110^{c}
SEM±	3.01	8.94	0.084	4.27	18.52	0.045

^{a,b,c}Means of mean effects or interaction within a column with different superscripts are significantly different ($p \leq 0.05$)

supplemented betaine or NH_4Cl without heat conditioning. Feed conversion ratio significantly (p<0.05) improved in all groups compared to the control group. The best FCR showed for birds exposed to heat conditioning with betaine (16.19% improving) supplementation followed by the heat conditioning group fed supplemented diet with NH_4Cl (13.83% improving). The main effect of heat conditioning and betaine or NH_4Cl supplementation showed that birds exposed to heat conditioning technique significantly (p<0.05) superior BW, FI and FCR compared to the control.

Physiological parameters: Results in Table 2 shows that pH, H/L and Hb had a significant improvement by exposure quail to heat conditioning at an early age compared with the control. The addition of either betaine or NH_4Cl to quail diets resulted in a significant improvement in blood measurements compared to the control. This effect was positively reflected on the interaction between early age of heat conditioning and the addition of either betaine or NH_4Cl to quail diets. The exposure of quail to early age of heat conditioning and the addition of betaine or NH_4Cl led to significant improvement in the level of T_3 and T_4. They were positively reflected on the interaction between the two factors.

As shown in Table 3, exposure of quail to early age of heat conditioning resulted in a significant improvement in total plasma proteins (TP) and plasma globulin (GLU) but

Table 2: Effect of treatments on haematological parameters and thyroid hormones of quail at 40 days of age

Items	pH	H/L	Hb	T_3	T_4
Main effects					
Heat conditioning (A)					
Non-conditioning	7.41[a]	0.59[a]	9.03[b]	4.29[b]	22.980[b]
Heat conditioning	7.31[b]	0.55[b]	9.74[a]	4.66[a]	24.120[a]
SEM±	0.022	0.011	0.174	0.089	0.379
Dietary supplementation (B)					
Without	7.45[a]	0.61[a]	8.86[b]	4.19[b]	22.590[b]
+Betaine	7.34[b]	0.55[b]	9.59[a]	4.61[a]	23.940[a]
+NH_4Cl	7.30[c]	0.56[b]	9.71[a]	4.61[a]	24.130[a]
SEM±	0.024	0.012	0.213	0.108	0.456
Interaction (A×B)					
Non-conditioning	7.57[a]	0.67[a]	8.45[b]	4.07[c]	21.360[b]
Non-conditioning+betaine	7.35[b]	0.55[b]	9.22[ab]	4.37[c]	23.570[a]
Non-conditioning+NH_4Cl	7.32[bc]	0.56[b]	9.42[a]	4.43[bc]	24.020[a]
Heat conditioning	7.32[bc]	0.56[b]	9.27[ab]	4.32[c]	23.820[a]
Heat conditioning+Betaine	7.33[bc]	0.54[b]	9.94[a]	4.86[a]	24.310[a]
Heat conditioning+NH_4Cl	7.28[c]	0.56[b]	10.00[a]	4.80[ab]	24.240[a]
SEM±	0.016	0.012	0.273	0.135	0.589

[a,b,c]Means of mean effects or interaction within a column with different superscripts are significantly different ($p \leq 0.05$), pH: Potential hydrogen, Hb: Blood haemoglobin, H/L: Ratio of heterophils/lymphocytes, T_4: Plasma thyroxin, T_3: Triiodothyronine

Table 3: Effect of treatments on plasma protein fractions and plasma aminotransferases of quail at 40 days of age

Items	TP	ALB	GLU	ALT	AST
Main effects					
Heat conditioning (A)					
Non-conditioning	4.130[b]	1.590	2.540[b]	7.280	50.770
Heat conditioning	4.650[a]	1.780	2.870[a]	8.110	52.330
SEM±	0.103	0.084	0.108	0.442	1.665
Dietary supplementation (B)					
Without	4.090[b]	1.840[a]	2.250[c]	7.250	50.000
+Betaine	4.480[a]	1.720[ab]	2.760[b]	7.670	51.580
+NH_4Cl	4.600[a]	1.490[b]	3.110[a]	8.170	53.080
SEM±	0.134	0.099	0.095	0.552	2.049
Interaction (A×B)					
Non-conditioning	3.840[c]	1.720[ab]	2.120[c]	6.830	48.000
Non-conditioning+betaine	4.370[b]	1.870[ab]	2.500[b]	7.170	51.330
Non-conditioning+NH_4Cl	4.200[bc]	1.190[c]	3.010[a]	7.830	53.000
Heat conditioning	4.340[b]	1.960[a]	2.380[bc]	7.670	52.000
Heat conditioning+betaine	4.600[ab]	1.570[b]	3.030[a]	8.170	51.830
Heat conditioning+NH_4Cl	5.010[a]	1.800[ab]	3.210[a]	8.500	53.170
SEM±	0.154	0.113	0.116	0.796	2.990

[a,b,c]Means of mean effects or interaction within a column with different superscripts are significantly different ($p \leq 0.05$), TP: Total plasma proteins, ALB: Plasma albumin, GLU: Plasma globulin, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase

Table 4: Effect of treatments on antioxidant status of quail at 40 days of age

Items	TAC	CAT	SOD
Main effects			
Heat conditioning (A)			
Non-conditioning	12.360[b]	5.74[b]	153.530[b]
Heat conditioning	13.470[a]	6.79[a]	162.340[a]
SEM±	0.278	0.303	1.426
Dietary supplementation (B)			
Without	11.710[b]	4.98[b]	150.590[b]
+Betaine	13.420[a]	7.07[a]	161.650[a]
+NH_4Cl	13.620[a]	6.72[a]	161.560[a]
SEM±	0.285	0.301	1.567
Interaction (A×B)			
Non-conditioning	10.580[c]	3.91[c]	142.580[c]
Non-conditioning+betaine	13.240[ab]	7.03[ab]	159.290[b]
Non-conditioning+NH_4Cl	13.260[ab]	6.28[ab]	158.730[b]
Heat conditioning	12.850[b]	6.06[b]	158.610[b]
Heat conditioning+betaine	13.590[ab]	7.15[a]	164.020[a]
Heat conditioning+NH_4Cl	13.970[a]	7.17[a]	164.380[a]
SEM±	0.287	0.331	0.458

[a,b,c]Means of mean effects or interaction within a column with different superscripts are significantly different ($p \leq 0.05$), TAC: Total antioxidant capacity. CAT: Catalase enzyme, SOD: Superoxide dismutase

the improvement was insignificant in plasma albumin (ALB), aspartate aminotransferase (AST) and alanine aminotransferase (ALT). The addition of either betaine or NH_4Cl significantly improved the level of plasma protein fractions compared to the control. But the improvement in the level of plasma aminotransferases was not significant. Interaction between the two factors was positive when compared with the control.

Table 4 reveals that early heat conditioning and the addition of betaine or ammonium chloride of quail led to a significant improvement of antioxidant status (TAC, CAT and SOD) compared with the control group.

DISCUSSION

The results of growth performance indicated that early heat conditioning technique and addition of betaine or ammonium chloride to quail diet significantly ($p<0.05$) improved performance of Japanese quail under hot climate. Exposing chicks to early age heat conditioning would help to induce thermo-tolerance and allow the birds to tolerate high environmental temperatures at a later growth stage. These findings are in agreement with those reported by Gouda *et al.*[8] and El-Moniary *et al*[9]. According to El-Moniary *et al.*[9] early age heat conditioning of broiler chicks reduces hyperthermia and mortality in older chicks when they are confronted by high environmental temperatures.

Betaine supplementation in quail diets improved growth performance. These findings are in agreement with Remus[26] who demonstrated that betaine supplementation to broiler diets significantly improved feed conversion by 6.3% at 49 days of age. Betaine acts as an osmolyte, helping maintain the bird's cellular water balance to protect cells and tissues from dehydration and osmotic inactivation by facilitating water retention in the body and also help to maintain both the bird's energy balance and feed intake. The bird's maintenance energy requirement is then reduced, despite osmotic stress and more energy is available for growth and production. Abd El-Gawad *et al.*[15] reported that betaine supplementation to broiler diets significantly improved performance. The best results occurred to productive performance of chicks were recorded using 1.5 g kg^{-1} added betaine. Wang[27] showed that average daily gain and feed: gain ratio improved linearly with increasing betaine levels in duck diets from 0-0.2%. Ratriyanto *et al.*[28] found that supplementation of betaine at 0.06 and 0.12% to quail diet increased nutrients utilization and tend to improve of performance and production. Betaine supplementation improves nutrient digestibility due to its osmoprotective properties, supporting intestinal cells and the growth of intestinal microbes[29]. Thus, betaine could be considered as a useful tool to increase bird's tolerance to high temperatures and minimize the deleterious effects of heat stress.

When environmental temperature is higher than the thermo-neutral zone, birds increase respiratory rate up to 10 times this leads to an excessive loss of carbon dioxide, resulted to increase blood pH (alkalosis), bird attempts to correct blood pH by excreting bicarbonates via urine. Bicarbonates are negatively charged ions that must be coupled with positively charged ions, such as potassium to be excreted in urine. Addition of ammonium chloride, potassium chloride and/or sodium bicarbonate have reduced blood pH and improved performance of broilers[30]. Amani *et al.*[31] showed that quail chicks exposed to heat conditioning (at 5 days of age) significantly consumed less feed than control group. Adding NH_4Cl significantly increased feed intake and improved feed conversion ratio. They also concluded that using heat conditioning and adding 0.05% NH_4Cl significantly improved quail performance and tolerate heat stress.

The results of physiological parameters showed that early heat conditioning and betaine or ammonium chloride supplementation improved the all measured parameters. Many researches explained that the mechanisms of the body thermoregulation (heat production and heat lost) of birds is complete after hatching. The exposure of birds to high temperatures at early age, especially the first week after hatching helps to resist the negative effects of thermal stress in the age of marketing. Heat conditioned chicks behaved like the chronically-stressed reflecting the importance of chick conditioning at an early age to adapt the waves of stress at marketing age[32,33,8]. It is well known that thyroid hormones play an important role in the thermal regulation of bird species and it is clear from the significant increase in thyroid hormones (T_3 and T_4) in quail affected by heat conditioning at early age of life. This occurred due to internal adaptation of heat resistance at marketing age, it is indicated by feed intake and increase in body weight compared to the control. These findings are in accordance with Lin *et al.*[34] and Hemid *et al.*[33]. Adapting birds to high temperature conditions as a result of heat conditioning at early age of life improved nutrients utilization and heat tolerance of chicks. Level of plasma protein fractions and plasma aminotransferases are a strong indicator of nutrient efficiency in metabolism, especially protein metabolism. These results are in close agreement with those of El-Wardany *et al.*[32]. Subjected birds to heat stress increased levels of reactive oxygen species in mitochondria, which leads to disturbance of balance between the oxidation and antioxidant defense systems causing lipid peroxidation, oxidative damages to proteins, DNA and biological molecules[34]. This explains the importance of increasing level of antioxidant enzymes including total antioxidant capacity such as glutathione peroxidase, superoxide dismutase, Catalase and etc. Yardibi and Turkay[35] found that the exposure of chicks to high temperatures at an early age led to an increase in antioxidant status activities when exposed to high temperatures in the late stages of life and this was agreed upon by the results of the present study.

Addition of betaine or ammonium chloride led to an improvement in all measurements whether it is individual or combination with early heat conditioning. Regarding to the addition of betaine under heat stress, betaine have two important physiological functions, a donor to the methyl group (CH_3) and a regulator of the osmosis (Zwitterions Dipolar). It has the ability to maintain the balance of cell fluids under conditions of heat stress[36]. This helps to increase cell water and improves cell physiological condition and blood parameters, such as blood pH, hemoglobin and number of red and white blood cells. Mashaly *et al.*[37] reported that reduction of lymphocyte during heat stress is due to increase of inflammatory cytokines which stimulate the hypothalamic production of corticotrophin releasing hormone under heat stress. These findings are in agreement with Nofal *et al.*[38] who showed that supplementation of betaine to broiler diet significantly decreased heterophile percentage but lymphocyte percentage significantly increased, whereas, H/L ratio significantly decreased. Gudev *et al.*[39] reported that adding betaine in diets significantly increased the lymphocyte percentage where as heterophile and H/L ratio significantly decreased compared to the control group.

Betaine is an important source of total protein, where the methionine deficiency under heat stress condition is compensated by granting the methyl group (CH_3) to regenerate methionine from the homocysteine required to form the protein. In this study, adding betaine to quail diet increased the level of total proteins, albumin and globulin in blood, these results are in agreement with Rao *et al.*[12]. At high ambient temperatures the activity of thyroid hormones declines to depress performance of birds. Based on the relationship of thyroid secretion rate to growth demonstrated in chickens, it is possible that the poorer performance in the control group of birds was due partly to the decreased T_3 activity[16].

The improvement in tested parameters with the addition of ammonium chloride is due to the acid effect of Cl^-, which works on the stability of electrolytes in blood, improves the pH and some of the other blood measurements such as hemoglobin and number of red and white blood cells[30]. This makes the bird live under internal stability as a result of controlling internal biological processes, which is evident in the functions of the thyroid gland. Since the increase of hormones T_3 and T_4 is good indication of the improvement of internal stability compared to the control[40]. The improvement in blood parameters and feed intake explains the improvement in the plasma protein fractions and plasma aminotransferases[41]. Ammonium ion in ammonium chloride plays an oxidized form (ammonia) role, which involved in the acid-base balance. This affects a lot of metabolic changes that help to resist heat stress by increasing total antioxidant capacity such as glutathione peroxidase and superoxide dismutase.

CONCLUSION

In general, based on the results of the present study, it could be concluded that using early-age heat conditioning of quail chicks and diet supplemented with betaine (1.5 g kg^{-1}) or ammonium chloride (0.5 g kg^{-1}) improve growth performance and blood parameters and could be recommended for alleviating the negative effects of heat stress.

SIGNIFICANCE STATEMENT

This study discovered that early-age heat conditioning and dietary supplementation of betaine or ammonium chloride can be significantly improved performance and physiological parameters of quail chicks rearing on hot climate. This study will help the researcher to uncover the critical area of using early-age heat condition and addition of betaine or ammonium chloride that many researchers were not able to explore. Thus, a new theory on combination of early heat conditioning and betaine or ammonium chloride supplementation and possibly other combinations, may be arrived at.

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