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The effect of vitamin C on egg shell quality under high environmental temperatures

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Summary

The effects of keeping hens in cold or intermittent or continuous heat on different criteria of shell quality such as deformation, breaking strength, specific gravity, shell thickness and shell percentage were determined.
Fluctuating temperatures of 85° F and 75–80 % R.H. for 10 hours during daylight and 65–70° F with 50–60 % R.H. for 14 hours at night, shell quality was the same as with cold environment, 55° F and 50–60 % R.H. A constant temperature of 85° F with 75–80 % R.H. (heat stress) depressed shell quality and calcium in blood plasma significantly as compared with cold.
In hot environment a supplement of 50 mg ascorbic acid Hoffman–La Roche per kg ration tended to improve shell quality as judged by deformation, breaking strength, shell thickness and percentage, and amount of shell deposited per eggs. Also blood plasma calcium rose significantly.

Introduction

One of the well known causes of losses in marketed eggs is poor quality of shells in summer. Many workers as Wilhelm (1940) and Romanoff and Romanoff (1949) have observed this fall in quality in summer. Shells are thickest and most uniform in winter and become thinner in spring and summer.
Bennion and Warren (1939) noted that egg shells seemed to be more fragile when the birds were subjected to high air temperatures. Warren and Schnepel (1940) obtained thinner egg shells almost immediately after experimentally increasing the environmental temperature from 20° C to 32.5° C. Shell thickness recovered after a subsequent decrease in temperature. They also observed that high humidity accentuated the effects of high temperature, and that the feed consumption of hens was 27 % less at 32.5° C than at 20° C.
Several workers have improved shell quality by supplementing the hens' diet with vitamin C during the hot season. Thornton and Moreng (1958; 1959) reported that shell thickness increased by supplementation of ascorbic acid in amounts of 5 mg, 10 mg and 20 mg per pound ration. This effect was more pronounced with high environmental temperature.
They added that ascorbic acid may have had an influence on the thyroid activity specially under high temperature. Bzowska and Schurch (1965) reported that vitamin C caused a statistically significant increase in weight of the thyroid gland in three groups (normal environmental temperature, heat stress and cold stress).

However Heywang and Kemmerer (1955) reported that supplementing an all-mash laying diet with 454 mg ascorbic acid per pound did not improve shell quality, as measured by the ratio of dried shell weight to whole egg weight. The data were obtained over two summers under uncontrolled ambient temperatures with average maxima of 101° to 104°F and average minima of 90° to 91°F. Heywang et al. (1964) also reported that supplemented ascorbic acid in concentrations of 10, 20 and 454 mg per pound feed had no appreciable effect on egg weight, shell thickness, or ratio of dried shell weight to whole egg weight. Results were similar at all three levels of ascorbate. We have studied the influence of vitamin C on shell quality in hens under heat stress in an attempt to clarify the earlier conflicting results. This study was a part of the Ph.D. research by the first author on 'Egg shell quality and microstructure as affected by vitamin C, other feed additives and high environmental temperatures' under the supervision of the second author.

Materials and methods

Experimental animals: 48 Single-Comb White Leghorn pullets about 7 months old were used in this study and replacements were kept available.
Housing: two rooms were available for this trial, one cold and the other hot. Both rooms were artificially lit, each with two incandescent 100W bulbs for 14 hours per day. The cold room was air-conditioned. Temperature in the room was 55° ± 5°F, relative humidity was 50 to 60 %. The hot room had four thermostatic electric blower-heaters each of 2 kW. The room was kept at 85° ± 5°F and was humidified by an electric boiler maintaining the room at about 50 % R.H. If higher humidity was required a hygrostatic electric sprayer raised R.H. to 75–80 %. Both rooms were ventilated by an exhaust fan. Each room had 24 hens in a battery equipped with automatic waterers.
Feed was offered in troughs; birds were fed to appetite. Since calcium percentage in a layer's ration certainly affects shell quality, it was kept at 2.75 %[1] instead of 2.25 % to avoid effect of calcium deficiency on shell strength.
The feed formula and composition are given in Table 1.

Experimental design

A survey of the experimental design is given in Table 2.

Control period
For a month at the start of the trial both rooms were kept the same in climate to check that the groups were the same and to allow statistical corrections for group differences.

Acclimatization (fluctuating temperature)
In this period climate was artificially regulated only by central heating of the building and additional gas-heating and an electric boiler for vapour supply in the hot room. The hot room was heated during the day only from 8.00 h to 18.00 h to 85°F and humidified to 75–80 % R.H.

[1] Official National Research Council (1966). Allowances have since been raised from 2.25 % to 2.75 %.

Table 1 Composition of all-mash laying ration

Ingredients	*Amount (kg)*
Ground yellow maize	37
Barley meal	20
Oatmeal	17.5
Wheat bran	2
Soya bean oilmeal (solvent-extracted)	5
Maize gluten feedmeal	3
Fishmeal (68 % protein)	4
Lucerne meal (18 % protein)	4
Vitamin B mixture [1]	1
Vitamins AD (750 IU D_3 + 2250 IU A/G)	0.2
Mineral mixture [2]	2
Precipitated chalk	4.4
Dicalcium phosphate ($Ca_2(PHO_4)_2.2H_2O$) with 23 % calcium	0.5
Manganese sulphate	0.007
Zinc sulphate	0.020
	100.627

Calculated energy and chemical components

Metabolizable energy	2650 kcal/kg
Net energy	1850 kcal/kg
Crude protein	15.1 %
Crude fibre	5.8 %
Calcium	2.75 %
Phosphorus	0.68 %
Manganese	75 mg/kg
Zinc	70 mg/kg
Methionine	0.24 %
Cystine	0.22 %
Lysine	0.65 %
Arginine	0.77 %

[1] *Contents of vitamin B mixture*

Riboflavin	200 mg/kg
Pantothenic acid	400 „
Nicotinic acid	1500 „
Choline	40000 „
Vitamin B12	0.5 „
Vitamin E	200 „
Vitamin K3	100 „

[2] *Contents of mineral mixture (%)*

Calcium carbonate	48.85
Calcium phosphate ($Ca_3(PO_4)_2$ + $CaHPO_4$ $2H_2O$ with 25 % Ca)	37.85
Sodium chloride	12.00
Zinc sulphate	0.3
Copper sulphate ($CuSO_4.5H_2O$)	0.2
Manganese sulphate	0.8

Heat stress

In this period the hot room was kept continuously at 85° F and 75–80 % R.H. Data were collected under the same conditions. The cold room was the control.

Vitamin C

In the last period of the trial the pullets in each room were randomly assorted into two groups of which one was given vitamin C with the feed and the other served as a control. Each group consisted of 12 pullets. Vitamin C in the form of l-ascorbic acid (Hoffman–La Roche) was added to the ration at 50 mg per kg after premixing. The ration was prepared weekly, stored in a cold basement and offered twice a day to cut down losses by oxidation. Stability was evaluated by estimating vitamin C photometrically in the ration (Anon., 1951).

The following determinations show the effect of storing the mixed feed with 100 mg/kg for one day, one week and two weeks at 65–70° F and 60 % R.H.

one day's storage	11.58 mg/100 g
	11.42 mg/100 g
one week's storage	13.48 mg/100 g
	14.85 mg/100 g
two weeks' storage	11.96 mg/100 g
	12.26 mg/100 g

These data show that any possibility of loss of vitamin C during the trial can be ruled out.

Sampling procedures
All eggs were collected hourly every day throughout the trial, placed in a humid cold container, to prevent losses by evaporation, until specific gravity had been estimated.

Estimations

Egg weight and specific gravity. Eggs were weighed in air and in water at 20° C every day at 16.00 h on a special Mettler balance type H3 to the nearest centigramme. During weighing in water, air bubbles were avoided to ensure the correct reading. Specific gravity was calculated as follows:
egg weight — egg weight in water = egg volume
specific gravity = egg weight/egg volume [2].

Deformation was measured with a special apparatus designed by Schoorl and Boersma (1962). This apparatus measures the bending of the egg under a load of 500 g. Deformation was measured at the equator in μm.

Breaking strength was measured with a specially constructed device. Pressure was applied to the blunt end of the egg by a bolt by means of granulated lead shot. As the lead was poured, the pressure on the shell increased. As soon as the shell broke, the flow of shot stopped automatically; the lead was then weighed on the balance and expressed in kilogrammes.

Shell thickness was measured to 0.01 mm with an outside micrometer with a pointed anvil and a rounded spindle in fresh shells at the blunt end, the equator and the pointed end of the shell (shell membranes included). Average shell thickness was obtained by averaging the thickness of the three different places.

Shell percentage was estimated by breaking the eggs, removing the contents and rinsing the shells carefully with luke-warm water to remove traces of egg white, drying at 105° C for 3 hours and weighing twice to constant weight to the nearest 0.01 mg. It was calculated as follows: shell percentage = (shell weight/egg weight) × 100.

Blood plasma calcium was estimated complexometrically in plasma from blood samples taken from the wing vein between 13.00 h and 15.00 h, only from hens which had

[2] For simplicity specific gravity was not corrected for temperature, so that all values are related to water temperature of 20° C.

Table 2 Summary of the experimental design

Date	*Period*	*Cold room*				*Hot room*			
		number of pullets		*temp. (°F)*	*% R.H.*	*number of pullets*		*temp. (°F)*	*% R.H.*
1–31 Dec. 1963	control	24		65–70	50–60	24		65–70	50–60
1 Jan. to 29 Febr. '64	acclimatization	24		55	50–60	24		85	75–80 [1]
								65–70	50–60 [2]
1–15 March 1964	transition	24		55	50–60	24		65–70	50–60
15–31 March 1964	no data							85	75–80
1–30 April 1964	heat stress	24		55	50–60	24		85	75–80
		no vit. C	vit. C			no vit. C	vit. C		
1 May to 30 June '64	vit. C stress	12	12	55	50–60	12	12	85	75–80

Both rooms were lit from 6.00 h to 20.00 h throughout the experiment.

[1] Heating was during daylight for 10 h by means of electric heaters.

[2] Normal heating was at night for 14 h by means of central heating of the building.

laid an egg on that day. Heparin was used as an anti-coagulant. Blood was taken monthly throughout the trial.

Results and discussion

Shell quality as affected by temperature and vitamin C additions

In Fig. 1, shell quality tends to fall throughout the trial, as assessed by all criteria, except shell thickness. This indicates that shell structure deteriorates with the hen's

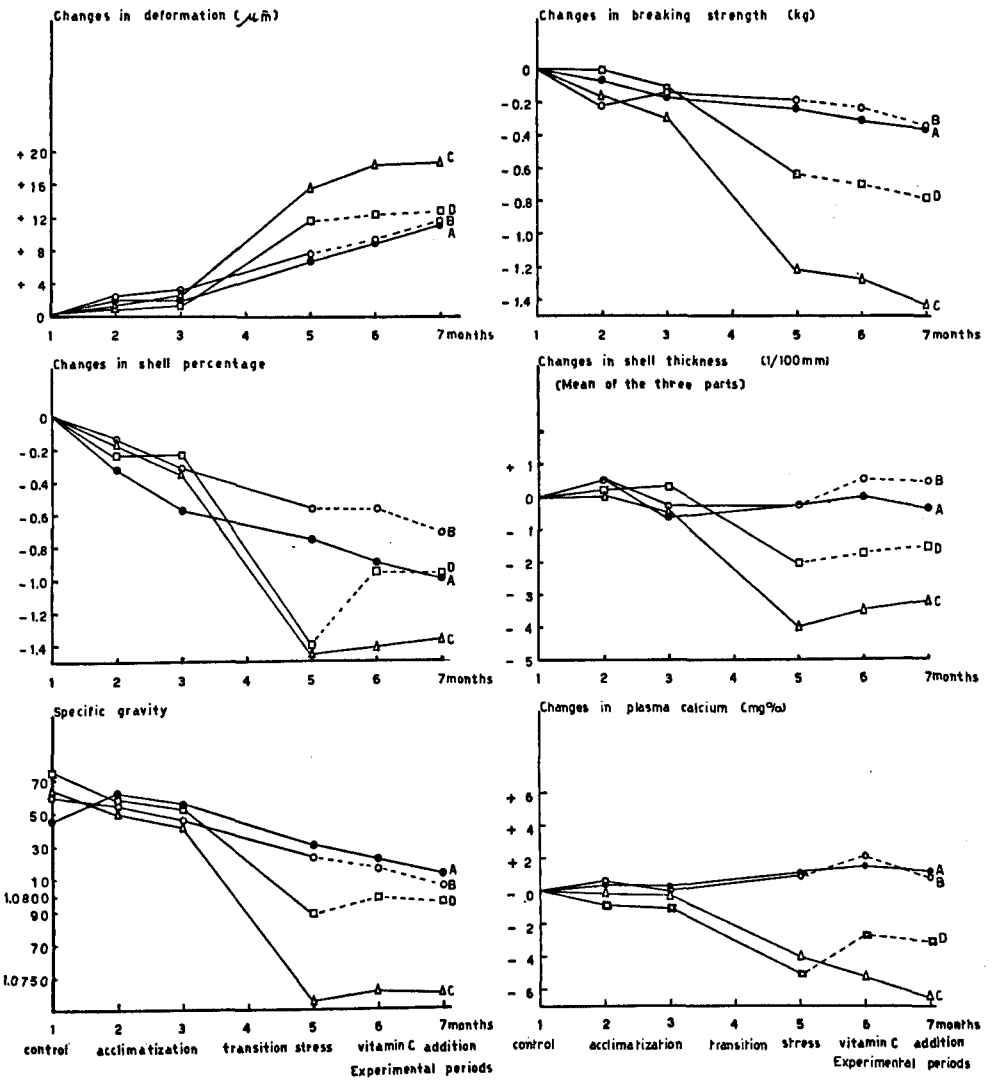

Fig. 1 Changes in criteria of shell quality (deformation, breaking strength, percentage shell, shell thickness, specific gravity) and plasma calcium.

● *A control (cold);* ○ *B treated (cold);* △ *C control (hot);* □ *D treated (hot);* - - - - *vitamin C addition.*

age but that the decline in shell thickness normally observed during summer might be an effect of heat rather than of age. In the acclimatization period, analysis of variance showed no clear effect of fluctuating temperature on the shell quality as assessed by deformation, breaking strength, percentage shell, shell thickness and specific gravity (Table 3). This observation agrees with the findings of Mueller (1961), who noticed that with fluctuating temperature hens laid significantly better shelled eggs than with constant 90° F environment.

The difference between heat stress and cold environment in shell quality (Table 3; Fig. 1) clearly shows that heat stress had a highly significant harmful effect on all criteria of shell quality. These findings agree with the work of Bennion and Warren (1933), Warren and Schnepel (1940), Wilhelm (1940) and Brant et al. (1953).

Vitamin C (Table 3; Fig. 1 and 2) had no significant effect on shell quality in the cold environment but analysis of covariance demonstrated a significant and highly significant improvement with vitamin C in the hot environment except for specific gravity. However, because of interactions already observed during stress, the effect of vitamin C must be interpreted carefully. The effect of vitamin C on shell percentage was significant but the effect on shell thickness, breaking strength and deformation was debatable. The significant increase in egg weight and in shell percentage with vitamin C in the hot environment indicates a material improvement in the amount of shell deposited per egg.

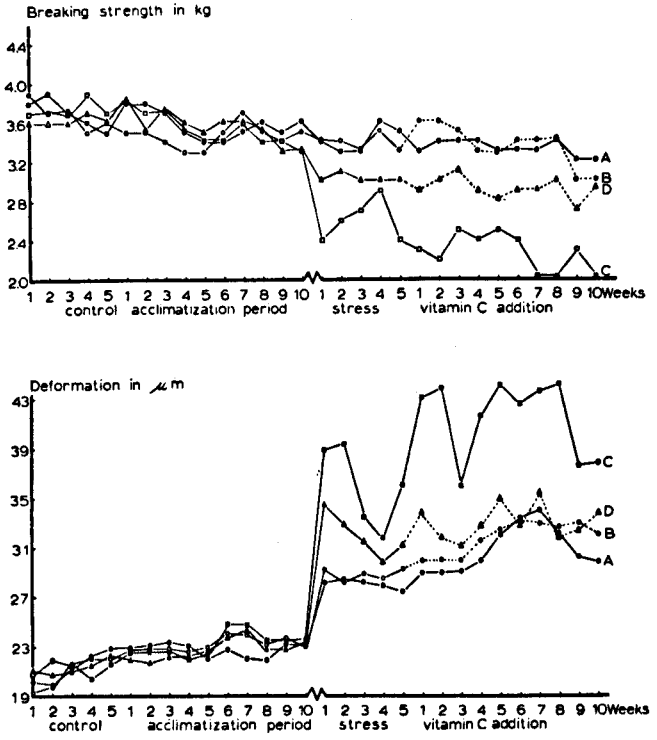

Fig. 2 Effect of environmental conditions and of vitamin C on breaking strength and deformation of the shell.

● A control (cold); ○ B treated (cold); △ C control (hot); □ D treated (hot); - - - - vitamin C addition.

Table 3 Analysis of variance and covariance showing the effect of vitamin C, temperature and their interactions on criteria of shell quality and on blood plasma calcium

Period	*Kinds of analysis*	*Calculated F values*								
		specific gravity (source of variation)			*deformation (source of variation)*			*breaking strength (source of variation)*		
		treat-ment	*temper-ature*	*inter-action*	*treat-ment*	*temper-ature*	*inter-action*	*treat-ment*	*temper-ature*	*inter-action*
Control	AV	0.98	1.11	0.60	0.01	0.00	0.02	0.84	0.06	0.00
Acclimatization	AV	0.05	0.15	0.49	0.01	0.41	0.24	0.88	0.67	1.97
Heat stress	AV	0.79	14.59***	1.98	1.00	16.15***	2.86*	1.96	19.86***	2.85*
Vitamin C	ACo	1.86	10.85***	4.06**	3.99*	14.35***	3.07*	12.71***	82.18***	5.83**

Period	*Kinds of analysis*	*Calculated F values*								
		mean shell thickness (source of variation)			*shell percentage (source of variation)*			*plasma blood calcium (source of variation)*		
		treat-ment	*temper-ature*	*inter-action*	*treat-ment*	*temper-ature*	*inter-action*	*treat-ment*	*temper-ature*	*inter-action*
Control	AV	0.42	0.00	1.30	0.44	0.17	1.02	0.13	0.28	0.15
Acclimatization	AV	0.21	0.37	1.46	0.01	0.22	0.63	1.90	0.87	0.06
Heat stress	AV	1.02	19.09***	6.87**	1.52	14.28***	3.33*	2.13	36.77***	0.03
Vitamin C	ACo	7.76***	71.39***	3.51*	4.39**	13.33***	1.25	4.49**	38.10***	2.96*

Degrees of freedom: treatments, temperature and interactions 1, error 44.
* F 10 % = 2.84; ** F 5 % = 4.06; *** F 1 % = 7.27.
AV = Analysis of variance; ACo = Analysis of covariance against control and acclimatization periods.

Fig. 2 shows more clearly the effect of vitamin C on breaking strength and deformation in the hot environment. Similar results were obtained by Thornton and Moreng (1958; 1959), Thornton (1960), Arscott et al. (1962) and Hunt and Aitken (1962).

Plasma blood calcium, shell quality and vitamin C in hot environment
The cold groups did not show any fluctuations in plasma calcium (Table 3; Fig. 1). Fluctuating temperature had no significant effect on plasma calcium. Mueller (1959) came to similar conclusions. He reported that serum calcium levels in the variable environments were significantly higher than those in the constantly hot environments. In the stress period high temperature and humidity caused a significant decrease in plasma calcium and a reduction in shell quality. Bennion and Warren (1933), Conrad (1939), and Warren and Schnepel (1940) came to the same conclusion. Vitamin C clearly increased plasma calcium in the hot environment significantly; this increase was reflected in shell deposition.

Conclusions

Heat and humidity depress both shell quality and plasma calcium. We suggest that poor shells and low blood plasma calcium are due to a lower metabolism of the hen resulting from reduced thyroxin output of the thyroid in hot weather. The work of Galpin (1938) supports this suggestion; he reported seasonal variation in weight of thyroid gland with the low point in July in the Scottish climate. Asmundsen and Pinsky (1935), Reineke and Turner (1942), Gutteridge and Pratt (1946) and Gutteridge and Novikoff (1947) concluded that a synthetic source of thyroxin (thyroprotein) and desiccated thyroid as a food supplement significantly improved shell quality during hot weather by increasing the metabolic rate.
Our work also showed that dietary vitamin C significantly improved shell quality of eggs from hens kept in hot environment. According to the literature, metabolic rate and deposition of shell constituents are related. If so, a reduced metabolic rate may be associated with inability of the hen to synthesize enough of certain compounds needed for shell formation.
Thornton (1961) showed that when hens were subjected to heat stress, ascorbic acid in the blood declined markedly, indicating that insufficient vitamin C was being synthesized. Since vitamin C has been shown to be involved in the utilization and metabolism of both organic and inorganic nutrients in bone; it seemed probable that its role in shell formation could be included in either one or both phases. We suggest that vitamin C takes effect through the thyroid gland. This can be confirmed by the work of Bzowska and Schurch (1965), who reported a statistically significant positive influence of the added vitamin C on weight of thyroid gland in three groups (normal environmental temperature, heat stress and cold stress) indicating a close relationship between thyroid activity and the vitamin C supply to the hen.
The reduction of both plasma calcium and egg shell quality in heat stress does not prove that reduction of calcium in blood plasma is the primary cause of reduced shell deposition. Because of the well known reduction of egg weight in hot conditions as confirmed in our trial, it could be that the increased respiratory rate under heat stress is accompanied by increased blood flow through the lungs and decreased blood flow through the oviduct, the latter resulting in a decreased deposition of egg and shell constituents.

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