

RESPONSE TO DIETARY ASCORBIC ACID SUPPLEMENTATION IN LAYING
HENS (*Gallus domesticus*) : EFFECT OF EXPOSURE TO HIGH
TEMPERATURE AND OCHRATOXIN A INGESTION.

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of

Graduate Studies

The University of Manitoba

by

Felix Miyoba Haazele

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of

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BY

FELIX MIYOBA HAAZELE

**A Thesis submitted to the Faculty of Graduate Studies of the
University of Manitoba in partial fulfillment of the requirements
for the degree of**

DOCTOR OF PHILOSOPHY

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ABSTRACT

Whereas situations associated with stressors are known to lower tissue levels of ascorbic acid, it remains questionable as to whether vitamin C status can be a limiting factor to production in the domestic chicken which has the capacity for endogenous synthesis of L-ascorbic acid. It is not clear as to whether a chicken has the capacity to respond to dietary supplements of ascorbic acid or whether environments exposing chicken to stressors induce a response. Responsiveness to dietary supplements of ascorbic acid would suggest that vitamin C could be a factor in production detriments in a species traditionally considered immune from such a deficiency due to adequate endogenous biosynthetic capacity. A series of experiments were conducted using Shaver 288 hens to investigate the effect of dietary supplements of synthetic L-ascorbic acid on egg production, hen condition, some plasma components and some egg quality characteristics through a year of production, under short-term exposure to high temperature (33 C or 35 C) and with short-term ochratoxin A ingestion at 25 C and 33 C.

Supplements of L-ascorbic acid (up to 60 ppm) in diets fed to hens under normal production conditions over a period of 40 weeks had no significant effect on feed intake, egg production, feed efficiency of egg production, egg weight, Haugh unit score, eggshell elasticity, body weight or mortality rate but tended to increase proportions of fresh yolk to whole egg. In hens subjected to 35 C over a 14-day period, dietary ascorbic acid supplements of up to 60 ppm gave limited benefits in counteracting the heat induced decline in egg

production but did not affect feed intake, egg weight, eggshell elasticity, Haugh unit score or proportions of fresh yolk to whole egg. When incorporated into the diet at 1,800 ppm, vitamin C prevented ($P \leq .05$) the decline in egg production caused by exposure to 35 C. Feed intake, egg weight, eggshell elasticity, body weight and body temperature were not significantly affected.

Supplying laying hens with diets containing 3 ppm ochratoxin A significantly ($P \leq .05$) reduced feed intake, egg production, body weights, plasma Na^+ , K^+ and total Ca^{++} concentrations, and increased eggshell elasticity, plasma Cl^- concentration and aspartate transaminase activity. The effects of ochratoxin A were less pronounced at 25 C compared to 33 C where a significant ($P \leq .05$) reduction in egg weights also occurred. All the negative effects of ochratoxin A, apart from body weight loss and reduction in feed intake, and increase in eggshell elasticity at 33 C, were either moderated or significantly ($P \leq .05$) reversed by 300 ppm dietary ascorbic acid supplementation. Results from these studies suggest that hens have the capacity to respond to dietary supplements of ascorbic acid, the response being non-specific, and support the hypothesis that environmental factors can affect vitamin C requirement. It is possible that environment, through some unknown mechanism, can render endogenous L-ascorbic acid biosynthesis inadequate to meet metabolic needs for vitamin C in the laying hen.

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FOREWORD

The preparation of this thesis followed a manuscript format. Manuscript I will be submitted to the "Journal of Applied Poultry Research". Manuscripts II and III will be submitted to the "Zambian Journal of Agricultural Science" and Manuscript IV will be submitted to "Canadian Journal of Animal Science". The authors of Manuscripts I, II and III are F.M. Haazele and W. Guenter, and Manuscript IV are F.M. Haazele, W. Guenter, R.R. Marquardt and A.A. Frohlich.

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INTRODUCTION

Under normal circumstances, a chicken has no dietary requirement for vitamin C, endogenous ascorbic acid (AA) biosynthesis being adequate in meeting metabolic needs (Emmett and Peacock, 1923; Hart *et al.*, 1925). However, a number of stressors, including exposure to high temperature, are known to result in tissue depletion of AA (Freeman, 1967; Kechik and Sykes, 1979; Cheng *et al.*, 1990), a condition associated with corticosteroid production (Freeman, 1970, 1980; Pardue and Thaxton, 1984) in response to a stressor. Exposure to high temperature is a common source of stress in production especially in tropical climates and is known to be detrimental to productivity. Exposing laying hens to a temperature of 35 C decreases feed consumption, egg production, egg weight and eggshell thickness (Tanor *et al.*, 1984), a situation which results in reduced egg mass output and marketable eggs due to increased egg breakage. Loss of eggs and profits resulting from poor shells is already a concern under normal production conditions. Anderson and Carter (1976) estimated down grading of two thirds of eggs produced in the United Kingdom, mostly due to poor shells, while Hamilton (1982) estimated eggshell breakage to account for a loss of 7 to 8% of total eggs laid in Canada and Roland (1988) estimated eggs lost or cracked in the United States to range from 13 to 20%. The effect of exposure to high temperature also disturbs general hen body condition as it affects respiratory frequency, body temperature (Darre and Harrison, 1987; van Kampen, 1988), haematology (McFarlane *et al.*, 1989), blood circulation (Darre and Harrison, 1987) and blood chemistry (Odom *et al.*, 1986).

Although the exact mechanism of tissue depletion of AA remains unknown, the process suggests a change in utilization, biosynthesis or transport of AA and a potential condition-induced need for dietary supplements of the vitamin. The implication is that AA deficiency could be a contributing factor to detriments observed under stress situations.

Literature on the effect of supplemental AA in laying hen diets gives inconsistent results, responses being achieved in different aspects under different conditions in some reports while no response was achieved in others. At normal temperature, Sullivan and Kingan (1962) achieved a response in eggshell quality, an observation similar to that made by Arscott *et al.* (1962), but only in diets with 3.0% calcium and not 2.25% calcium. Herrick and Nockels (1969) on the other hand achieved a response in albumen quality with prolonged feeding of the vitamin. Ascorbic acid supplements were also effective in reversing vanadium induced reductions in albumen quality (Benabdeljehl and Jensen, 1990) and NaCl induced eggshell defects (Balnave *et al.*, 1991). On the contrary, Pepper *et al.* (1961) did not achieve any response in diets deficient or adequate in calcium and Rowland Jr. *et al.* (1973) found no response in bone mineralization, serum calcium or egg production. Long-term supplements of AA in pullets not only failed to improve egg weight, shell thickness and Haugh unit score, but also had the negative effect of delaying the onset of laying (Dorr and Nockels, 1971).

Under high ambient temperature, responses to vitamin C supplementation were noted in hen body temperature regulation, metabolic activity and eggshell quality (Ahmad *et al.*, 1967), eggshell quality and plasma calcium (El-Boushy and van Albada, 1970), albumen quality and egg production in low protein diets (Chen and Nockels, 1973), egg production and eggshell quality (Kechik and Sykes, 1974) and albumen quality (Cheng *et al.*, 1990). Contrary to reports above, other reports did not notice any responses in egg production and

eggshell quality (Harms and Waldroup, 1961) or egg weight and eggshell quality (Heywang *et al.*, 1964) with diets low or adequate in calcium or shell quality alone (Lyle and Moreng, 1968) with diets adequate in calcium.

In spite of evidence of a potential to respond to dietary AA, the hen's ability to respond to dietary AA supplements or stress induced response remain questionable such that current feed formulations make no consideration for the vitamin. Clarification of this subject may be of importance in production if the response is substantial enough to affect production, especially in tropical climates where laying hens experience high temperatures over the greater part of the year. With this in mind, experiments were conducted to further investigate the effect of dietary AA supplement in laying hens considering production, egg quality and general hen condition. The aim of the studies was to attempt to establish the ability of laying hens to respond to dietary supplements of AA and to demonstrate a stress induced response to dietary AA using high temperatures and ochratoxin A (OA). Rather than look for overt signs of deficiency, a marginal deficiency was assumed manifesting itself in production changes resulting from changes in vitamin C's diverse biochemical functions. It was hoped that this work would help to assess the benefit of dietary AA supplements in counteracting production detriments induced by high temperature. It is of interest to note that early work by Lind, which led to the discovery of vitamin C's antiscorbutic property, recognized that a variety of stress factors encountered during sea voyages accentuated the severity of scurvy (Roddis, 1950).

LITERATURE REVIEW

Vitamin C

Chemistry and Synthesis of Ascorbic Acid

Following the demonstration by Lind in the 1700's of the value of citrus juices in the treatment of scurvy, vitamin C has been shown to be the anti-scorbutic factor. It was identified as a single crystalline compound in 1932 by King and Waugh and by Szent Gyorgy and Svirbely, and structure identification and synthesis of the compound was achieved in 1933 by several organic chemists (King, 1961). Vitamin C is a derivative of the hexose glucose, and possesses activity both as L-ascorbic acid or the partly oxidized product dehydroascorbic acid. The fully reduced form and products of the two oxidation steps are given in Appendix 1. Crawford (1982) gives a comprehensive account of the various laboratory methods used to synthesize L-ascorbic acid. To date, a number of methods of synthesis have been demonstrated which fall in three categories. Synthesis can be achieved by coupling C₁ and C₅ carbon fragments, coupling C₂ and C₄ carbon fragments or conversion of a C₆ carbon chain into the correct oxidation state and stereochemical configuration.

Currently, synthesis from glucose by carbon-chain inversion is the industrial method for the production of L-ascorbic acid but efforts to further improve the process continue.

In the evolution of animals, biosynthetic capacity started in the kidney of amphibians and reptiles, was transferred to the liver in some animals and lost in others (Chaudhuri and Chatterjee, 1969). The chemical reactions and enzymes involved in the biosynthetic process of L-ascorbic acid are given in Appendix 2. The inability of scorbutic animals to synthesize vitamin C is due to the lack of the last enzyme in the process, gulonolactone oxidase (Lehninger, 1982). Under normal conditions, a chicken has the capacity for sufficient endogenous synthesis of L-ascorbic acid to meet metabolic needs (Emmett and Peacock, 1923; Hart *et al.*, 1925) so that dietary supplies of the vitamin are considered unnecessary.

Metabolism of Vitamin C

No reports could be found on the absorption mechanism of vitamin C in avian species, however, some differences in mechanisms of absorption between scurvy prone and non-scurvy prone animals have been shown in mammals. Stevenson and Brush (1969) reported passive absorption of AA in the non-scurvy prone rat while the scurvy prone guinea pig absorbed AA by an active transport system characteristic of a Na⁺ dependent, gradient coupled mechanism. The rate of dehydroascorbic acid absorption was very slow and susceptible to a concentration gradient created by AA, suggesting a passive process. Rose *et al.* (1988) noted a net transepithelial absorption of dehydroascorbic acid *in vitro* with most substrate appearing in the reduced form (AA) in the jejunum of the guinea pig but not in that of the rat. However, both tissues took up dehydroascorbic acid across the basolateral surface

and reduced it, maintaining a substantial level of AA in the mucosa. If the above results from rat and guinea pig studies hold true for all scurvy prone and non-scurvy prone animals, the absorption of AA in the chicken would be a passive process while that of dehydroascorbic acid would be of minor importance only affecting levels of AA within intestinal tissue.

Pardue *et al.* (1984) achieved elevated plasma AA at a concentration of 250 ppm or greater upon supplying chicks 5, 50, 100, 250, 500, 1,000 or 2,000 ppm AA in drinking water. There was a significant increase in plasma AA within 4 hours at 1,000 ppm, maximum values occurring after 8 hours and high plasma AA values were maintained for 16 hours after removal of supplemented water. Hence, increases in plasma AA following supplementation are transient such that constant plasma AA levels can only be maintained by continuous ingestion. Once absorbed, AA is distributed to all body tissues. Ascorbic acid is found in liver, spleen, kidney, adrenal gland, brain, skeletal muscle and cardiac muscle, the highest concentrations being in the adrenal and other glandular tissues (Kuether *et al.*, 1944).

There are many metabolic end products of AA, but only a few have been identified. In man, administered C^{14} AA was excreted in urine chiefly as AA, diketo-L-gulonic acid and oxalic acid (Hellman and Burns, 1958). Baker *et al.* (1966) identified AA, dehydroascorbic acid and oxalate as urinary excretory products in man. Ascorbate sulphate in human urine (Baker *et al.*, 1971) and 2-methyl-L-ascorbic acid (Blaschke and Hertting, 1971) have also been identified as excretory products. A 30% reduction of dehydroascorbic acid to AA in isolated rat and guinea pig kidney tubules was reported by Rose (1989), suggesting that some of the AA found in urine is a product of this reduction process.

It is not clear as to whether animals are capable of oxidizing vitamin C to CO_2 . Hellman and Burns (1958) could not detect any C^{14} in respiratory CO_2 from men after intravenous administration of C^{14} AA but Baker *et al.* (1966) reported respiratory CO_2 as one

of the end products of AA metabolism in man although it was little and confined to a few hours after ingestion of the vitamin. In the guinea pig, AA oxidation to CO₂ was dependent upon the amount of AA fed as a single supplement and influenced by environmental stress (Tillotson, 1980). Kallner *et al.* (1985) reported oxidation of up to 30% of ingested AA in man and suggested that the formation of CO₂ was due to a presystemic effect as a result of microbiological or chemical degradation of AA in the intestine. This suggestion appears likely as it would explain lack of CO₂ detection in subjects where AA was administered by injection (Hellman and Burns, 1958), why little CO₂ was released and confined to a few hours after ingestion (Baker *et al.*, 1966) and why oxidation to CO₂ was dependent upon the amount of AA fed as a single supplement (Tillotson, 1980).

Biochemical Functions of Vitamin C

Biochemical functions of vitamin C are not clearly known, the most accepted function is as a cofactor in the enzymatic hydroxylation of proline of the collagen of connective tissue (Lehninger, 1982). Activation of the enzyme prolylhydroxylase requires AA as a reducing agent in an enzymatic system involving molecular oxygen, Fe²⁺ and α -ketoglutarate (Omaye *et al.*, 1982). Vitamin C is also thought to be part of the hydroxylating system involved in the production of α -hydroxy- β -N-trimethyl-L-lysine, an intermediate in carnitine biosynthesis (Hulse *et al.*, 1978). The mechanism requires α -ketoglutarate and Fe²⁺ and resembles that in collagen synthesis. Hence, daily supplementation of 200 mg AA increased urinary carnitine excretion in healthy elderly men and the urinary carnitine/creatinine ratio was positively correlated to leucocyte AA (Davies *et al.*, 1987).

There is strong evidence that vitamin C is involved in the transformation of cholesterol to bile acids. Supplemental AA reduced blood cholesterol in rats and rabbits (Sokoloff *et al.*, 1967), and cholesterol was found to accumulate in the blood serum and liver of guinea pigs with chronic latent vitamin C deficiency, there being a direct correlation between vitamin C concentration in the liver and the rate of cholesterol transformation to bile acids (Ginter, 1973). Ginter *et al.* (1979) found the highest rate of cholesterol transformation to bile acids and lowest concentration of total lipids cholesterol and triglycerides in serum and liver of guinea pigs fed .5% AA compared to 0% and .05% AA in diets with high level of saturated fatty acids or with .2% cholesterol.

In guinea pigs deficient in AA but without clinical scurvy, the rate of excretion of bile acids and the size of the bile acid pool were reduced by about 50%, and the activity of cholesterol 7 α -hydroxylase was also reduced without reductions in food intake (Harris *et al.*, 1979). In work by Holloway and Rivers (1984), there was an elevation in plasma cholesterol in guinea pigs with a chronic AA deficiency and an increase in bile acid pool with excess supplies of AA. Horio *et al.* (1989) reported hypercholesterolemia due to a depression in bile acid synthesis in OD rats, a scorbutogenic rat mutant, fed cholesterol containing purified diets. In studies with chicks, dietary AA at 1,000 ppm ameliorated plasma cholesterol increase in response to a 30 minute exposure to 43 C (Pardue *et al.*, 1985). Apart from involvement in cholesterol transformation to bile acid, vitamin C may be involved in lipid metabolism at other levels. Ascorbic acid was effective in reducing blood triglycerides and increasing lipoprotein lipase activity in rabbits and rats (Sokoloff *et al.*, 1967) and Tsai *et al.* (1973) reported that AA was a tissue factor required for Mg²⁺ ATP-dependent inactivation of the hormone sensitive lipase.

In guinea pigs, a dose of 50 mg AA per day resulted in a rise in specific activities

of cytochrome P-450 and b_5 (Sutton *et al.*, 1983), and chronic deficiency caused a depression in hepatic cytochrome P-450 levels (Holloway and Rivers, 1984). Omaye *et al.* (1982) suggested that the association of AA to cytochrome P-450 may be the explanation for reported declines in the activity of various drug-metabolizing enzymes, *in vitro*, in tissues from guinea pigs with AA deficiency. In experiments with rats and guinea pigs, La Du and Zannoni (1961) and Knox and Goswami (1961) reported AA to be part of the enzyme system, p-hydroxyphenylpyruvic acid oxidase, involved in p-hydroxyphenylpyruvate oxidation in tyrosine metabolism.

Vitamin C may have a role in cellular iron conservation by retarding ferritin degradation through reduction of lysosomal autophagy of the protein which increases cellular iron availability (Bridges, 1987). There is also experimental evidence to suggest that vitamin C is involved in some forms of immune response. While Little and Edgar (1971) found dietary AA at 110 to 220 ppm ineffective in limiting detrimental effects of coccidia (*P. gallinaceum*) infections, McCorkle *et al.* (1980) reported that 1% AA in chicken diets did not influence cell mediated immunity and antibody response to sheep red blood cells (T-dependent) but enhanced response to a T-independent antigen (*Brucella abortus*). They suggested that AA modulates B-cells (lymphocytes) but not T-cells. van Niekerk *et al.* (1989), concluded that effects of supplemental AA on immuno responsiveness in chicks were related to the quality of husbandry, length of supplemental feeding, age of chicks, endogenous-exogenous balance of AA and its relationship with corticosterone.

Vitamin C and its Interaction With Other Nutrients

There is a possibility for interaction between vitamin C and vitamin E through their common anti-oxidation properties. Chen and Thacker (1987) reported increases in red blood cell spontaneous haemolysis, liver thiobarbituric acid values (an indication of lipid peroxidation), and activities of glutamate-oxaloacetate transaminase, pyruvate kinase and creatine phosphokinase plus lowered vitamin E and glutathione peroxidase activities in plasma of rats fed a vitamin E and vitamin C free diet. Tube-feeding vitamin C to these rats achieved a partial reversal effect on liver thiobarbituric acid value, plasma vitamin E levels, and the activities of plasma pyruvate kinase, glutamate-oxaloacetate transaminase and creatine phosphokinase. In rats and guinea pigs fed poly-chlorinated biphenols (PCB), a combination of vitamin E and AA was most effective in reversing growth depression and rise in thiobarbituric acid reactive substances (Kawai-Kobayashi and Yoshida, 1986), another indication of a possible relationship between vitamin C and vitamin E. In a more recent study, Igarashi et al. (1991) demonstrated the synergistic action of vitamin E and C using a rat mutant unable to synthesize vitamin C. These results suggest that vitamin C has a sparing effect on vitamin E and that the two vitamins interact in protecting against lipid peroxidation. However, the turn-over rate of vitamin E in guinea pigs was not affected by AA nutrition (Burton *et al.*, 1990), casting doubts on the sparing effect relationship.

The ability of AA to form radicals which can interact with mineral ions provides a potential for effects on mineral absorption and utilization. Although considerable interaction is known to occur between ascorbate radicals and some mineral ions, especially iron and copper, nothing is obvious about the nutritional consequences of this interaction. It has been reported that AA will enhance the absorption of iron and selenium and reduce the absorption

of copper, nickel and manganese but has little effect on zinc and cobalt (Solomons and Viteri, 1982) and the absorption of zinc and calcium at normal dietary levels is not affected by AA (Sandstrom and Cederblad, 1987). Contrary to the report above, Mykkanen and Mutanen (1986) did not find a direct intestinal interaction between AA and seleno-methionine and sodium selenite in chicks. They concluded that the reaction of AA with selenium was lost under normal feeding conditions. At the cellular level AA was reported to retard ferritin degradation by reducing lysosomal autophagy of the protein thereby increasing the availability of cellular iron (Bridges, 1987).

Vitamin C and Stress

Exposure to stress has long been known to result in a reduction in levels of AA in animal tissues (Sayers *et al.*, 1945, 1946). In the chicken, reductions in AA have been observed in the adrenal gland after handling (Freeman, 1967), in blood, intestinal tissue, liver and adrenal glands with infection by intestinal coccidia *Eimeria acervulina* (Kechik and Sykes, 1979) and in blood and adrenal glands upon exposure to high temperature (Cheng *et al.*, 1990). In young chicks, depletion and repletion of AA can occur within a short time (Freeman, 1967). It is suspected that the repletion process involves the bursa because bursectomy impairs adrenal AA repletion (Freeman, 1970). Evidence of involvement of the bursa in AA repletion was also presented by Nir *et al.* (1975) who reported adrenal AA depletion in bursectomized chicks with adrenal cortical trophic hormone (ACTH) administration, immersion in cold water or starvation while intact chicks did not show any depletion for all stimuli. The bursa has been largely known as an organ involved in immune

response, however, evidence of bioconversion of C-19 and C-21 steroid substrates to metabolites by supernatant prepared from bursa of Fabricius indicates the tissue is capable of metabolizing steroids (Bedrak *et al.*, 1971). This observation suggests that the bursa is at least involved in some other function besides immune response.

There is experimental evidence linking tissue AA depletion under stress to cortical steroid production. Administration of ACTH stimulated depletion of AA in bursectomized chicks (Freeman, 1970; Nir *et al.*, 1975) and administration of cortisol lowered plasma AA in broiler cockerels (Pardue and Thaxton, 1984). Mature hens grown on diets containing 150, 300, or 3,000 ppm AA had an increased corticosterone response to ACTH which increased with increasing AA concentration (Schmeling and Nockels, 1978). However, hens fed 3,000 ppm had a smaller body pool of corticosterone and the turnover rate tended to be slower. Chicks given glucagon had an increase in plasma corticosterone if they were injected with AA at 200 mg/kg (Freeman, 1980).

While a relationship between stress, corticosteroid release and tissue AA depletion is evident, the exact nature of the relationship remains unknown. Depletion of tissue AA suggests a change in either utilisation, biosynthesis or transport of AA or any combination of these. A change in biosynthesis is however unlikely since corticosterone was reported to have no effect on renal enzyme activity associated with AA synthesis (Dieter, 1969). A change in AA transport is a possible explanation to tissue AA depletion since uptake of ascorbic acid in the adrenal cortex was reported to be an energy-dependent process inhibited by several cortical steroids in the presence of glucose (Sharma *et al.*, 1963). de Nicola *et al.* (1968) reported aldosterone to be the most potent inhibitor of AA uptake while corticosterone was least and that the transport of AA into the adrenal gland required Na^+ , K^+ and Ca^{++} while inhibition required a protein. However, this does not explain why AA administration

should result in increased corticosterone response to ACTH and a decrease in corticosterone turnover rate as reported by Schemeling and Nockels (1978) or an increase in plasma corticosterone as reported by Freeman (1980). The association of tissue AA depletion to stress response is very strong, Siegel (1971) concluded that adrenal AA depletion was a classical stress response in fowls.

Effect of High Ambient Temperature on Laying Hen Production and Egg Quality

The detrimental effects of high temperature on productivity in laying hens have been well known for decades. High temperature was reported to reduce egg weights and egg quality more than fifty years ago (Benion and Warren, 1933; Warren and Shnepel, 1940). Exposing laying hens to 35 C for as little as 3 or 4 days after keeping them at 18 C decreased feed consumption, egg production, egg weight and eggshell thickness (Tanor *et al.*, 1984). Exposure to elevated temperature will reduce feed intake within a day of change in temperature (Jones *et al.*, 1976) while eggshell quality deteriorates within hours of temperature change (Miller and Sunde, 1975). During exposure to 35 C, there is an increase in respiratory frequency and body temperature (Darre and Harrison, 1987; van Kampen, 1988), increase in cardiac output and decrease in total peripheral resistance and mean blood pressure (Darre and Harrison, 1987).

Hens subjected to a series of temperature increments (29.4 C, 35.0 C and 43.3 C) also show decreased oxygen consumption (Ahmad *et al.*, 1967), alkalosis develops 1 hour after the start of exposure to 35 C followed by a decline in blood pH as lactate and pyruvate levels increase (Odom *et al.*, 1986). There are haematological changes associated with

exposure to high temperature as McFarlane *et al.* (1989) reported an increase in percent heterophil and decrease in percent lymphocyte and basophil in chicks exposed to 30.4 C or 34.8 C.

A number of factors most likely affect the response of hens to elevated temperature including breed or strain, age, duration of exposure, pre-exposure temperature and hen nutrition status. Among White Leghorns, New Hampshires and Delawares, White Leghorns were least affected by exposure to high temperatures (Ahmad *et al.*, 1967), suggesting that breed or strain may indeed be a factor. Okumura *et al.* (1988) found the effect of temperature on egg production to be dependent on the age of laying hens, older hens being more susceptible than young hens.

A constant temperature of 29.4 C at 80% relative humidity resulted in depressed shell quality and plasma calcium while a fluctuating temperature of 29.4 C at 75 - 80% relative humidity during the day and 19.4 - 21.1 C at 50 - 60% relative humidity for 14 hours at night did not affect shell quality (El-Boushy and van Albada, 1970). Cyclic temperatures of 26.7 - 35.6 C (averaging 31 C) had a less pronounced effect than a constant 31 C on egg production, egg specific gravity and eggshell thickness although it had similar detriments on feed consumption, egg weight and plasma calcium (de Andrade *et al.*, 1977). Compared to a constant 23.9 C, exposing hens to cyclic temperature ranges of 15.6 to 37.7 C and 21.1 to 37.7 C had no effect on egg production, feed efficiency and body weights but affected feed intake, egg weights and eggshell thickness (Emery *et al.*, 1984). Exposure to a 24 hour linear cycle which ranged from 24 to 35 C, compared to a constant 21 C, did not affect egg production and eggshell strength but resulted in reduced body weight gains, reduced feed intake and reduced egg weights (Deaton *et al.*, 1986). The results of the above studies demonstrate that the performance of laying hens and the quality of the eggs that are produced

are markedly affected by high ambient temperature. The detrimental effects from exposure to high temperatures tend to be more severe when the temperature is constant as opposed to a cyclic or fluctuating temperature even when the upper limit of the cyclic temperature exceeds the constant high temperature. Hens kept at 30 C failed to acclimatise to 38 C exposure, as was determined by feed intake, oxygen consumption and rectal temperature, if they were exposed to a lower temperature (5 or 20 C) and allowed *ad libitum* feeding prior to exposure to 38 C (Sykes and Fataftah, 1986). Acclimatization to exposure to intermittent heat stress was also lost or reduced if dietary energy was increased during exposure to heat and delayed if hens kept at 30 C were subjected to 10 C prior to heat stress exposure (Sykes and Salih, 1986). Sykes and Salih (1980) concluded that heat tolerance is influenced by changes in energy metabolism brought about by dietary or environmental changes.

The cause of production detriments in hens under elevated temperature is not exactly known. El-Boushy and van Albada (1970) speculated on lowered metabolism due to reduced thyroxin output accompanied by a reduction in blood calcium to be part of the cause. However, a reduction in thyroxin output appears unlikely since exposing chicks to 32 C did not affect their iodine requirement for growth (Rogler and Parker, 1978). El-Boushy and van Albada (1970) further stated that the situation of lowered metabolism was also accompanied by a reduction in blood flow through the oviduct, which reduced the rate of synthesis of egg components. This speculation was partly in agreement with that made by de Andrade *et al.* (1977) who suggested a reduction in blood circulation in the internal organs due to increased peripheral circulation to be the cause of poor shells. On the contrary, Darre and Harrison (1987), using calculated values from mean blood pressure and cardiac output, reported a reduction in total peripheral resistance in hens exposed to 35 C.

Miller and Sunde (1975) explained the cause for poor eggshells to be a reduction in

plasma CO_2 due to hyperventilation during panting. This was thought to reduce HCO_3^- availability for eggshell formation and/or increase renal excretion of calcium bases which reduced available calcium for shell formation. A reduction in plasma CO_2 as a cause for poor eggshells was supported by the observation that use of carbonated drinking water in hens at 35 C partially relieved temperature induced eggshell thinning (Odom *et al.*, 1985). A change in acid-base balance accompanied by a reduction in blood ionized calcium was reported by Odom *et al.* (1986), an observation consistent with reports above. Furthermore, a change in ion concentrations during exposure to high temperature was suggested from a lack of response to injecting Ca^{2+} or Na^+ at 37 C, when the two injected at 28 C caused hypothermia and hyperthermia, respectively (Maki *et al.*, 1988). It was thought that the two states of temperature exposure would have resulted in similar responses if they had identical body ion concentrations.

Cohen *et al.* (1972) produced alkalosis with NaHCO_3 , NaSO_4 , CH_3COONa and NaH_2PO_4 at constant dietary Cl^- and acidosis with CaCl_2 , FeCl_3 , KCl and CholineCl at constant dietary Na^+ . Their results showed that blood pH and HCO_3^- are a function of the Na to Cl ratio. However, no benefits could be realised in eggshell thickness, albumen quality, egg production or egg weight when hens in hot weather were fed sodium bicarbonate, but a reduction in rough shelled eggs was achieved (Ernst *et al.*, 1975). Furthermore, Hamilton and Thompson (1980) did not find a relationship between acid-base balance and eggshell strength and Odiba *et al.* (1981) found different dietary levels of NaCl and NaHCO_3 to have no effect on eggshell deformation or thickness.

The reduction in feed intake associated with exposure to high temperature has been suggested to contribute to some of the detrimental effects observed in hens under heat stress. This reduction is rapid, occurring within a day of change in temperature (Jones *et al.*, 1976).

Miller and Sunde (1975) observed that the reduction in egg weights in hens under heat stress appeared to be directly related to feed intake while the deterioration in eggshell quality was too immediate to be directly related to feed intake. They suggested that the cause to poor shells was either unrelated to feed intake or dependent on another factor or factors along with reduced feed intake. This observation was in agreement with that made by Emery *et al.* (1984) who reported that pair feeding hens at a constant 23.9 C with those at cyclic 15.6 to 37.7 C and 21.1 to 37.7 C resulted in heavier eggs with thicker shells. They concluded that reductions in egg weights and eggshell thickness under cyclic temperatures were not simply a result of reduced feed intake but was also due to a direct effect of heat stress.

However, the involvement of feed intake as a contributing factor to some of the detriments observed under high temperature has been demonstrated. In work by de Andrade *et al.* (1977) feeding a high nutrient density diet, 10% more energy and 25% more of other nutrients above what was considered adequate to meet NRC recommendations, increased egg production at high temperatures. Scott and Balnave (1988) also achieved limited benefits in egg production by increasing energy and protein intakes at high temperatures. Increased metabolizable energy and crude protein intake (11.0 to 12.5 MJ/kg and 13.8 to 18.3%, respectively) at temperatures between 25 and 35 C increased egg mass output to equal values at 10 - 24 C but not at 6 - 16 C temperature ranges.

The limited benefit of increasing energy and protein intake was also reported by Marsden *et al.* (1987) where high dietary protein concentrations were required to maintain egg output at high temperatures, within the range 15 - 27 C, but high protein concentrations in diets failed to sustain egg output at 30 C. Although dietary energy affected egg weight and egg output, there was no interaction with temperature and feeding a high protein-high energy diet did not maintain egg weight or egg output at 30 C. In diets with energy levels at 2671,

2853 and 2992 kcal ME/kg, dietary energy had no effect on feed consumption or egg production at 35 C (Jones *et al.*, 1976). However, increases in dietary energy to 3371 kcal ME/kg and calcium to 6.5% partially helped to maintain egg production, egg weight and eggshell deformation (Tanor *et al.*, 1984).

Donoghue *et al.* (1989) suggested involvement of the endocrine system in the production decline in laying hens exposed to heat stress after observing a reduction in luteinizing hormone levels in hens exposed to 35 C. No other work could be found in the literature on this aspect of poultry physiology. In cows, heat stress during summer months did not affect the frequency of pre-ovulatory increase in luteinizing hormone when day temperatures reached 47 C (Vaught *et al.*, 1977). However, both base line and peak luteinizing hormone levels were reduced by exposing heifers to a diurnal temperature of 21-34 C (Miller and Alliston, 1974) or a constant temperature of 33.5 C (Madan and Johnson, 1973).

Measures Attempted to Improve Egg Production and Quality Under High Temperature

Breeding and Selection

Genetic differences do occur in susceptibility to heat stress for poultry, although no commercial hen strain has been specifically bred or selected for tolerance to heat. The White Leghorn is more tolerant to heat than New Hampshires and Delawares (Ahmad *et al.*, 1967). In more recent work, dwarf and naked-neck genes have been studied for productivity under

high ambient temperatures and appear to possess some potential. Dwarf naked-neck homozygotes had no reduction in feed intake, gave greater egg weights, greater egg mass output and better feed efficiency than normal feathered dwarf at 30 C (Bordas and Merat, 1984). Khan *et al.* (1987) reported 12% more egg production and 11.7% less mortality in dwarf genotypes compared to normal bodied siblings when they were subjected to a cyclic temperature of 21.1 to 45.5 C. In a study conducted in a tropical environment by Katongole *et al.* (1990), the Dwarf gene reduced laying intensity and egg size but had better feed efficiency. The Naked Neck gene on the other hand improved egg size but resulted in lower feed efficiency due to increased feed consumption. These researchers concluded that the Naked Neck and Dwarf genes may individually have practical relevance to increased production under a tropical climate.

Nutrition Modifications

Under the assumption that high temperature prevents hens from consuming adequate feed or specific nutrients, modifications in feeding practices and diet formulations have been attempted to improve egg production and quality under high ambient temperatures. The use of wet feed or dry and wet feed combinations increased dry matter intake at 33.3 C, but albumen quality and eggshell weights were still depressed (Tadtiyanant *et al.*, 1991). Jones *et al.* (1976) reported dietary energy to have no effect on feed consumption and egg production in hens subjected to 35 C. Whereas dietary energy affected egg weight and egg output in work by Marsden *et al.* (1987), there was no interaction with temperature and feeding a high protein high energy diet failed to maintain egg weight and egg output at 30

C. However, Pell and Polkinghorne (1986) reported that increases in dietary protein and energy during hot summer months increased egg weights and egg mass production. Furthermore, increases in energy and calcium intake partially helped to maintain egg production and egg weight, and prevented eggshell deformation in hens exposed to 35 C for 3 days (Tanor *et al.*, 1984). It appears that increasing energy, protein and calcium intake has limited benefits in counteracting detriments induced by high temperatures.

Supplying linoleic acid in diets can increase egg weights under normal production conditions (Guenter *et al.*, 1971) when body reserves are depleted of linoleic acid (Balnave and Weatherup, 1974). However, linoleic acid in excess of the requirement for normal body functioning is not specifically required for maximum egg size (Shannon and Whitehead, 1974). It is not known whether egg weight changes induced by exposing laying hens to high temperature can be affected by linoleic acid supplements.

Use of carbonated drinking water for hens subjected to 35 C was reported to help relieve temperature induced eggshell thinning by Odom *et al.* (1985), presumably by counteracting temperature induced changes in acid-base balance and increasing HCO_3^- availability for eggshell formation.

Supplemental Vitamin C in Laying Hen Nutrition

Supplemental Vitamin C in Laying Hens Housed Under Normal Temperatures

Reports in the literature on supplements of vitamin C in diets of laying hens give inconclusive results, but suggest a need for supplementation of the diet with this vitamin. Thornton and Moreng (1959) reported increases in eggshell thickness when a laying hen diet was supplemented with 22 ppm AA. Egg production, egg size and interior egg quality were not affected by AA supplement. Sullivan and Kingan (1962) also reported improvements in eggshell quality assessed as egg specific gravity or eggshell thickness when a diet was supplemented with 55 ppm AA. The effect of AA on the rate of egg production was inconsistent and of little magnitude. These reports imply that supplemental AA has an influence on the eggshell formation process, facilitating production of stronger eggshells. Arscott *et al.* (1962) reported a response to dietary supplements of AA in eggshell thickness only when diets had 3.00% calcium but not 2.25% calcium, suggesting that adequate calcium is required for the vitamin C effect to show. This observation may explain why dietary supplementation of AA at 44 ppm to a diet containing a low concentration of calcium could not improve eggshell quality (Hunt and Aitken, 1962). These observations suggest that levels of dietary calcium may be a factor affecting eggshell quality in laying hens fed supplemental AA. Prolonged feeding (7 X 28 day periods) of diets supplemented with AA at 2600 ppm did not affect eggshell thickness, rate of egg production, egg weight, feed efficiency or gain in body weight, but there was an improvement in Haugh unit score and survival rate (Herrick and Nockels 1969). The responses in Haugh unit score and survival rate suggest involvement

of the vitamin in other processes, probably reflected due to higher levels of vitamin feeding or extended feeding period.

Ascorbic acid has also been reported to prevent egg quality deterioration induced by excessive intake of NaCl and vanadium. In work by Benabdeljelil and Jensen (1990) reductions in albumen quality induced by feeding 10 ppm vanadium were prevented by supplementing diets with 100 or 500 ppm AA. Balnave *et al.* (1991) reported preventive effects by AA against an increase in eggshell defects caused by excess NaCl in drinking water.

Contrary to the findings above, Pepper *et al.* (1961) did not achieve any response in egg production, feed efficiency, egg weight, egg specific gravity or haugh units by supplementing diets that contained 1.0, 2.5 or 4.0% calcium with 33 ppm AA. In work by Dorr and Nockels (1971), long term supplements of AA at 1,200 or 3,300 ppm in growing pullets not only failed to affect egg weight, shell thickness or Haugh unit score, but had the negative effect of delaying the onset of laying. Dietary AA supplements in the range of 22 to 1,000 ppm, fed for periods of 3 days to 4 weeks in spent hens, had no effect on tibia breaking strength, serum calcium, egg production or tibia ash (Rowland Jr. *et al.*, 1973). This lack of response shown by Rowland Jr. *et al.* (1973) may be viewed as an indication that AA is not involved in the utilization of calcium *per se*, although it may be argued that this situation may not necessarily hold true for all age groups since the observation was made in spent hens.

Supplemental Vitamin C in Laying Hens Housed Under High Temperature

There are a greater number of reports of responses to AA when it was supplemented to hens exposed to high temperatures. Thornton and Moreng (1959) reported greater feed intake and O₂ consumption in hens supplemented with 22 ppm AA in diets during exposure to high temperature. Somewhat in agreement with the observation above, Ahmad *et al.* (1967) reported benefits in maintaining body temperature and metabolic activity (assessed by O₂ consumption) when hens subjected to a series of temperature increments (29.4 and 35.0 C) were supplied diets containing 44 ppm AA. Lyle and Moreng (1968) also reported moderation in the increase in the hen's body temperature associated with exposure to 29 C when the diet was supplemented with 44 ppm AA. These reports imply enhanced metabolic activity and body temperature regulation with AA supplement during exposure to high temperatures. During hot summer months, supplementing laying hen diets with AA at 25, 75 or 400 ppm sustained greater egg production (Perek and Kendler, 1962, 1963). Hunt and Aitken (1962) also achieved an improvement in the rate of egg production when hens exposed to high temperature were supplemented with 44 ppm AA. Likewise, Ahmad *et al.* (1967) reported AA supplement at 44 ppm to be helpful in maintaining production in hens subjected to a series of temperature increments (from 21 to 29.4 and 35.0 C).

In work by Kechik and Sykes (1974) weekly changes in egg yield which were associated with exposure to 32.2 and 33.3 C showed a numerical improvement when diets were supplemented with 100 or 500 ppm AA, an observation supportive of reports above. The positive effects of AA supplement on egg production is consistent with enhanced metabolic activity implied in earlier reports. Although egg production, egg size and interior egg quality were not affected by supplementing diets with 22 ppm AA (Thornton and

Moreng, 1959), an improvement in eggshell quality was achieved, a response reported under normal temperatures. Benefits in eggshell thickness were also realised by supplementing diets with 44 ppm AA in hens subjected to temperature increments from 21 to 29.4 and 35.0 C (Ahmad *et al.*, 1967).

Eggshell quality improvements achieved by supplementing diets with 50 ppm AA, in hens subjected to 29 C, occur over the entire eggshell (EL-Boushy *et al.*, 1968). Hence the effect of AA is not confined to a particular part or component of the shell, which suggests the involvement of the metabolic rate as it would result in a more general effect. An improvement in eggshell quality which was achieved by a dietary supplement of 50 ppm AA during exposure to high temperature was also associated with an increase in plasma calcium (EL-Boushy and van Albada, 1970). This report implies a change in calcium utilization with AA supplement and contradicts observations made by Rowland *et al.* (1973) with spent hens at normal temperature. Benefits in eggshell quality from supplementing AA were also evident from reductions in eggshell deformation and percent cracked eggs when hens at 32.2 and 33.3 C were fed diets containing 100 or 500 ppm AA (Kechik and Sykes, 1974). Cheng *et al.* (1990) did not achieve any improvement in eggshell quality by supplementing hen diets with 100 or 200 ppm AA at 31.1 C, but observed increases in Haugh units at 200 ppm, a response reported by Herrick and Nockels (1969) under normal temperature.

Thornton (1960) reported a relationship between dietary protein and response to AA supplement under elevated environmental temperatures. Dietary AA at 44 ppm was effective in maintaining eggshell thickness in a 13% protein diet but not in a 17% protein diet, a possible reflection of influence of nutritional stress on the effect of AA. Chen and Nockels (1973) also reported a dietary protein-AA relationship in responses in internal egg quality and production. These workers also demonstrated that hen strain and age were factors involved

in determining response to AA supplementation, suggesting that a number of factors may be involved in determining the response of laying hens to supplemental AA.

Dietary AA appears to have some beneficial effects on general bird condition in hens kept at high temperature. A vitamin C supplement (25, 75 and 400 ppm) during hot summer months gave beneficial effects on survivability (Perek and Kendler, 1962; Perek and Kendler, 1963) and supplements at 100 or 500 ppm AA resulted in non-significant improvement in changes in body weights associated with exposure to 32.2 and 33.3 C (Kechik and Sykes, 1974). Responses in general bird condition further supports the suggestion of involvement of metabolic rate as mentioned earlier.

In contrast to the above reports, several researchers have reported that the supplementation of diets with AA did not counteract the detrimental effects of exposure to high temperature in laying hens. Supplementing diets containing 2.5 or 4.6% calcium with 22 ppm AA had no effect on either the rate of egg production or eggshell thickness in hens reared under hot weather (Harms and Waldroup, 1961). Similarly, Heywang *et al.* (1964) did not achieve any appreciable effects on egg weight or eggshell thickness when hens reared under hot weather were fed 2.25% or 4.25% calcium diets supplemented with 22 to 1,000 ppm sodium ascorbate. In diets adequate in calcium and all other nutrients, dietary AA at 44 ppm could not counteract the decrease in eggshell thickness associated with increasing temperatures (Lyle and Moreng, 1968).

Ochratoxin A

Chemistry of Ochratoxin A and its Metabolism

Ochratoxins are toxic metabolites from moulds, which constitute a group of related derivatives of isocoumarin with L- β -phenylalanine (Krogh, 1987). Ochratoxin A (OA) was first isolated from a culture of *Aspergillus ochraceus* in South Africa in the mid 1960's. It has a chloride group on the isocoumarin ring and may carry a methyl group on the carboxyl end of phenylalanine in its methyl ester form (van der Merwe *et al.*, 1965). In Canada, OA has been reported in commercial wheat (Scott *et al.*, 1970; Prior, 1976) and corn (Hamilton *et al.*, 1982). Marquardt *et al.* (1988) reported that 11.3% of twelve hundred blood samples of pigs slaughtered in western Canada contained greater than 10 ng per millilitre OA. Injecting rats with OA showed that it binds to blood serum albumin (Chang and Chu, 1977) and is mainly excreted in urine unchanged or as its metabolites, with faecal excretion being of little significance (Chang and Chu, 1977; Storen *et al.*, 1982). Among the identified metabolites excreted, ochratoxin α (isocoumarin ring) makes up the greater part while 4-hydroxy-ochratoxin A constitutes a smaller part (Storen *et al.*, 1982). In vitro studies with pig, human and rat liver microsomes showed that 4-hydroxy-ochratoxin A is produced in a hydroxylation process involving cytochrome P₄₅₀ (Stormer and Pedersen, 1980).

Ochratoxin A Toxicosis

Ochratoxin A is an inhibitor of RNA synthesis (Heller and Rosenthaler, 1978). Specifically, it has been reported to lower total renal mRNA in rats (Meisner *et al.*, 1983), protein synthesis in bacteria (Heller and Rosenthaler, 1978; Bunge *et al.*, 1978) and mitochondrial ATP synthesis (Meisner and Chan, 1974; Wei *et al.*, 1985), and catalytically enhance lipid peroxidation (Rahimtula *et al.*, 1988; Omar *et al.*, 1990; Aleo *et al.*, 1991). Inhibition of specific enzyme systems by OA has also been reported. Bacteria β -galactosidase and cAMP stimulation of protein kinase (Heller and Rosenthaler, 1978), rat renal phosphoenolpyruvate carboxykinase (PEPCK) (Meisner and Meisner, 1981; Meisner *et al.*, 1983) and rat renal gluconeogenesis (Meisner and Meisner, 1981) are inhibited by OA.

Ochratoxin A Toxicosis in Poultry

In poultry, OA toxicity was observed to result in enlarged kidneys, increased uric acid excretion and decreased plasma K^+ in broiler chicks (Huff *et al.*, 1975), increased serum uric acid in poults and nephropathy in laying hens (Hamilton *et al.*, 1982). In day old chicks, isolates of *Aspergillus ochraceus* caused extensive microscopic hepatic injury consisting of either fatty changes or necrotic foci with the severity of the effect being directly related to the concentration of OA in the diets (Doupnik, Jr. and Peckham, 1970). Signs of acute OA toxicity in day-old chicks include microscopic acute nephrosis, hepatic degeneration or focal necrosis, enteritis, growth suppression and death (Peckham *et al.*, 1971). Severe

damage to the kidneys and liver leading to death was reported in laying hens by Choudhury *et al.* (1971). Chronic exposure of pullets to OA by daily intramuscular injections at .25 or .5 mg per kilogram body weight results in disruption in kidney functioning within 10 days which is reversible by discontinued administration (Glahn *et al.*, 1989).

The LD₅₀ for OA is not clearly known, however, Dwivedi and Burns (1986) quoted oral administration LD₅₀ values of 2.14, 3.60 and 3.3 mg per kilogram body weight for day old broiler chicks, 3 week old broiler chicks and day old White Leghorn cockerels, respectively. Choudhury *et al.* (1971) reported severe mortality during the first 6 weeks when 14 week old Single Comb White Leghorn hens were fed diets containing 2 or 4 ppm OA. On the contrary, Prior and Sisodia (1978) found no effect on mortality when 26 week old White Leghorn layers were fed diets containing up to 4 ppm OA for six weeks. Daily intramuscular injections of OA (.25 to .5 mg per kilogram body weight) over a period of 10 days in 10 week old Single Comb White Leghorn pullets produced acute ochratoxicosis but allowed the pullets to remain sufficiently healthy for renal function analysis (Glahn *et al.*, 1989).

Based on reported body weights and feed intakes, daily OA ingestion at 4 ppm was approximately .24 mg per kilogram body weight for Choudhury *et al.*, (1971) and approximately .27 mg per kilogram body weight for Prior and Sisodia, (1978). The difference in observed responses between Choudhury *et al.* (1971) and Prior and Sisodia (1978) was probably due to differences in ages, environmental or nutritional factors. Hens in the study by Glahn *et al.* (1989) were able to withstand higher levels of OA due to a shorter period of exposure. Results presented here suggest that laying hens will survive short term exposure to OA at .5 mg per kilogram body weight and prolonged exposure at up to .27 mg per kilogram body weight. Ochratoxin A also depresses egg production (Choudhury

et al., 1971; Prior and Sisodia, 1978; Hamilton *et al.*, 1982), feed efficiency (Choudhury *et al.*, 1971), egg weights (Prior and Sisodia, 1978) and eggshell quality (Hamilton *et al.*, 1982).

Potential Ochratoxin A - Ascorbic Acid Interaction

Ochratoxin A catalytically enhances lipid peroxidation (Rahimtula *et al.*, 1988; Omar *et al.*, 1990; Aleo *et al.*, 1991) while vitamin C has a protective effect against lipid peroxidation (Kawai-Kobayashi and Yoshida, 1986; Chen and Thacker, 1987; Igarashi *et al.*, 1991). Furthermore one of the metabolites of OA, 4-hydroxy-ochratoxin A, is produced in a hydroxylation process involving cytochrome P-450 (Stormer and Pedersen, 1980). Hutchison *et al.* (1971) reported 4-hydroxy-ochratoxin A at 40 mg per kilogram body weight to be nontoxic in rats and concluded that the microsomal hydroxylation represented a detoxification reaction. The activity of cytochrome P-450 has been shown to be influenced by AA status. Supplements of AA increase the activity of cytochrome P-450 (Sutton *et al.*, 1983) while a deficiency reduces hepatic cytochrome P-450 levels (Holloway and Rivers, 1984). From these relationships, it can be suggested that AA status will influence the rate of lipid peroxidation by OA and the formation of 4-hydroxy-ochratoxin A while a disruption in liver and kidney functioning by OA could in turn interfere with AA biosynthesis.

**MANUSCRIPT I: LONG-TERM DIETARY ASCORBIC ACID
SUPPLEMENTATION AND LAYING HEN PRODUCTION PERFORMANCE**

ABSTRACT

Six-hundred and forty Shaver 288 hens were randomly assigned to five test diets in groups of 16 hens, eight replications per treatment. The five test diets consisted of a wheat-soybean meal basal diet supplemented with 0, 15, 30, 45 or 60 ppm ascorbic acid. The test diets were fed over a period of 40 weeks, production performance being assessed in 10 consecutive 28-day periods with daily high and low temperatures being recorded. Although temperature was significantly ($P \leq .05$) correlated to feed intake, egg production, feed efficiency of egg production, eggshell elasticity and Haugh unit score, correlation coefficients were low (.42, -.48, .63, -.29 and -.42, respectively). Feed intake, egg production, feed efficiency of egg production, egg weight, proportions of fresh yolk to whole egg and Haugh unit score were mainly influenced by hen age. Period was significantly ($P \leq .05$) correlated to all parameters above, correlation coefficients being .80, -.96, .80, .96, .88 and -.94 for feed intake, hen-day egg production, feed efficiency of egg production, egg weight, yolk percent and Haugh unit score, respectively. Eggshell elasticity fluctuated and did not reveal a clear trend over the 40 weeks. The correlation coefficient of period to eggshell elasticity was low (.41) though significant ($P \leq .05$). Supplementing ascorbic acid to the diet had no significant effect on feed intake, egg production, feed efficiency of egg production, egg weight, Haugh unit score, eggshell elasticity, body weight gain or mortality rate but tended to increase proportions of fresh yolk to whole egg. It was concluded that no significant benefit in production is realised from dietary AA supplements of up to 60 ppm in laying hens under normal production conditions in a temperate climate.

KEY WORDS: ascorbic acid, supplement, hen, performance.

INTRODUCTION

Under normal circumstances, a chicken has no dietary requirement for vitamin C (Emmett and Peacock, 1923). Livers from chicken raised on a vitamin C free diet will contain enough vitamin C to cure scurvy in a guinea pig (Hart *et al.*, 1925), showing adequacy of the chicken's biosynthetic capacity. However, stress is known to result in reductions in tissue levels of ascorbic acid (AA) suggesting a potential need for dietary supplementation of the vitamin under such situations. Reductions in AA were reported in the adrenal gland after handling (Freeman, 1967), in blood, intestinal tissue, liver and adrenal glands with infection by intestinal coccidia (Kechik and Sykes, 1979) and in blood and adrenal glands upon exposure to high temperature (Cheng *et al.*, 1990).

Reports in literature suggest a potential for response to dietary supplements of AA on production especially under high temperature. At normal temperature, Sullivan and Kingan (1962) reported the effect of supplementing diets with 55 ppm AA on egg production to be inconsistent and of little magnitude. Furthermore, Rowland Jr. *et al.* (1973) found no effect on egg production and Herrick and Nockels (1969) found no effect on egg production and feed efficiency from supplementing diets with AA at concentration ranges of 22 to 2,600 ppm. Worse still, long-term supplementation of ascorbic acid at 1,200 or 3,300 ppm in growing pullets had a negative effect of delaying the on-set of laying (Dorr and Nockels, 1971). Dietary supplementation of AA at concentration ranges of 22 to 55 ppm improved eggshell quality in diets adequate in calcium (Thornton and Moreng, 1959; Sullivan and Kingan, 1962; Arscott *et al.*, 1962) but could not prevent eggshell deterioration induced by low dietary calcium at 44 ppm (Hunt and Aitken, 1962). Herrick and Nockels (1969) did not achieve an improvement in eggshell quality with AA supplementation at 2,600 ppm but

reported an improvement in Haugh unit scores. Contrary to reports above, Pepper *et al.* (1961) and Dorr and Nockels (1971) did not achieve any response to dietary AA supplements at concentrations of 33 ppm and 1,200 to 3,300 ppm, respectively, in either eggshell quality or Haugh unit scores.

In hens exposed to high temperature, Ahmad *et al.* (1967) reported an improvement in egg production from a dietary supplement of 44 ppm AA and Kechik and Sykes (1974) reported improvements in weekly changes in egg yield and body weights which were associated with exposure to high temperature when diets were supplemented with 100 or 500 ppm AA. Dietary AA supplements in the concentration range of 22 to 500 ppm were also effective in improving eggshell quality (Thornton and Moreng, 1959; Ahmad *et al.*, 1967; El-Boushy *et al.*, 1968; El-Boushy and van Albada, 1970; Kechik and Sykes, 1974) and Haugh unit scores (Cheng *et al.*, 1990). On the contrary, Harms and Waldroup, (1961) found no effect on either the rate of egg production or eggshell thickness from supplementing diets with 22 ppm AA for hens reared in hot weather. Heywang *et al.* (1964) found sodium ascorbate supplements from 22 to 1,000 ppm to have no appreciable effect on egg weight or eggshell thickness in hens fed 2.25% or 4.25% calcium diets under hot weather. Furthermore, Lyle and Moreng (1968) reported that supplemental AA at 44 ppm did not counteract the decrease in eggshell thickness associated with exposure to high temperature. These inconsistencies revealed above suggest that there may be situations when laying hens respond to dietary supplements of ascorbic acid.

With the assumption of a potential condition-induced response to dietary ascorbic acid over a production year, especially changes in environmental temperature, an experiment was conducted to study the effect of long-term dietary AA supplementation on production in laying hens under normal conditions in a temperate climate.

MATERIALS AND METHODS

Six-hundred and forty Shaver 288 pullets at point of lay were randomly placed in cage units in groups of four, in a typical temperate climate layer house. Cages, which were equipped with automatic watering cups, allowed an area of 415 sq. cm per hen. The lighting program was set to provide 14 h of light over a 24 h period. At the age of about 24 weeks, 4 adjacent cage units sharing a common feeder were randomly assigned to either of five test diets in eight replications, giving a total of 128 hens per test diet. The five test diets consisted of a wheat-soybean meal basal (Table 1) as a control and basal diet supplemented with 15, 30, 45 or 60 ppm protected-coated L-ascorbic acid.¹ Test diets were fed over a period of 40 weeks, new batches of feed being prepared every second week and production performance was assessed in 10 consecutive 28-day periods over the 40 weeks.

Daily egg production and daily high and low temperatures were recorded while feed intake was determined at weekly intervals. Egg weights and eggshell elasticity² were determined on a random sample of eight eggs from each replication picked on two consecutive days at the end of each 28-day period while fresh yolk weights and albumen height³ were measured on a random sample of four of the eight eggs. Eggshell elasticity was measured at the equator region of intact eggs that had no signs of cracks. Haugh unit score, an index for albumen firmness, was calculated from the formula: $Hu = 100 \times \log_{10} [\text{albumen height (mm)} - (5.675 \times (30 \times \text{egg weight (g)} - 100)) \div 100 + 1.9]$ (Haugh, 1937). Total

¹*L-Ascorbic acid crystals, Lot No. 31902. Colborn-Dawes (Hoffmann-La Roche).
Cambridge, Canada.*

²*Measured with Marius deformation apparatus, Marius, Utrecht, Holland.*

³*Measured with Albumen Height Guage, Queensboro Instruments, Ottawa, Canada.*

TABLE 1. Basal diet composition (Experiment 1)

Ingredient	% of diet
Ground wheat	73
Soybean meal	13
Alfalfa meal	2
Tallow	1.4
Dicalcium phosphate	1
Limestone	5.6
Oyster shell	2.5
Vitamin premix ¹	1.0
Mineral premix ²	.5
Calculated analysis:	
Metabolizable energy (kcal/kg)	2963
Crude protein (%)	16.80
Lysine (%)	.83
Methionine (%)	.31
Calcium (%)	3.40
Available Phosphorus (%)	.30

¹Vitamin premix formulated to supply per kilogram of diet: 8250 IU vitamin A; 1000 IU vitamin D₃; 0.0112 mg vitamin B₁₂; 5.46 IU vitamin E; 500 mg DL-methionine; 2.2 mg riboflavin; 4.4 mg Ca-pantothenate; 6.6 mg niacin and 95.5 mg choline.

²Mineral premix formulated to supply 99 mg Mn; 40 mg Zn and 4.78 g NaCl (.007% I) per kilogram of diet.

weights of a day's egg production in each replication were also recorded on two consecutive days half-way through each 28-day period. Hen body weights were taken at the start and end of the entire 40-week experimental period. Mortality was recorded at time of occurrence and all hens that died were submitted to a veterinary laboratory for necropsy. Data for each period was analyzed in a General Linear Model procedure, with planned comparisons of class means being done in cases that approached significance and Pearson correlation coefficients over 40 weeks determined for selected variables (SAS, 1986). Tests of significance were made at $P \leq .05$ in all the procedures.

RESULTS AND DISCUSSION

Figure 1 shows a plot of recorded laying-house temperature ranges for daily highs and lows over the entire experimental period, the cross-lined area represents laying house temperature ranges. Day 1 on the plot represents 5th May while day 280 represents 9th February of the following year. Although outside summer temperatures were high, usually reaching upper 20's and lower 30's and winter temperatures very low (reaching -20's), there were little differences between summer and winter months in recorded laying house temperatures due to modifications by ventilation fans in summer and heaters in winter. However, the temperature record does not exactly reflect the extent of exposure to heat stress since humidity was not taken into consideration. On average, summer months had greater daily temperature ranges but daily highs rarely exceeded 29 C. Summer temperatures were influenced by environmental temperatures while winter temperatures were mainly influenced by the heating system.

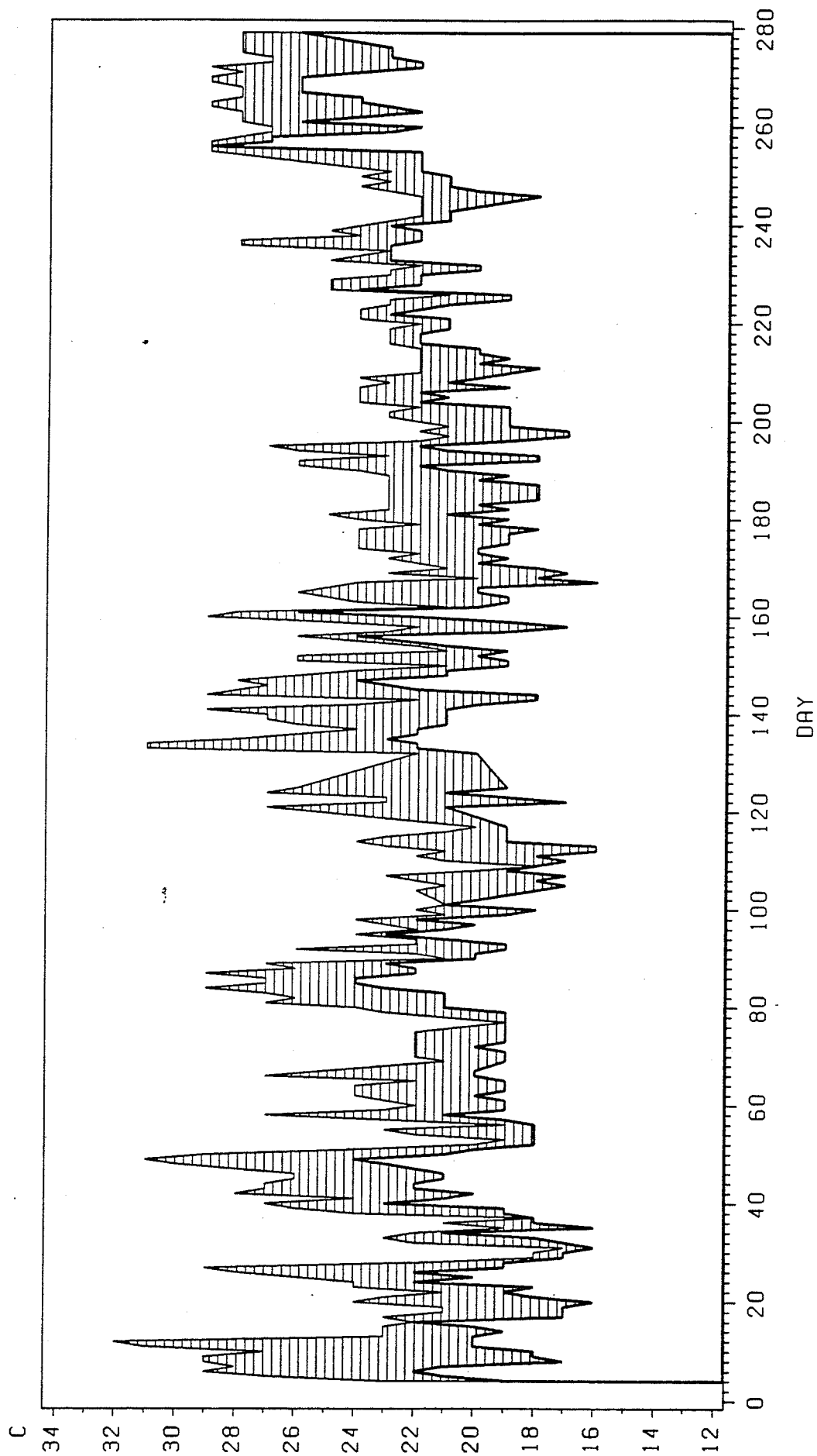


Figure 1. Recorded daily high and low laying house temperatures (C) over 40 weeks. Day 1 is May 5 whereas day 280 is February 9 of the following year (Experiment 1).
 [Days and corresponding periods: 1-28 = 1, 29-56 = 2, 57-84 = 3, 85-112 = 4, 113-140 = 5, 141-168 = 6, 169-196 = 7, 197-224 = 8, 225-252 = 9, and 253 - 280 = 10]
 [Days and corresponding seasons: 1-45 = spring, 46-140 = summer, 141-230 = fall and 231-280 = winter]

Plots of daily feed intake assessed over 28-day periods against periods are shown in Figure 2. There were no significant differences in feed intake between treatment groups in any of the 10 periods, showing that supplements of AA had no significant effect on feed intake. Feed intake increased over the 40-week duration due to increases in hen body weights with advancing age. The correlation between feed intake and period (age) was high ($r = .80$) and significant. Temperature had very little influence on feed intake, the correlation coefficient of feed intake to average high temperature was low (.42) though significant. The positive correlation between temperature and feed intake resulted from increased feed intake that occurred at older age, when the average of high temperatures tended to be higher. It is likely that temperatures in the current study were too low to induce a reduction in feed intake. Reported temperature induced reductions in feed intake occurred at a constant 35 C (Jones *et al.*, 1976; Tanor *et al.*, 1984) or cyclic temperature ranges with an upper limit of 35 C or above (de Andrade *et al.*, 1977; Emery *et al.*, 1984; Deaton *et al.* 1986). This result suggests that there is no response in feed intake from dietary supplements of AA of up to a level of 60 ppm in hens under normal production conditions.

Figure 3 shows plots of mean period egg production as hen-day percent egg production against time. Hens reached peak production (93-96%) in the second period which was followed by a gradual decline, reaching 72-75% in the tenth period. Temperature did not appear to have a major influence on egg production, the trend was normal, being determined by hen age. The correlation coefficient of hen-day egg production percent to the average high temperature was low (-.48) though significant, whereas that to period (age) was high and significant (-.96). Reductions in egg production were reported in hens exposed to a series of temperature increments of 29.4 and 35 C (Ahmad *et al.*, 1967) and a constant 35 C for 3 or 4 days (Tanor *et al.*, 1984), while a cyclic range of 26.7 to 35.6 C had a milder

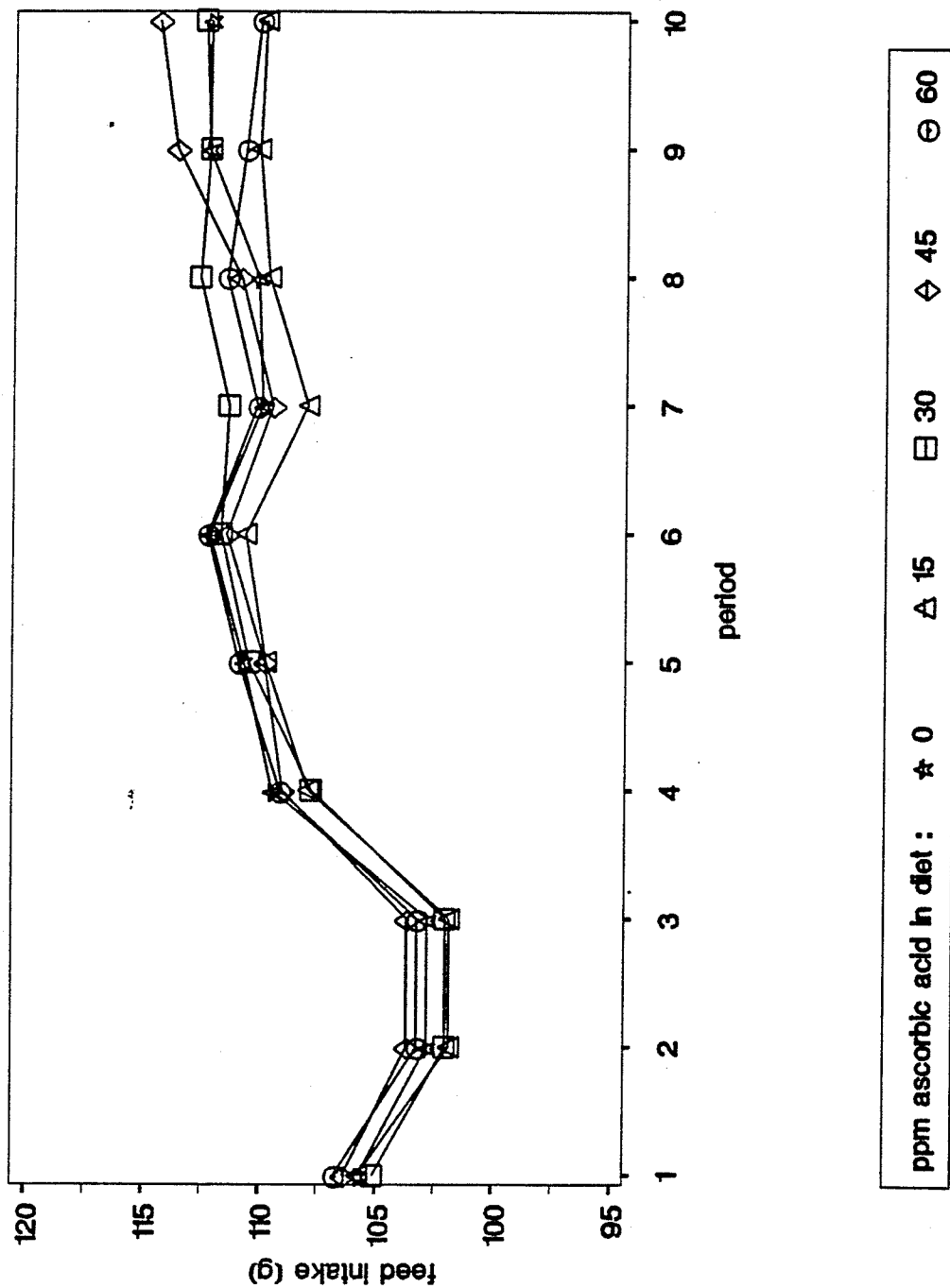


Figure 2. Mean feed intake (g/hen/day), over four-week periods, for hens fed different dietary levels of synthetic L-ascorbic acid (Experiment 1).

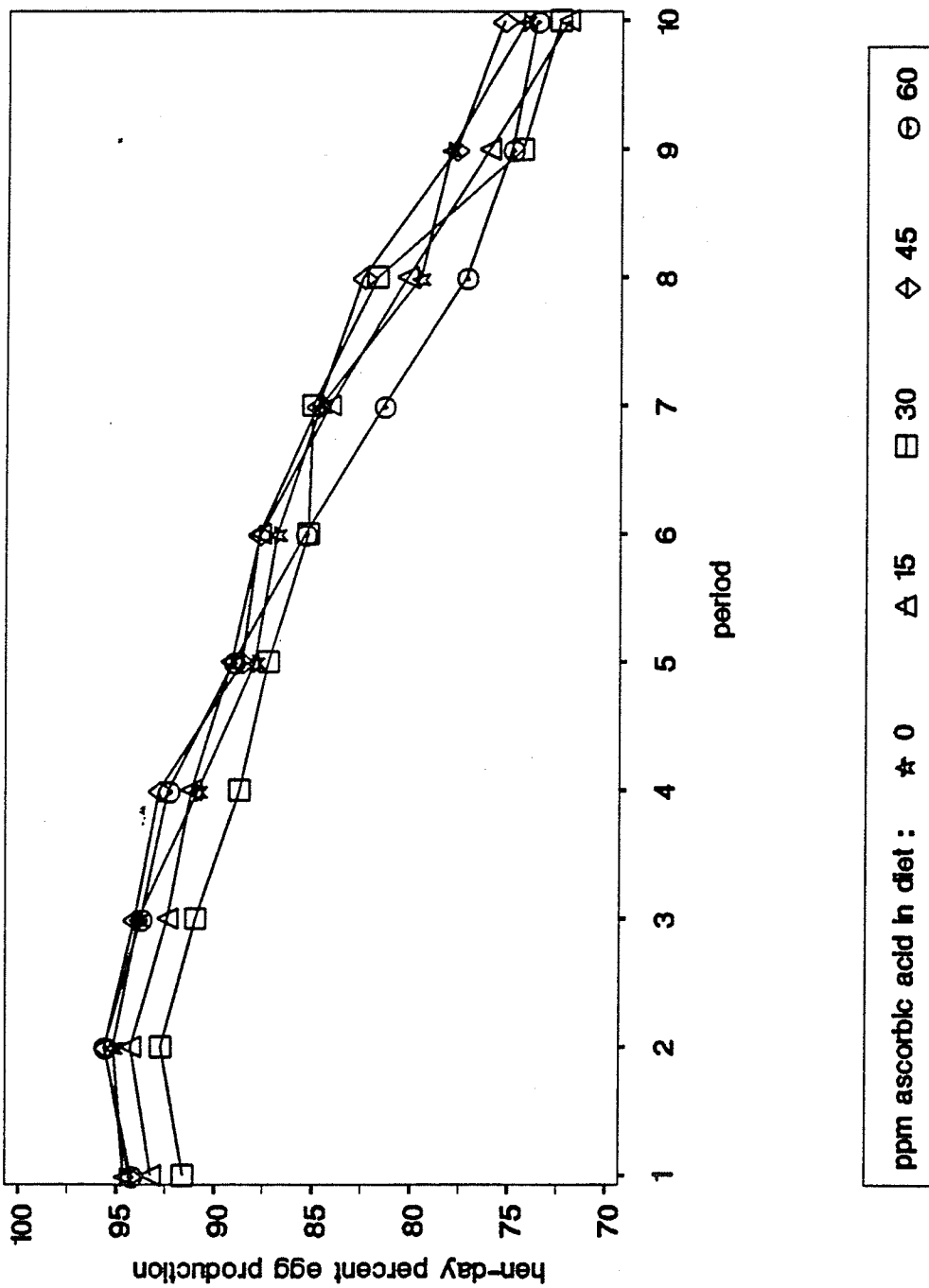


Figure 3. Mean Hen-day percent egg production over each period, determined from the total number of eggs produced over the four-week period as percent of hen days, for hens fed different dietary levels of synthetic L-ascorbic acid (Experiment 1).

effect than a constant 31 C (de Andrade *et al.*, 1977). Hens exposed to cyclic temperature ranges of 15.6 to 37.7 C and 21.1 to 37.7 C (Emery *et al.*, 1984) or 24 to 35 C (Deaton *et al.*, 1986) did not show reductions in egg production. These reports suggest that egg production is most affected by constant high temperatures, rather than cyclic, as was the case in the present study. Furthermore, high temperatures in the current study rarely exceeded 30 C which is the temperature range reported to induce reductions in egg production.

There were no significant differences in egg production among treatment groups in any of the periods suggesting that a response to dietary AA was not achieved. In agreement with this observation, Thornton and Moreng (1959) did not observe any improvement in egg production when laying hen-diets were supplemented with 22 ppm AA under normal temperatures. However, Sullivan and Kingan (1962) reported a small and inconsistent response when they supplemented diets with 55 ppm AA. This minor discrepancy in observations may be a reflection of differences in environments in different studies as response to dietary AA is assumed to be environment related.

Feed efficiency of egg production declined with age, the rate of decline being more pronounced at an older age (Figure 4). This trend was a reflection of increased feed intake and decreasing egg mass production with age. There were high and significant correlations between feed efficiency of egg production and period, feed intake and hen-day percent egg production, showing that age was indeed the main factor determining efficiency. Correlation coefficients of feed efficiency of egg production to period, feed intake and hen-day percent egg production were .80, .75 and -.90, respectively.

Temperature had little influence on feed efficiency, having a moderate positive but significant correlation coefficient of .63 to feed efficiency. The higher average of high temperatures at an older age is a contributing factor in this relationship as suggested earlier.

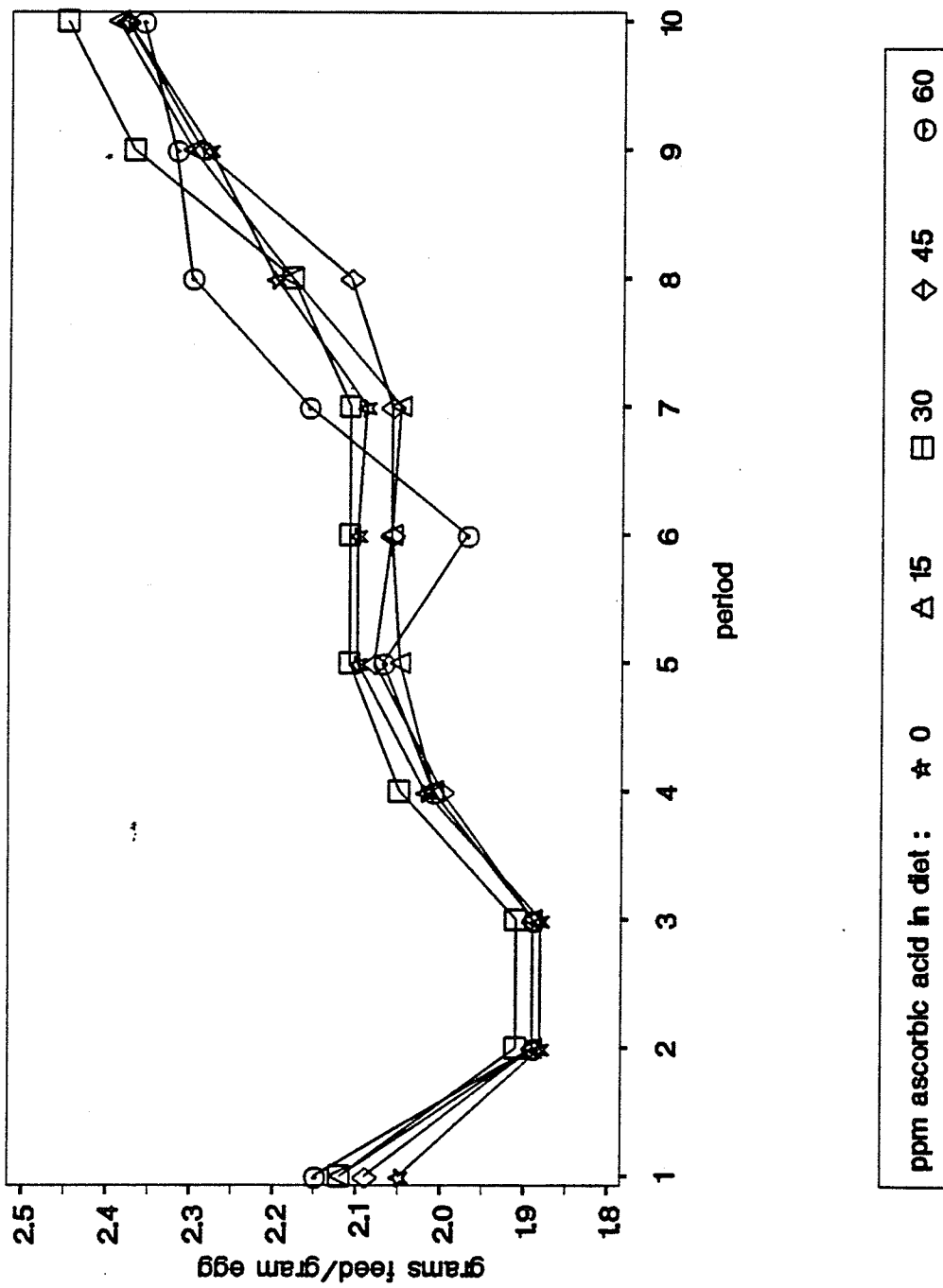


Figure 4. Mean feed efficiency of egg production over four-week periods for hens fed different dietary levels of synthetic L-ascorbic acid (Experiment 1).

In work by Emery *et al.* (1984) exposing laying hens to cyclic temperatures with an upper limit of 37.7 C did not affect feed efficiency. High temperatures in the current study were lower, rarely exceeding 30 C, and therefore less likely to affect feed efficiency. Dietary supplements of AA did not affect feed efficiency as there were no significant differences in feed efficiency among treatment groups in any of the periods. The observation made in the current study supports the report by Pepper *et al.* (1961) who reported a lack of response in feed efficiency in hens fed diets supplemented with 33 ppm AA under normal ambient temperatures.

Egg weight increased with age, a much greater rate of increase occurring to peak egg production followed by an intermediate rate in mid periods and a much lesser rate in later periods (Figure 5). Egg weight averaged 53.8 g in the first period and increased to 63.9 g by the tenth period. The observed trend was expected since egg weight increases with increasing hen body size and age, and relates well to the observed increase in feed intake with advancing hen age. The correlation coefficient of egg weight to period was high and significant (.96). There was no evidence that temperature had an effect on egg weight, the correlation coefficient of egg weight to average high temperature was not significant. High temperature induced reductions in egg weight were reported at constant temperatures of 31 C (de Andrade *et al.*, 1977), 35 C (Tanor *et al.*, 1984) and cyclic temperatures of 26.7 to 35.6 C (de Andrade *et al.*, 1977), 15.6 to 37.7 C (Emery *et al.*, 1984) and 24 to 35 C (Deaton *et al.*, 1986). It is likely that temperatures in the current study were too low to induce a reduction in egg weight since daily high temperatures (Figure 1) were lower than constant temperatures and upper limits of cyclic temperatures in the reports above. There were no significant differences in egg weight among treatment groups showing that supplementation of AA to the diet had no significant effect on egg weight. In agreement with

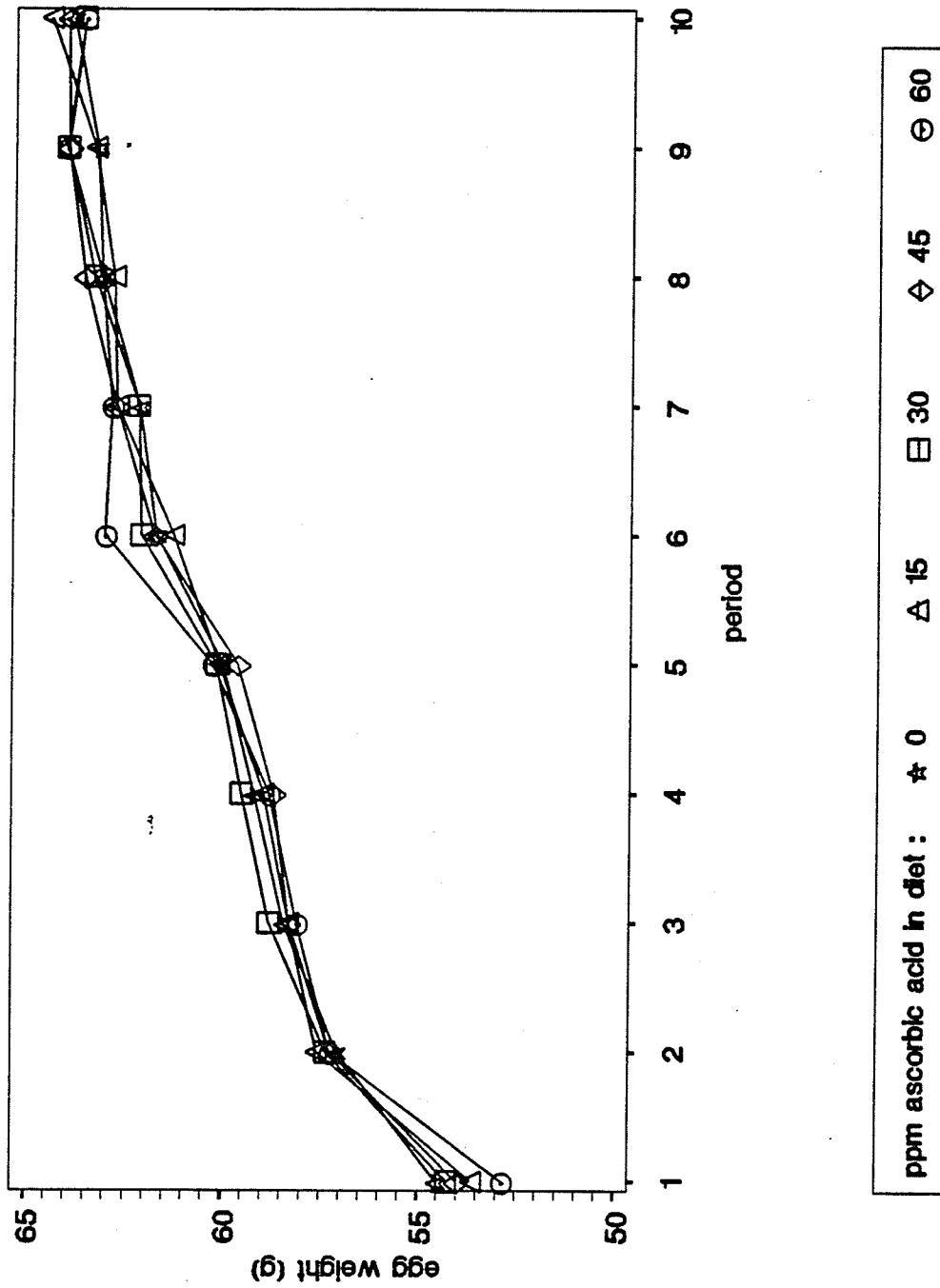


Figure 5. Mean egg weight, determined from egg collections made on two consecutive days half-way through each four-week period, for hens fed different dietary levels of synthetic L-ascorbic acid (Experiment 1).

the current observation, long-term supplements of AA in laying hen diets at 2,600 ppm (Herrick and Nockels, 1969) and 1,200 or 3,300 ppm (Dorr and Nockels, 1971) at normal temperature had no effect on egg weight. These results suggest that egg size is not affected by dietary supplements of AA in hens under normal conditions.

The proportion of fresh yolk to whole egg increased from period one to six, after which increases were not very evident (Figure 6). There was no evidence that temperature had an influence on percent fresh yolk to whole egg. Proportions of yolk to whole egg were to a great extent influenced by egg size which was in turn influenced by hen age. There were high and significant correlations between yolk proportions and egg weight to period but not temperature. Correlation coefficients of percent fresh yolk to egg weight and period were .93 and .88, respectively. Hens on the control diet tended to have the least yolk proportions in all periods and planned comparisons of class means showed that controls had significantly less percent yolk than the average of the supplemented groups, being $.8 \pm .3\%$, $.7 \pm .3\%$, $.8 \pm .3\%$, $.7 \pm .3\%$ and $.8 \pm .3\%$ less than the highest mean in periods 5, 6, 7, 9 and 10, respectively. Unless treatments caused a change in albumen quantity, the current results would suggest that prolonged supplements of AA had a tendency to result in increases in yolk size. No report could be found in the literature with this type of response. Association of AA to lipid metabolism, the major component of yolk material, has been reported for carnitine biosynthesis (Hulse *et al.*, 1978), lipoprotein lipase activity (Tsai *et al.*, 1973) and cholesterol transformation to bile acids (Ginter, 1973; Ginter *et al.*, 1979; Harris *et al.*, 1979; Holloway and Rivers, 1984; Horio *et al.*, 1989). However, it is difficult to speculate on the mechanism that would lead to the observed response with information available from the current study.

There was a reduction in Haugh unit scores from period one to ten, an indication of

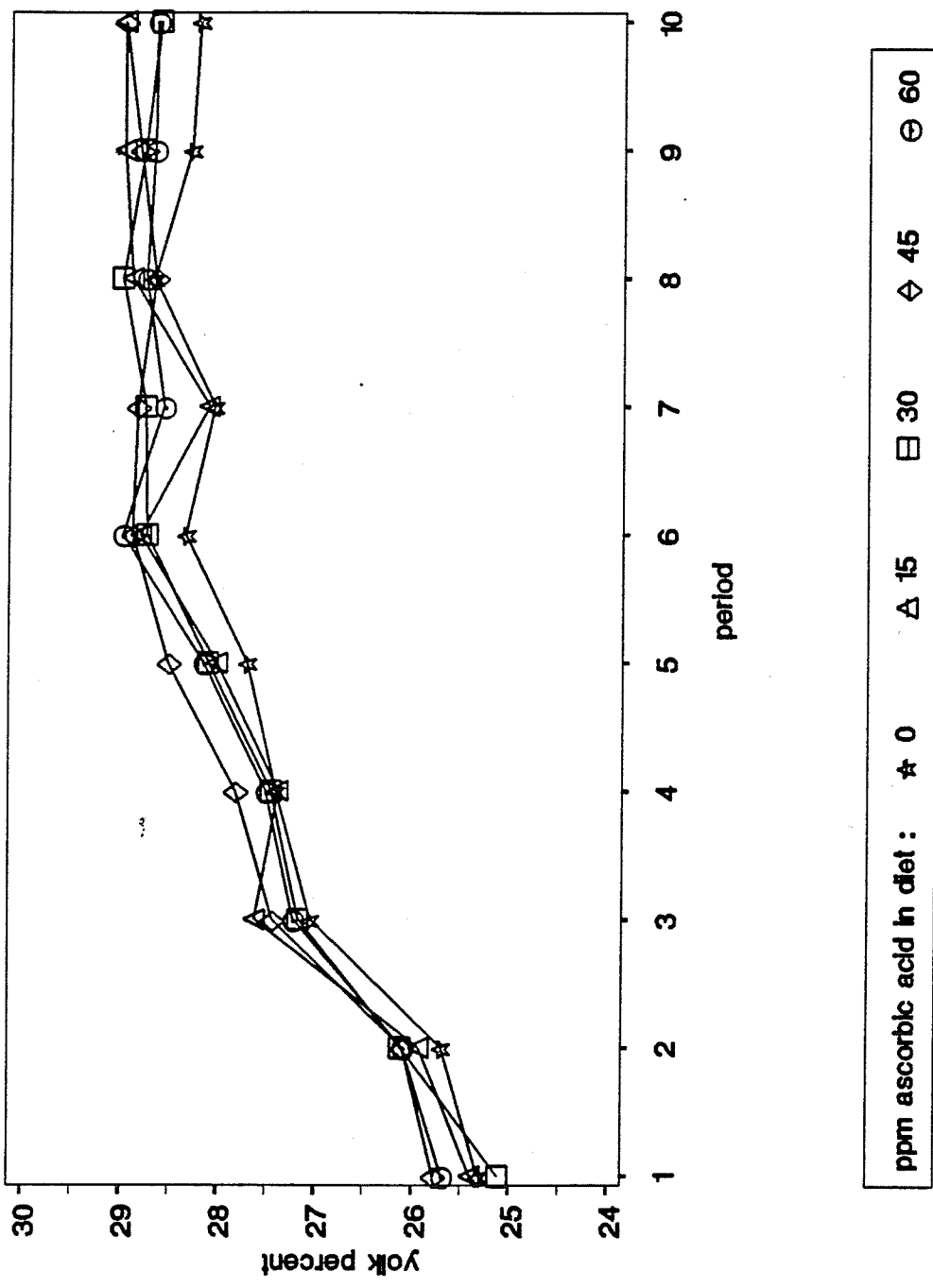


Figure 6. Mean yolk weight as a percentage of whole egg weight, determined on a random sample of 32 eggs picked on two days at the end of each four-week period, for hens fed different dietary levels of synthetic L-ascorbic acid (Experiment 1).

a reduction in albumen firmness with advancing hen age (Figure 7). Haugh unit scores averaged 90.9 in period one and 79.1 in period ten. The correlation coefficient of period to Haugh unit score was high (-.94) and significant. Temperature had little influence on Haugh unit scores. The correlation coefficient of temperature to Haugh unit score was low though significant (-.42). Supplemental dietary AA had no effect on Haugh unit scores, there being no significant difference between treatment groups in Haugh unit scores in any period. Hence, supplementing AA acid had no effect on albumen firmness and most likely chemical composition since albumen firmness is thought to be determined by composition.

In agreement with the current observation, Dorr and Nockels (1971) did not observe any effect on Haugh units from long-term supplements of AA at 1,200 or 3,300 ppm for growing pullets under normal temperature conditions. However, Herrick and Nockels (1969) reported an improvement in Haugh unit scores from prolonged feeding of diets supplemented with AA at 2,600 ppm in hens under normal temperature. In hens exposed to 31.1 C, supplementing diets with 200 ppm AA also resulted in an increase in Haugh unit scores (Cheng *et al.*, 1990). Temperatures in the current study reached 31 C only on 3 days in 40 weeks so that a temperature induced response to AA in Haugh units was not likely. The difference between the observation in the current study and that made by Herrick and Nockels (1969) may be explained by differences in levels of AA in diets. The level of AA used by Herrick and Nockels (1969) was more than forty times the level of the highest concentration in the current study. The level of dietary inclusion of AA in the current study was probably not high enough to induce a significant response. Chen and Nockels (1973) reported a dietary crude protein and AA interaction for internal egg quality response which was also influenced by hen age and strain. Diets in the current study had 16.8% crude protein but the crude protein content of the diet used by Herrick and Nockels (1969) was not

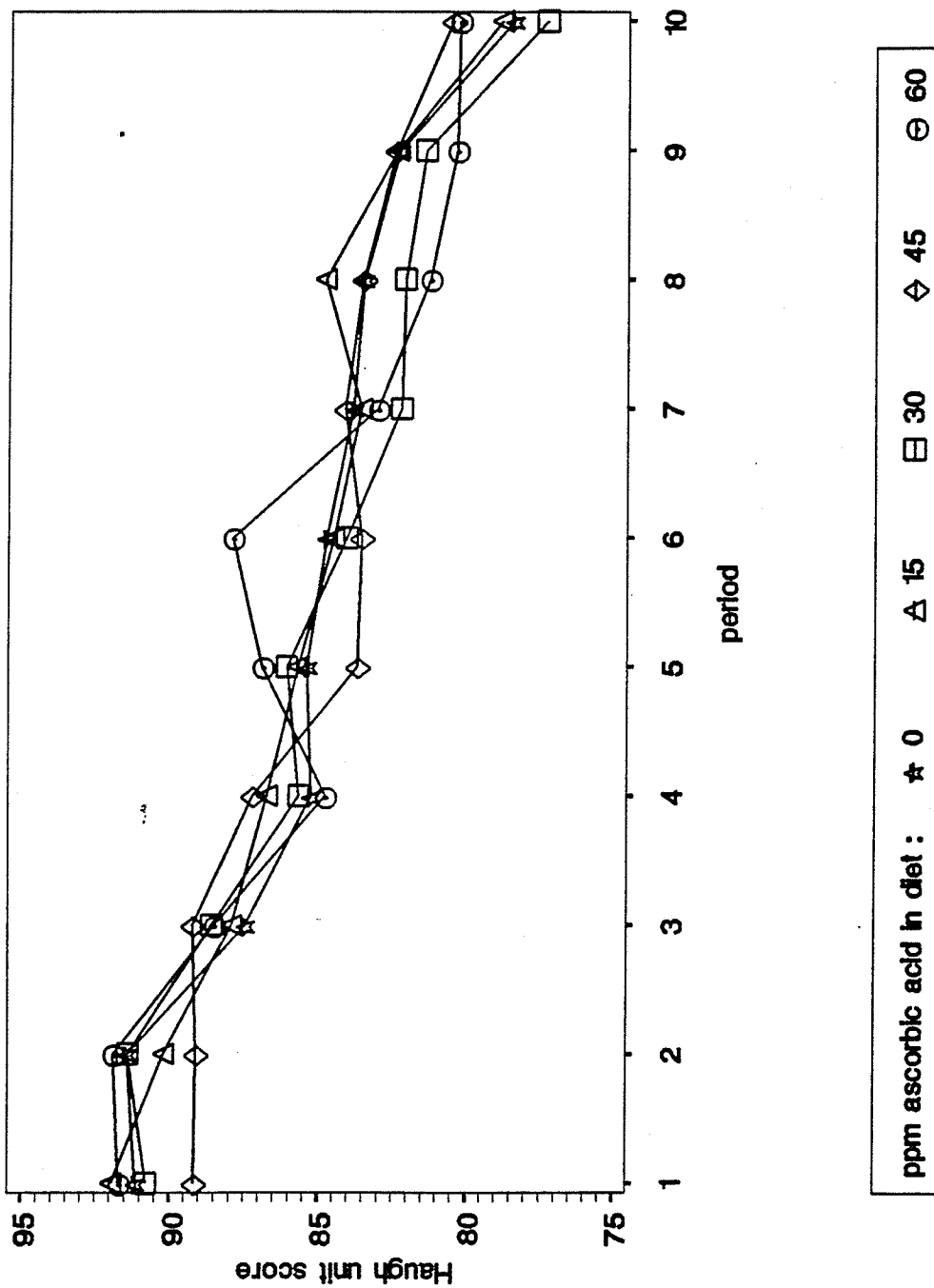


Figure 7. Mean Haugh unit scores, determined on a random sample of 32 eggs picked on two consecutive days at the end of each four-week period, for hens fed different dietary levels of synthetic L-ascorbic acid (Experiment 1).

given. Differences in strains and perhaps dietary protein levels may be other possible explanations to observed contradictions.

Eggshell elasticity fluctuated considerably in all treatments, decreasing over two or three periods after an increase over another two or three periods (Figure 8). Both temperature and period had little influence on eggshell elasticity. Though significant, correlation coefficients of eggshell elasticity to temperature and period were low, (-.29 and .41, respectively). El-Boushy and van Albada (1970) reported reductions in eggshell quality in hens exposed to a constant 29.4 C but not at cyclic temperatures of 19 to 29.4 C, a range close to that in the current study. Furthermore, de Andrade *et al.*, (1977) reported a milder effect on eggshell quality from exposure to a cyclic temperature of 26.7 to 35.6 C compared to a constant 31 C and Deaton *et al.* (1986) found no effect on eggshell quality from exposing hens to a cyclic temperature of 24 to 35 C. Reductions in eggshell quality were reported in hens exposed to a constant 35 C (Tanor *et al.*, 1984) and cyclic 15.6 to 37.7 C or 21.1 to 37.7 C (Emery *et al.*, 1984). These reports partially agree with current findings, suggesting that eggshell quality is most affected by constant high temperatures rather than cyclic and that upper limits of cyclic temperatures must exceed that attained in the current study to induce an effect. Since diets were formulated and mixed to supply identical levels of dietary Ca and P throughout the 40 weeks, fluctuations in eggshell elasticity in the current study suggest some form of control mechanism in shell formation, phases of restricted shell deposition being followed by greater shell deposition, a process most likely to be related to bone calcium depletion and repletion.

There were no significant differences in eggshell elasticity between treatment groups in any period, showing that AA supplement had no effect on eggshell quality. This observation contradicts reports by Thornton and Moreng (1959) and Sullivan and Kingan

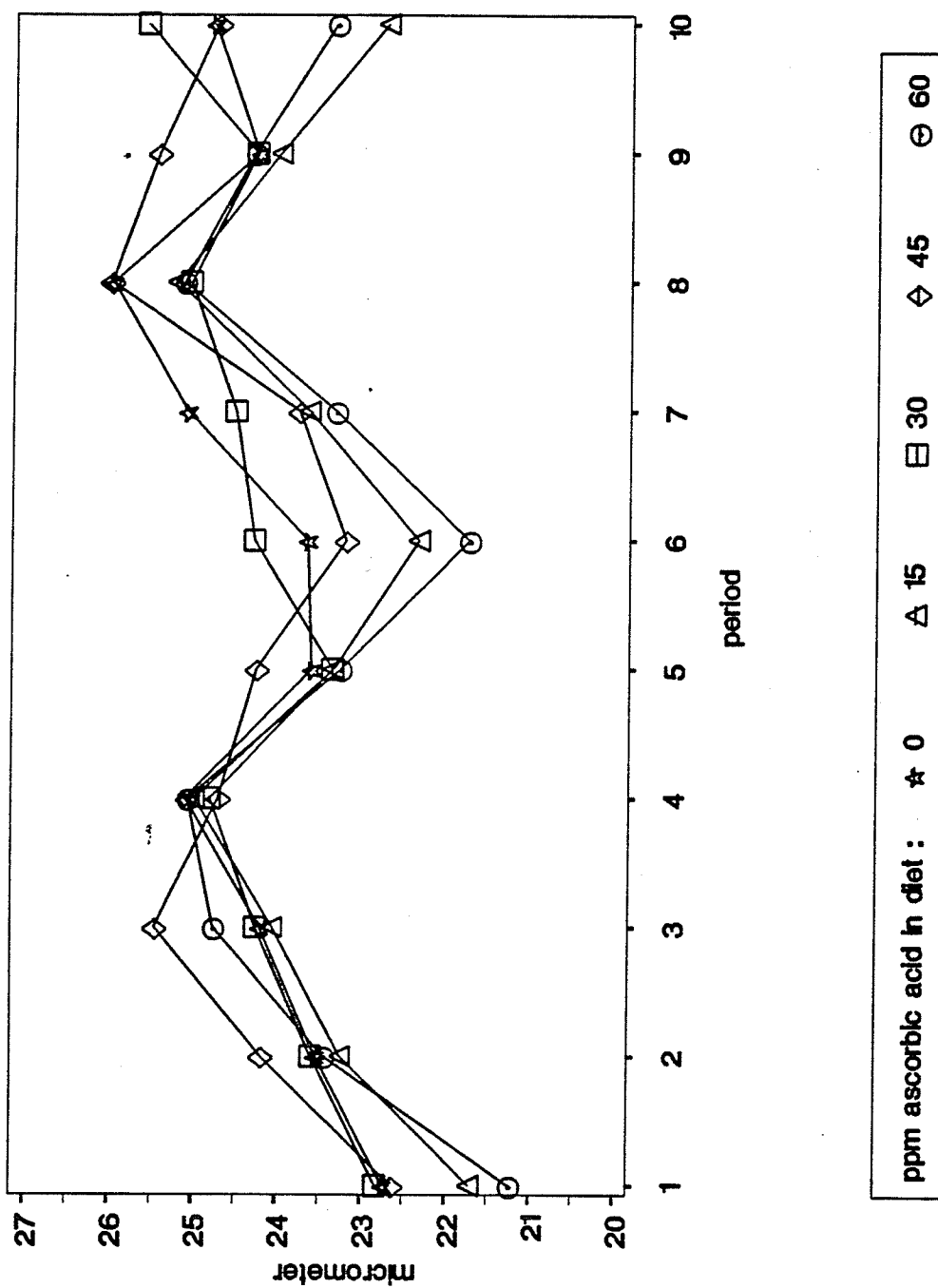


Figure 8. Mean eggshell elasticity, determined on a random sample of 16 intact eggs picked on two consecutive days at the end of each four-week period, for hens fed different dietary levels of synthetic L-ascorbic acid (Experiment 1).

(1962) who reported improvements in eggshell quality in hens when they were fed diets supplemented with 22 and 55 ppm AA, respectively. However, the current observation is in agreement with those made by Herrick and Nockels (1969) and Dorr and Nockels (1971) who reported a lack of response in eggshell quality to dietary AA supplements at concentrations of 2,600 ppm and 1,200 or 3,300 ppm, respectively, in hens under normal temperature. The current observation contradicts reports in which the level of AA used fell within the range tested while agreeing with reports where levels used were well in excess of the tested range. Apart from possible differences in environments in the different experiments, no other explanation can be given for these contradictions.

Mean body weight gains and percent mortality over the 40-week duration are given in Table 2. All treatment groups had gains in body weights and there were no significant differences between groups. A total of 3.3% mortality was recorded for the entire flock over the 40-week period. Of all hens that died, 90.5% died from laying related complications which included blow-outs, internal laying, fatty livers and one case of cage layer paralysis. There was no evidence that treatments had an influence on mortality. In work by Herrick and Nockels (1969) feeding diets with 2,600 ppm AA to pullets, under normal temperatures over a period of 196 days, did not affect body weight gain but improved survival rate. The level of AA used by these researchers is well in excess of the highest level used in the current study which might explain the difference in response in survival rate. It is also realised that mortality rate is influenced by many environmental factors, and reflection of a response in survival rate will in turn be affected by potential mortality. Thus, a situation which is not conducive to high mortality may fail to show a response in survival rate while one conducive to high mortality may. This is another possible explanation to the difference in observations between Herrick and Nockels (1969) and the current study.

TABLE 2. Mean body weight gains and mortality rate (Experiment 1)

<u>Treatment (mg AA¹/kg)</u>	<u>Weight gain (Kg)</u>	<u>%mortality</u>
0	.20	3.1
15	.24	1.6
30	.22	3.1
45	.22	4.7
60	.21	3.9

¹Ascorbic acid

It was concluded that temperatures in the current study had little effect on egg production. Production was mainly influenced by hen age, and supplementing diets with AA up to a level of 60 ppm did not affect feed intake, egg production, feed efficiency of egg production, egg weight, Haugh units, eggshell elasticity, body weight gains or mortality rate but tended to increase the proportions of fresh yolk. Hence under normal production conditions, in a temperate climate, no significant benefit in production was realised from dietary AA supplements of up to 60 ppm. However, the possibility of dietary AA influence on production and thus ability of hens to respond to dietary AA was suggested by the observed tendency toward an increase in yolk proportion. More research is necessary to verify the observed effect on yolk proportion and identify the components involved.

**MANUSCRIPT II: ASCORBIC ACID SUPPLEMENT IN DIETS
FED TO HENS UNDER HIGH AMBIENT TEMPERATURE**

ABSTRACT

Two experiments were conducted to study the effect of dietary ascorbic acid supplements on production in hens housed at high temperatures. In each experiment, twenty-four Shaver 288 hens in their first year of laying were housed in individual cages in a temperature-controlled chamber. In Experiment 2, hens were subjected to a cyclic temperature regime (25 C - 14 h, 35 C - 10 h) and fed one of four dietary treatments (wheat-soybean meal basal diet supplemented with 0, 20, 40 or 60 ppm ascorbic acid) in six replications of one hen each over a 14-day period. In Experiment 3, hens were subjected to a constant temperature of 35 C and fed one of three dietary treatments (wheat-soybean meal basal diet supplemented with 0, 60 or 600 ppm ascorbic acid) in eight replications of one hen each over a 14-day period. In each experiment, test diet feeding and exposure to high temperature followed a 14-day basal diet feeding and performance assessment period under normal temperature.

Exposure of ascorbic acid supplemented feed to 35 C over a 24 h period resulted in a possible maximum ascorbic acid oxidation of 14%. Exposing hens to cyclic temperatures had less severe detrimental effects on production than constant temperature even though feed intake was impaired to the same degree. Feed intake and eggshell quality were most susceptible to high temperature but the reduction in eggshell quality at high temperature was not entirely due to the reduction in feed intake. Dietary ascorbic acid supplement gave limited benefits in counteracting the heat induced decline in egg production but not feed intake, egg weight, eggshell strength and albumen quality. The results suggest that AA supplementation may minimize egg production declines associated with exposure to high temperatures.

KEY WORDS: ascorbic acid, supplement, hens, temperature.

INTRODUCTION

Chicken have no dietary requirement for vitamin C, endogenous biosynthesis of ascorbic acid (AA) being adequate in meeting metabolic needs under normal circumstances (Hart *et al.*, 1925). However, exposing chicken to stress situations results in reductions in tissue levels of AA (Freeman, 1967; Kechik and Sykes, 1979; Cheng *et al.*, 1990), a condition suggestive of a potential need for dietary supplements of the vitamin under such situations. Exposure to high temperature is one of the common sources of stress and is known to be detrimental to production in laying hens. Laying hens subjected to 35 C for as little as 3 days after keeping them at 18 C experienced decreases in feed consumption, egg production, egg weight and eggshell thickness (Tanor *et al.*, 1984). Literature reports on the effectiveness of AA supplements in laying hen diets in improving egg production or quality under high temperatures are inconsistent. Ahmad *et al.* (1967) reported benefits in egg production and eggshell thickness from supplementing 44 ppm AA in diets of hens subjected to temperature increments from 21 to 29.4 and 35.0 C. Somewhat in agreement with this report, Kechik and Sykes (1974) reported a numerical improvement in changes in egg yield and reductions in eggshell deformation and percent cracked eggs which were associated with exposure of laying hens to 32.2 and 33.3 C with dietary supplements of 100 or 500 ppm AA. El-Boushy *et al.* (1968) and El-Boushy and van Albada (1970) achieved improvements in eggshell quality by supplementing hens subjected to 29 C with 50 ppm AA, observations which agree in part with reports above. In more recent work, Cheng *et al.* (1990) reported an increase in Haugh units resulting from a supplement of 200 ppm AA in hens subjected to 31.1 C.

In contrast to reports above, a dietary supplement of 22 ppm AA had no effect on

either the rate of egg production or eggshell thickness (Harms and Waldroup, 1961) and 22 to 1,000 ppm sodium ascorbate had no appreciable effects on eggshell thickness (Heywang *et al.*, 1964) in hens reared in hot weather. Furthermore, dietary AA supplement at 44 ppm could not counteract the decrease in eggshell thickness associated with exposing hens kept at 21 C to a temperature of 29 C (Lyle and Moreng, 1968).

Supplementing AA in laying hen diets may be of interest in poultry production in warm tropical climates if such a practice will counteract some of the detrimental effects induced by heat stress. The current study was conducted to further study the effect of high ambient temperature on laying hens and to investigate the response to dietary AA in egg production and egg characteristics.

MATERIALS AND METHODS

In each of two experiments, twenty-four Shaver 288 hens in their first year of laying, selected for high laying activity and good eggshell quality, were placed in individual cages constructed in a temperature-controlled chamber.¹ Water was supplied by automatic watering cups connected to a tank outside the chamber and lights were set to provide 14 h of light. The chamber temperature was maintained at 25 C and hens were allowed a 14-day adaptation period during which they were fed a wheat-soybean meal diet (Table 3). Following the adaptation period, hens were continued on the basal diet and production performance and egg characteristics assessed over another 14 days. Feed was stored at room temperature in

¹ENCONAIRE systems Ltd. Winnipeg, Canada.

TABLE 3. Composition of the basal diets (Experiments 2 and 3)

Ingredient	Percent of diet	
	Experiment 2	Experiment 3
Ground wheat	72	74
Soybean meal	14	13
Alfalfa meal	2	-
Tallow	1.5	2
Dicalcium phosphate	.6	1.2
Limestone	5.9	5.8
Oystershell	2.5	2.5
Vitamin premix ¹	1	1
Mineral premix ²	.5	.5
Calculated analysis:		
ME (Kcal/kg)	2963	2794
Crude protein (%)	16.8	16.9
Lysine (%)	.83	.71
Methionine (%)	.31	.28
Calcium (%)	3.40	3.49
Available Phosphorus (%)	.30	.33

¹Vitamin premix formulated to supply per kilogram of diet: 8250 IU vitamin A; 1000 IU vitamin D₃; 0.0112 mg vitamin B₁₂; 5.46 IU vitamin E; 500 mg DL-methionine; 2.2 mg riboflavin; 4.4 mg Ca-pantothenate; 6.6 mg niacin and 95.5 mg choline.

²Mineral premix formulated to supply 99 mg Mn; 40 mg Zn and 4.78 g NaCl(.007% I) per kilogram of diet.

a room adjacent to the chamber and was only exposed to the chamber temperature during the 24 hours of feeding time.

Ascorbic acid determination

The concentration of AA in samples was determined by spectrophotometry on a, Gilford² model 2400, spectrophotometer. Plasma was obtained from blood samples by centrifuging at 10,000 g for 15 minutes at 4 C. The extraction and determinations were carried out immediately using the procedure described by Sauberlich *et al.* (1976) modified to suit available laboratory equipment. Ascorbic acid was extracted from plasma samples with chilled 1.5% metaphosphoric acid on ice for 10 minutes and clear extracts obtained by centrifuging at 15,000 g for 15 minutes at 4 C. Extracts were buffered to pH 2.3 with a sodium citrate buffer and then reacted with 2,6-dichloroindophenol. The chemical reaction of the test is given in Appendix 4. The absorbance of the resulting mixture was read at 518 nm within a minute of initiating the reaction. The concentration of AA in samples was then determined from a standard curve of known concentrations. In feed analyses, the procedure was applied to extracts obtained by filtering feed samples washed with chilled 1.5% metaphosphoric acid. The absorption spectrum of 2,6-dichloroindophenol dye at acid pH and two sample standard curves are given in the Appendices 3 and 5, respectively.

²*Gilford Instruments, Oberlin, Ohio, USA.*

Experiment 2

Experiment 2 was conducted to study the effect of dietary AA supplement on egg production and eggshell quality in hens subjected to cyclic high temperature. After performance assessment at 25 C, individual hens were randomly assigned to one of four basal diet portions supplemented with 0, 20, 40 or 60 ppm AA³ giving a total of six replications per treatment. Chamber temperature was set at 35 C (at approximately 30% relative humidity) for 10 h during light hours and 25 C during the rest of the day. The temperature was set to rise at first light reaching 35 C within 30 minutes and took approximately 30 minutes to fall to 25 C after the 10 h of high temperature exposure. Cyclic temperature exposure and feeding of test diets was carried out over a period of 14 days, new feed being added to feeders every morning to minimize potency loss of the vitamin due to exposure to high temperature. Daily feed intake and egg production were recorded and all eggs produced were weighed. Eggshell elasticity at the equator region of the egg was determined on all intact eggs that had no visible cracks using a Marius deformation apparatus.⁴ At the end of the test ration feeding period, blood samples were collected by cardiac puncture and immediately placed in heparinized vacutainers standing on ice for individual bird determinations of plasma AA.

³*L-ascorbic acid crystals, Lot No. 31902. Colborn-Dawes (Hoffmann-La Roche).
Cambridge, Canada.*

⁴*Marius Instruments, Utrecht, Holland.*

Experiment 3

Experiment 3 was conducted to study the effect of dietary AA supplement on egg production, eggshell quality and interior egg characteristics in hens subjected to constant high temperature. Individual hens were randomly assigned to one of three basal diets supplemented with 0, 60 or 600 ppm AA, giving a total of eight replications per treatment after a 14-day pre-test period at 25 C. The chamber temperature was set at a constant 35 C (approximately 30% relative humidity) for the entire 14-day test period. Prior to the beginning of the experiment, blood samples were collected by cardiac puncture and immediately placed on ice in heparinized vacutainers for plasma AA determinations. After feeding test diet, all hens were fed the basal diet, the chamber temperature was reduced to 25 C and performance was further assessed for a week. Daily feed intake, egg production, egg weights and eggshell elasticity were assessed as described in Experiment 2. All eggs were broken for albumen height measurements, using an Albumen Height Gauge⁵, and yolk weight determinations. Haugh unit score, an index for albumen firmness was calculated from the formula: $Hu = 100 \times \log_{10} [\text{albumen height}(\text{mm}) - (5.675 \times (30 \times \text{egg weight}(\text{g})^{.37} - 100)) \div 100 + 1.9]$ (Haugh, 1937). After separation from albumen, yolks were rolled on paper towels to ensure maximum removal of albumen before weighing. To assess the extent of oxidation of AA in feed after a 24 h exposure to 35 C, samples of feed were analyzed for AA content before and after exposure and percent oxidation estimated.

Both experiments were conducted in accordance with the principles and guidelines presented in the "Guide to the Care and Use of Experimental Animals."⁶ Statistical analysis

⁵*Queensboro Instruments, Ottawa, Canada.*

⁶*Canadian Council on Animal Care, vol.2, 1984.*

for production and egg parameters was carried out in a split-plot design with birds as the main plot and week of assessment as the subplot (SAS, 1986), all tests of significance being made at $P \leq .05$.

RESULTS

Exposure of laying hens to both cyclic and constant high temperature resulted in changes in hen behaviour which included a reduction in activity, spreading of wings, increased water intake and panting, all of which tended to be most evident in the early days of exposure. Results of production parameters are given in plots where phase 0 represents the pre-test period, phases 1 and 2 are the two weeks of test diet feeding and phase 3 is the post-test period.

Experiment 2

Exposing laying hens to a cyclic 10 h 35 C temperature during light hours and 14 h 25 C temperature during the rest of the day resulted in significant reductions in feed intake in all treatment groups. The addition of AA to the diet up to a maximum of 60 ppm had no significant effect on feed intake (Figure 9). Egg production, egg weight and egg mass were not significantly affected by either exposure to cyclic high temperature or the addition of AA to the diet (Figures 10, 11 and 12, respectively). Eggshell elasticity was significantly increased when hens were exposed to high temperature but there was no significant difference among treatment groups (Figure 13).

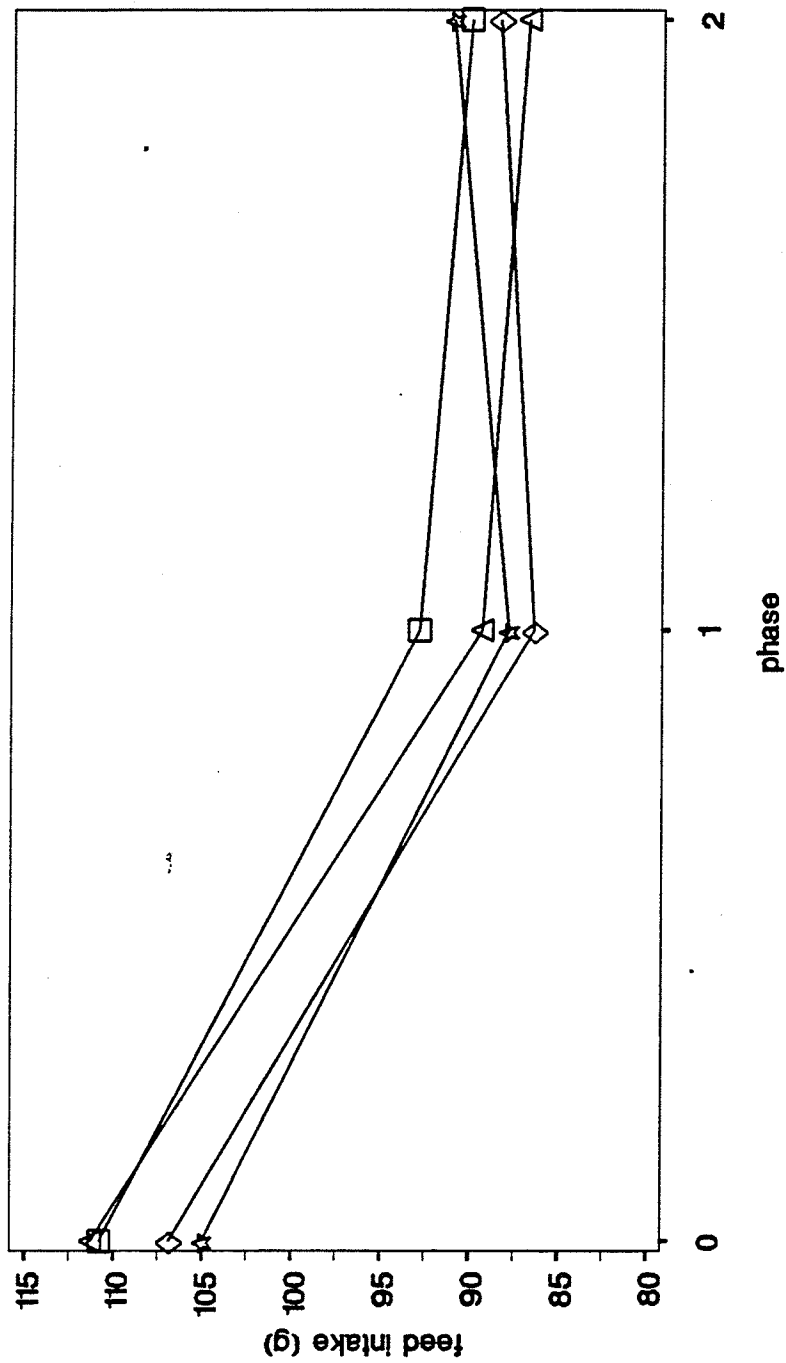


Figure 9. Mean feed intake (g/hen/day) at 25 C during basal-diet feeding (phase 0) and at a cyclic 35 C (10 h) - 25 C (14 h) when treatment groups were fed diets containing different levels of synthetic L-ascorbic acid (phases 1 & 2). A phase represents a 7-day period (Experiment 2).

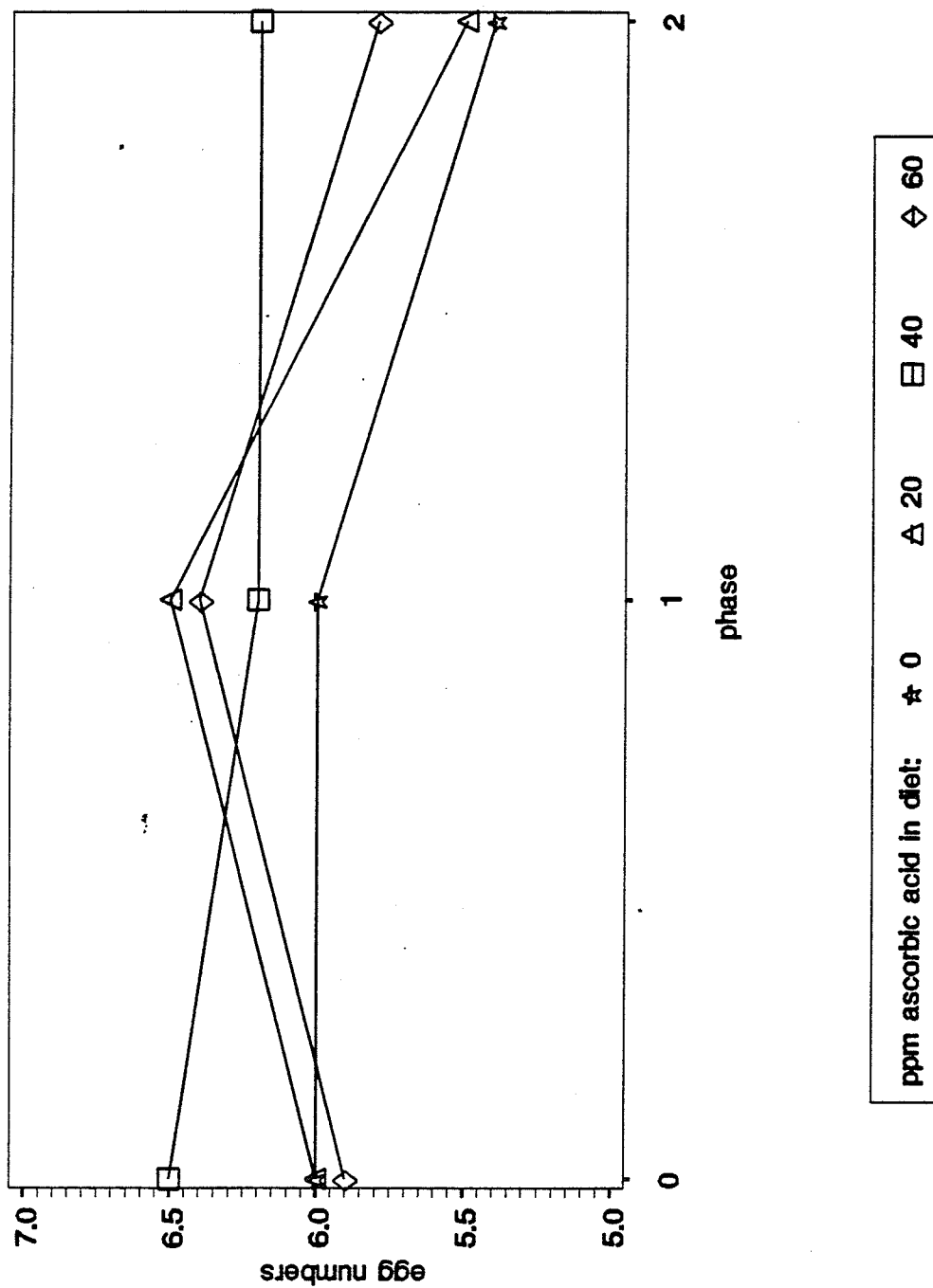


Figure 10. Mean egg numbers per hen at 25 C during basal-diet feeding (phase 0) and at a cyclic 35 C (10 h) - 25 C (14 h) when treatment groups were fed diets containing different levels of synthetic L-ascorbic acid (phases 1 & 2). A phase represents a 7-day period (Experiment 2).

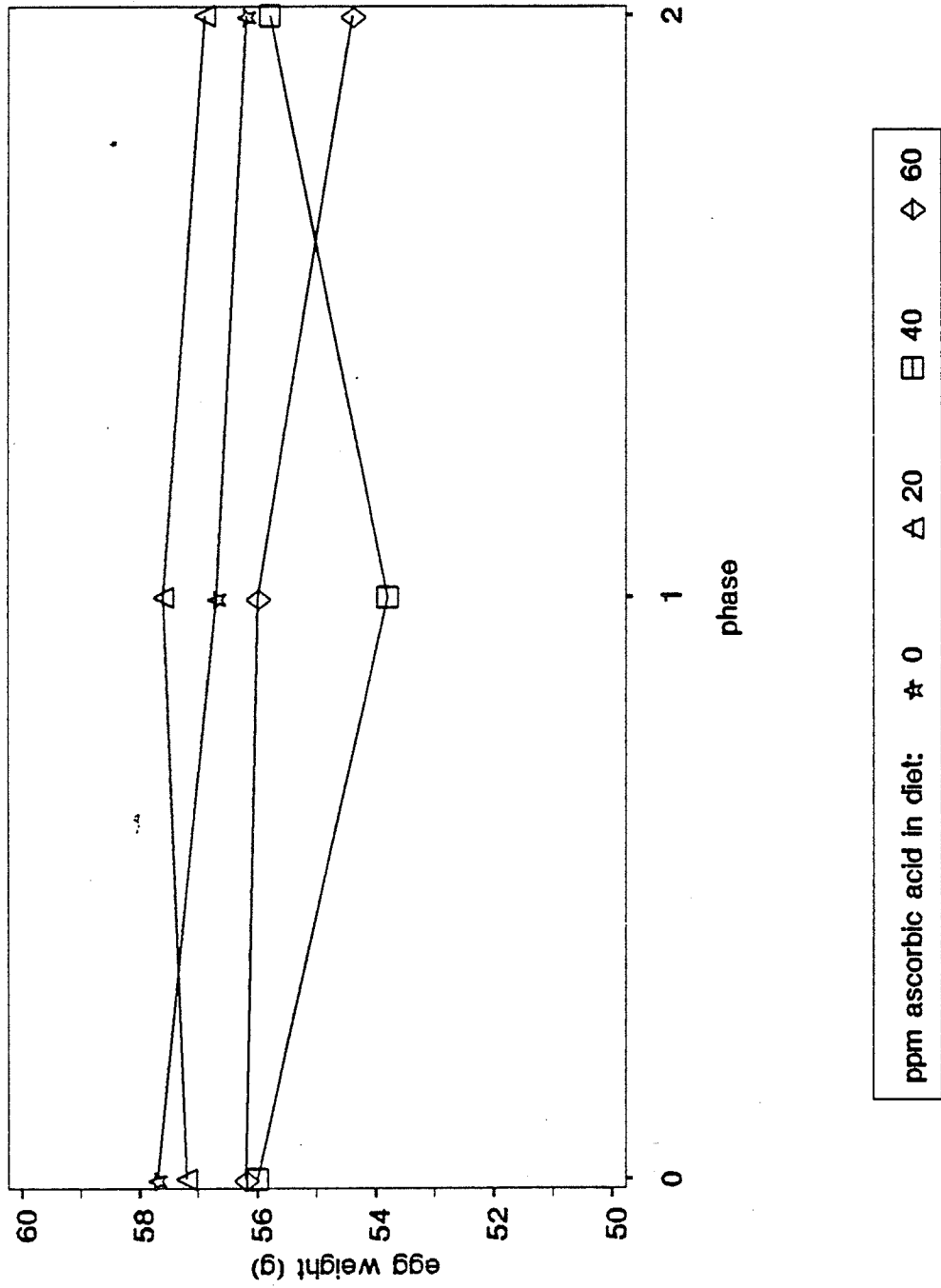


Figure 11. Mean egg weight at 25 C during basal-diet feeding (phase 0) and at a cyclic 35 C (10 h) - 25 C (14 h) when treatment groups were fed diets containing different levels of synthetic L-ascorbic acid (phases 1 & 2). A phase represents a 7-day period (Experiment 2).

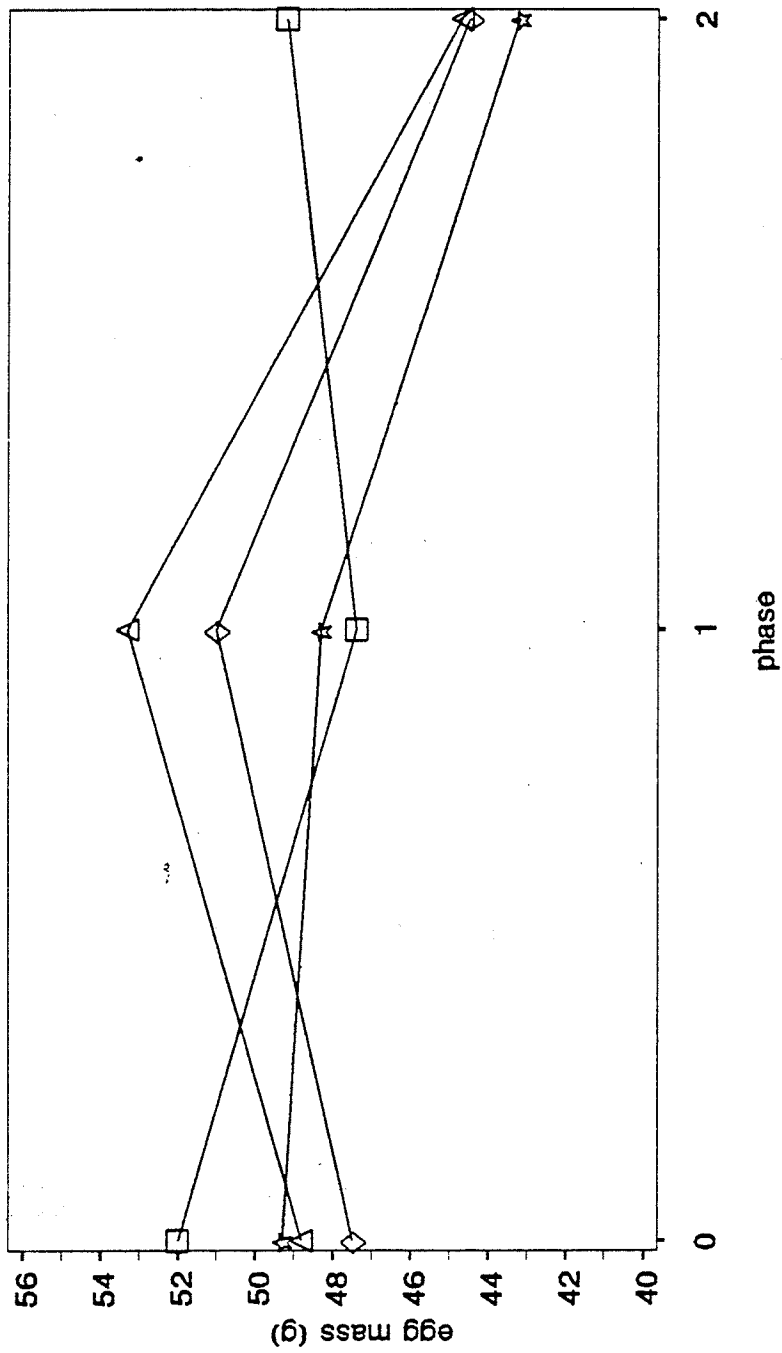


Figure 12. Mean daily egg mass per hen at 25 C during basal-diet feeding (phase 0) and at a cyclic 35 C (10 h) - 25 C (14 h) when treatment groups were fed diets containing different levels of synthetic L-ascorbic acid (phases 1 & 2). A phase represents a 7-day period (Experiment 2).

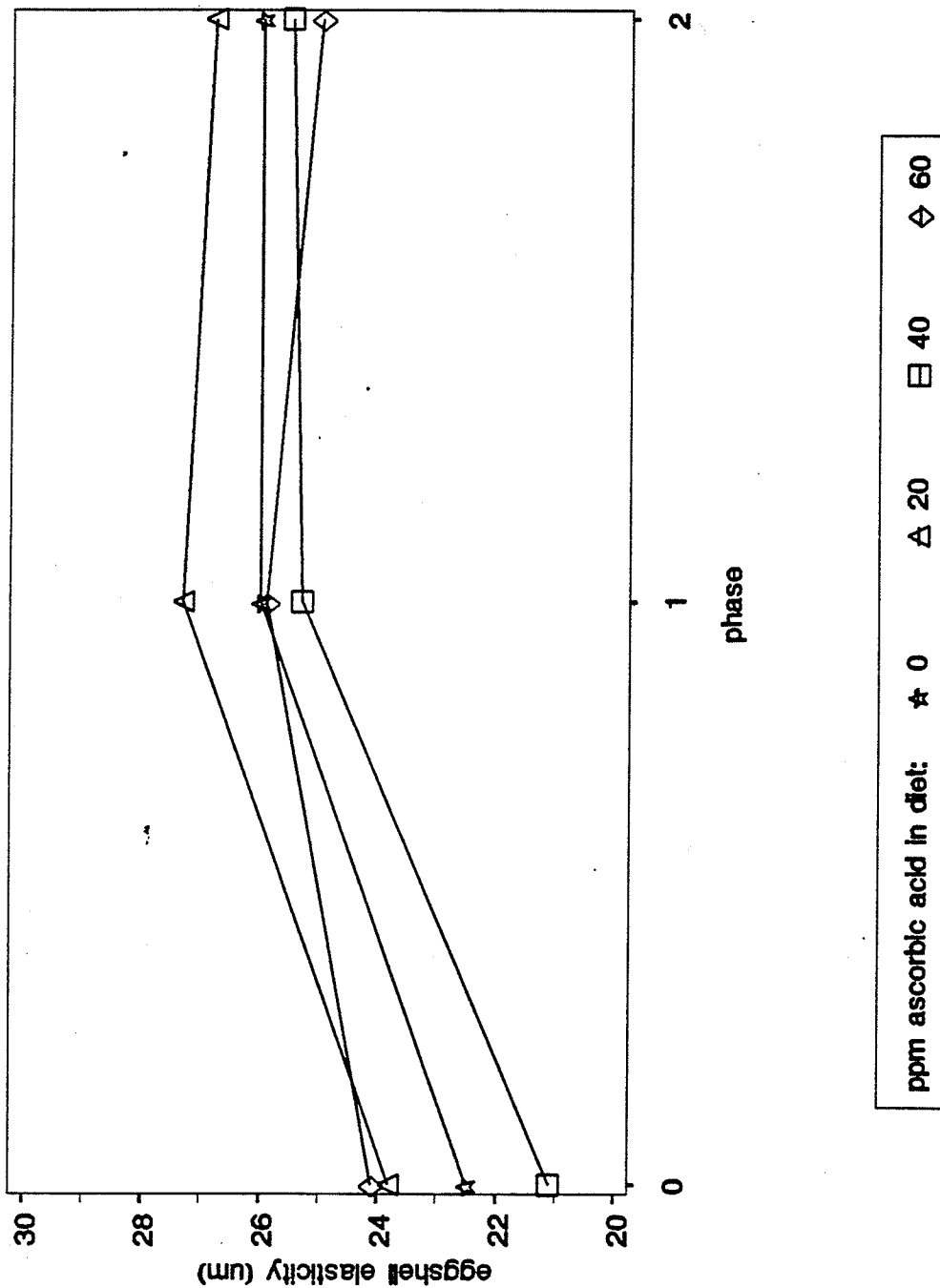


Figure 13. Mean eggshell elasticity at 25 C during basal-diet feeding (phase 0) and at a cyclic 35 C (10 h) - 25 C (14 h) when treatment groups were fed diets containing different levels of synthetic L-ascorbic acid (phases 1 & 2). A phase represents a 7-day period (Experiment 2).

In the AA determination procedure, the applicability of the spectrophotometer available in detecting 2,6-dichloroindophenol light absorption and peak absorbance at acid pH were tested. Appendix 3 shows that maximum absorbance was achieved at around 518 nm, the wavelength reported in literature as appropriate for the determination. Two sample standard curves of concentration ranges 0 to 70 μg AA per ml are given in Appendix 5. Curves were obtained from one standard preparation run at two different times with the same dye preparation. The shift in the curve was due to a difference in light detection by the spectrophotometer at two different times. One plasma extract determined in 15 repeated runs gave a mean AA concentration of $9.43 \pm .33$ μg per millilitre. Although the accuracy of the method was not tested, it was considered adequate to reflect changes in AA due to dietary supplements. Determinations of plasma AA gave mean values of 24 ± 4 , 47 ± 5 , 53 ± 6 and 54 ± 2 μg per ml for controls, 20, 40 and 60 ppm AA diets, respectively.

Experiment 3.

High levels of plasma AA in controls of Experiment 2 necessitated further determination of plasma AA in hens prior to feeding of the test diets. Eleven plasma samples taken from eleven different hens at 25 C gave a mean plasma AA concentration of $5.45 \pm .1$ μg per ml. Exposing laying hens to a constant 35 C resulted in significant reductions in feed intake with only a moderate recovery in the week after exposure. The addition of AA to the diet up to a level of 600 ppm had no significant effect on feed intake (Figure 14). High temperature caused significant reductions in egg production, both as numbers and egg mass, while AA had no significant effect on either parameters (Figure 15 and 16, respectively). Production however tended to be numerically less in the control group during the weeks of

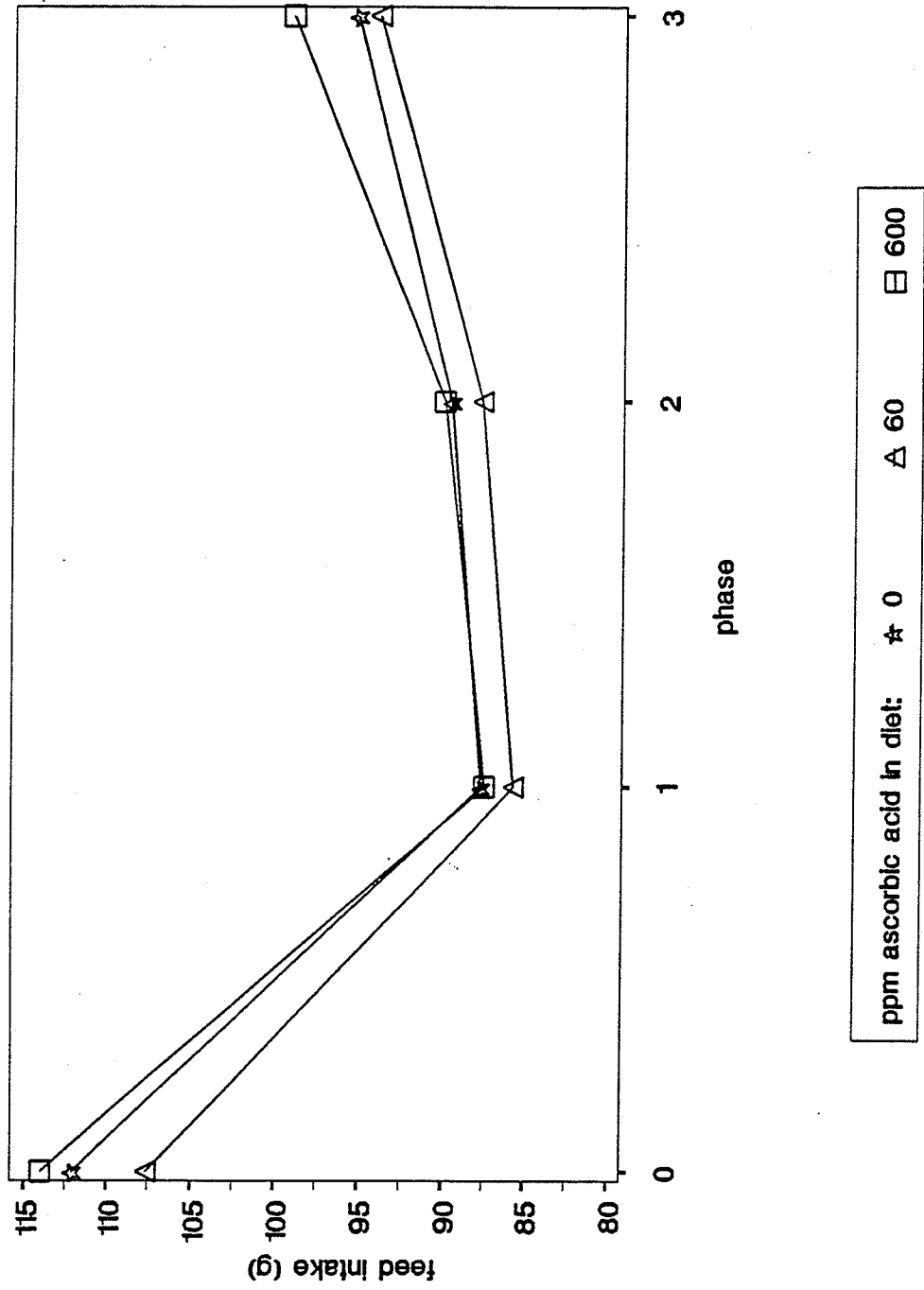


Figure 14. Mean feed intake per hen per day at 25 C during basal-diet feeding (phase 0), at constant 35 C when treatment groups were fed diets containing different levels of synthetic L-ascorbic acid (phases 1 & 2) and at 25 C during basal-diet feeding post supplementation (phase 3). A phase represents a 7-day period (Experiment 3).

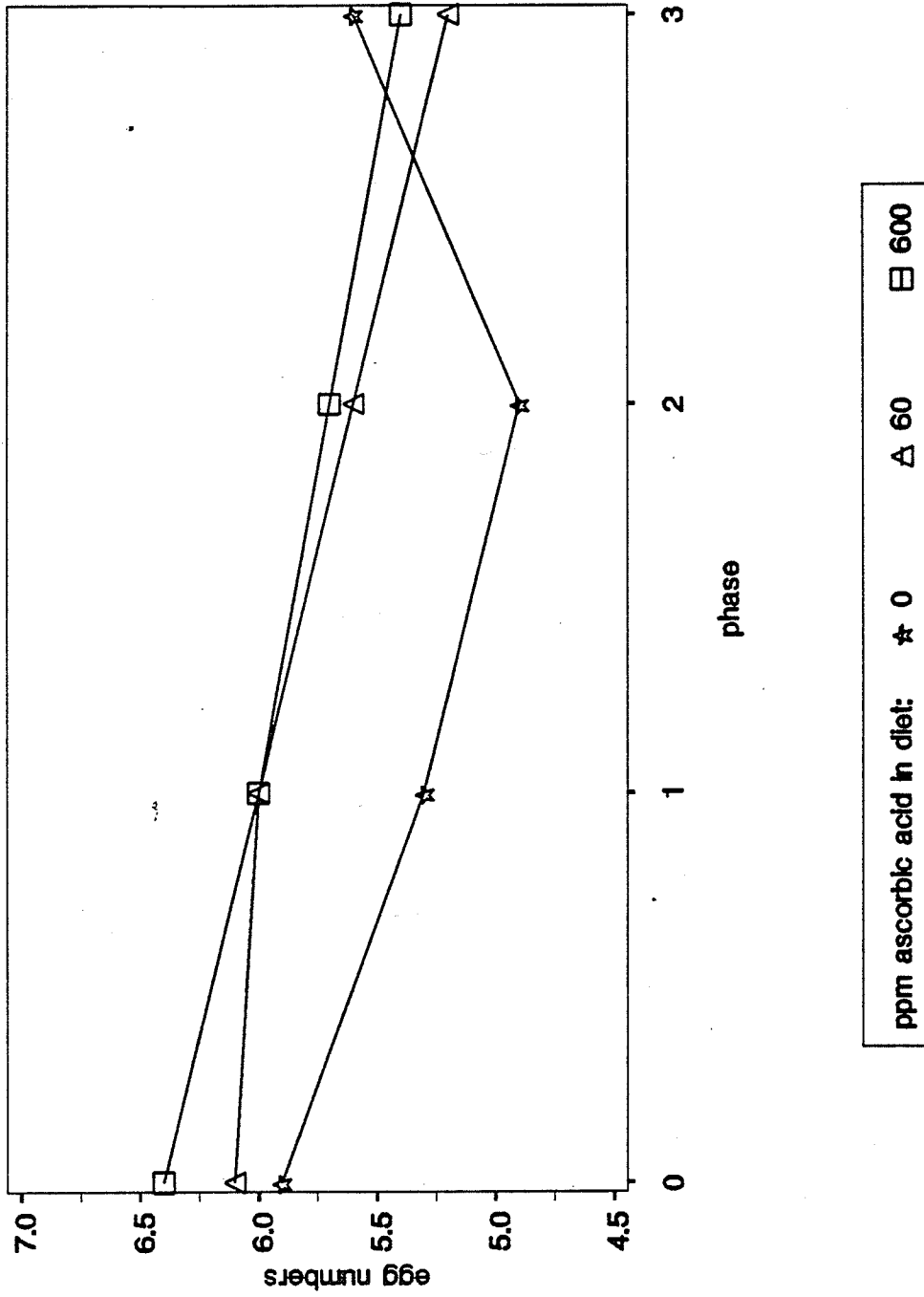


Figure 15. Mean egg numbers per hen at 25 C during basal-diet feeding (phase 0), at constant 35 C when treatment groups were fed diets containing different levels of synthetic L-ascorbic acid (phases 1 & 2) and at 25 C during basal-diet feeding post supplementation (phase 3). A phase represents a 7-day period (Experiment 3).

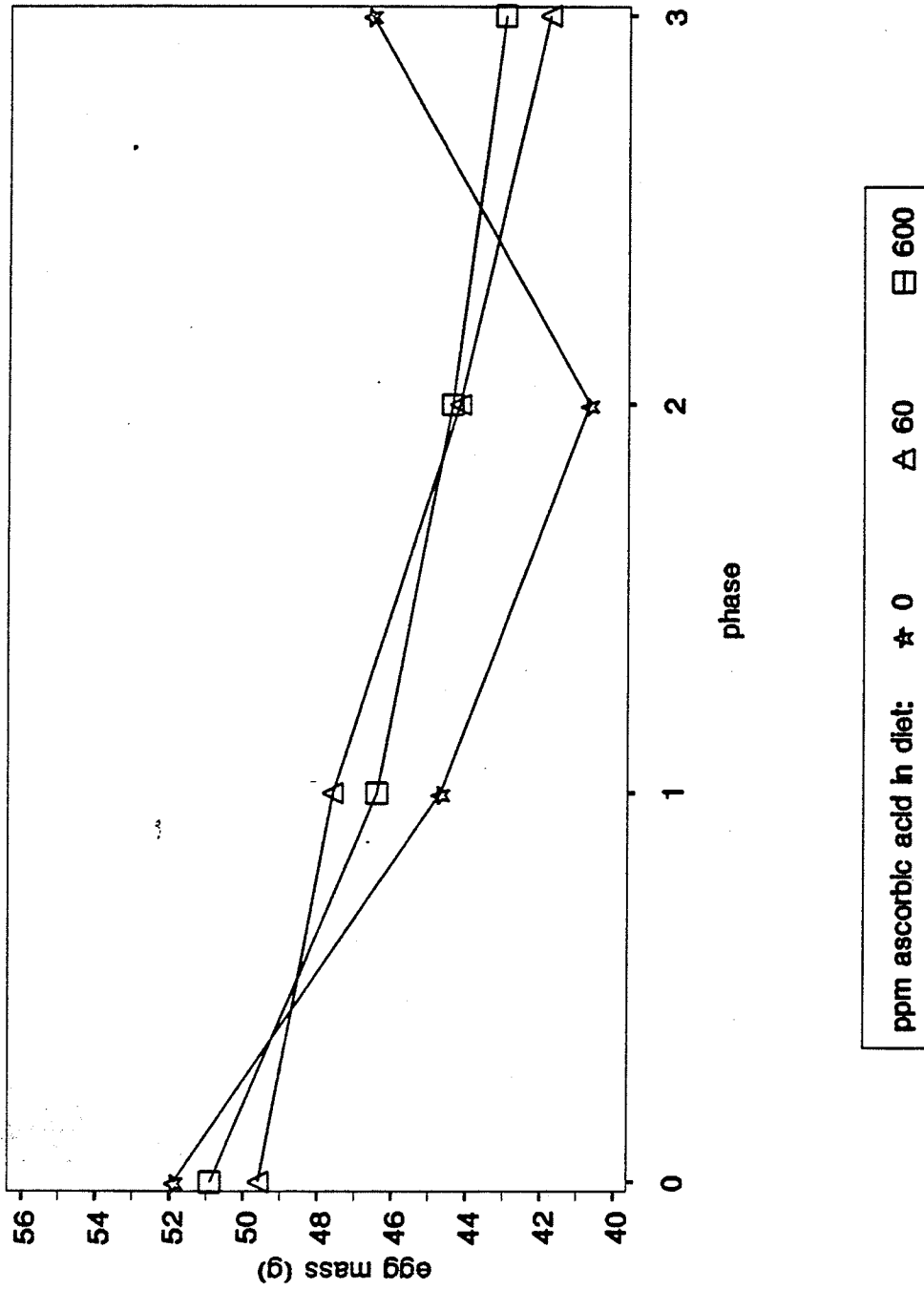


Figure 16. Mean daily egg mass per hen at 25 C during basal-diet feeding (phase 0), at constant 35 C when treatment groups were fed diets containing different levels of synthetic L-ascorbic acid (phases 1 & 2) and at 25 C during basal-diet feeding post supplementation (phase 3). A phase represents a 7-day period (Experiment 3).

exposure to high temperature compared to hens on 60 and 600 ppm AA. Numerical ranking also suggests that hens on the control recovered in the week after exposure while supplemented groups had a continuing decline.

Egg weight was significantly reduced by exposure to high temperature, there being no noticeable recovery in the week post-exposure (Figure 17). The addition of AA to the diet had no significant effect on egg weight. Eggshell elasticity was significantly increased by exposure to high temperature but showed a numerical improvement in the second week and complete recovery was achieved within the first week after exposure (Figure 18). No significant effect on eggshell elasticity was achieved from the addition of AA to the diet. Haugh unit score was significantly reduced by exposure to high temperature, reductions being numerically greater in the second week of exposure (Figure 19). Only the control group achieved complete recovery in the week after exposure to high temperature. In contrast, the group on 60 ppm AA only had a numerical improvement over the second week mean while those on 600 ppm had a continuing decline. The addition of AA to the diet had no significant effect on Haugh unit score in hens exposed to high temperature. The proportion of fresh yolk to whole egg significantly increased with exposure to high temperature and declined in the week after exposure (Figure 20). The addition of AA to the diet had no significant effect on proportions of fresh yolk to whole egg.

Analysis of feed samples showed that exposing AA supplemented feed to 35 C for a 24 h period resulted in a maximum possible AA oxidation of only 14%.

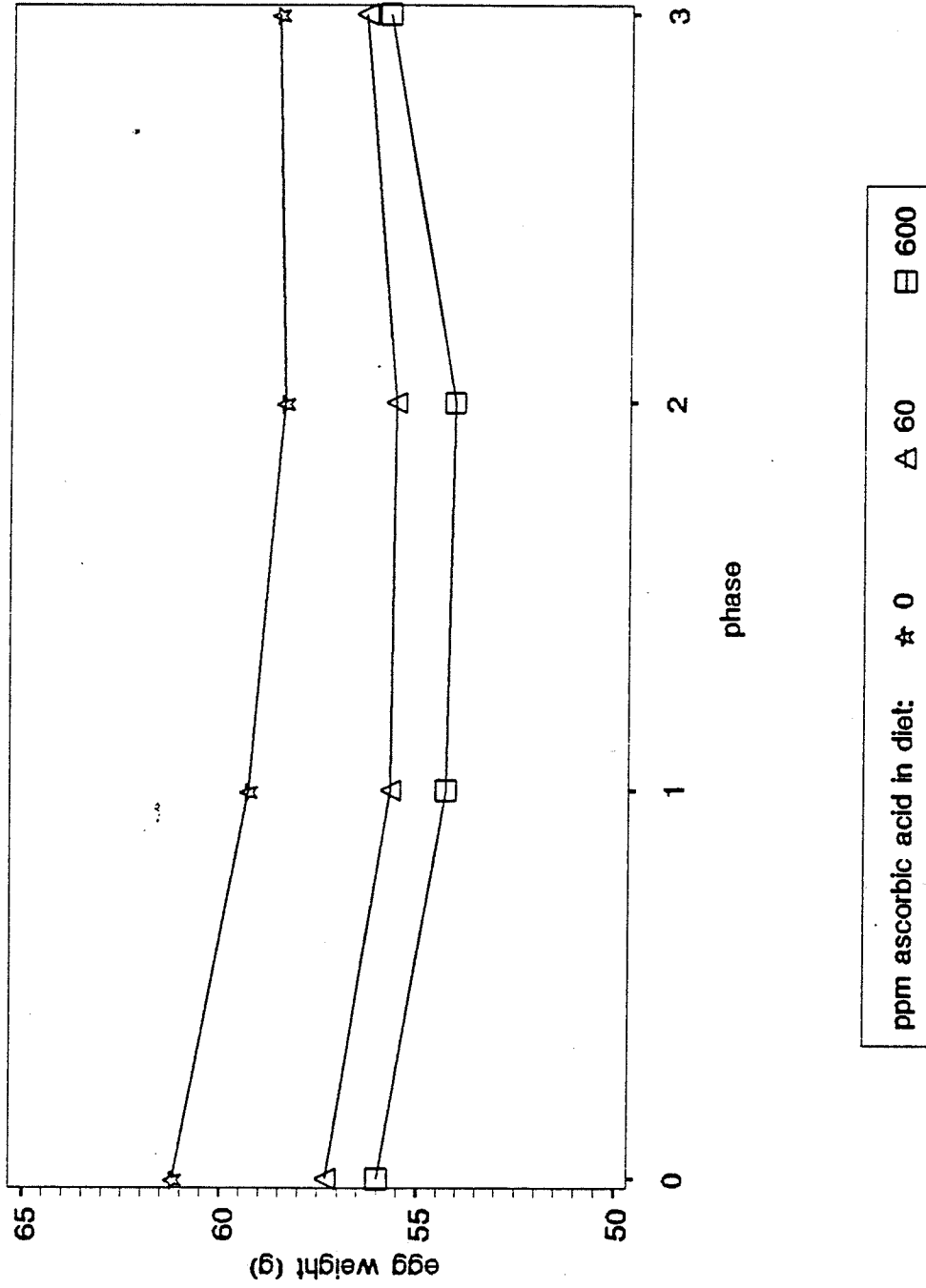


Figure 17. Mean egg weight at 25 C during basal-diet feeding (phase 0), at constant 35 C when treatment groups were fed diets containing different levels of synthetic L-ascorbic acid (phases 1 & 2) and at 25 C during basal-diet feeding post supplementation (phase 3). A phase represents a 7-day period (Experiment 3).

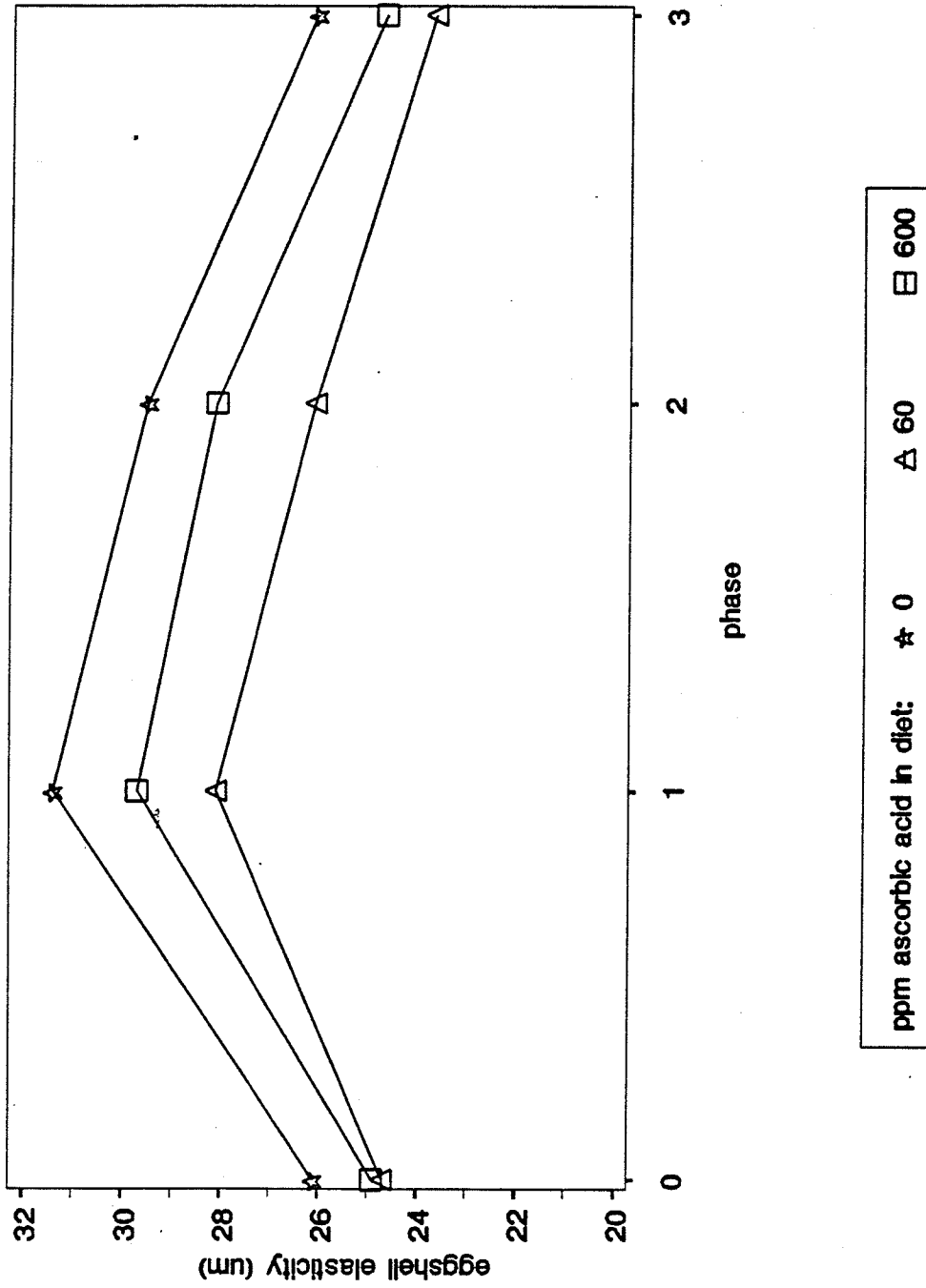


Figure 18. Mean eggshell elasticity at 25 C during basal-diet feeding (phase 0), at constant 35 C when treatment groups were fed diets containing different levels of synthetic L-ascorbic acid (phases 1 & 2) and at 25 C during basal-diet feeding post supplementation (phase 3). A phase represents a 7-day period (Experiment 3).

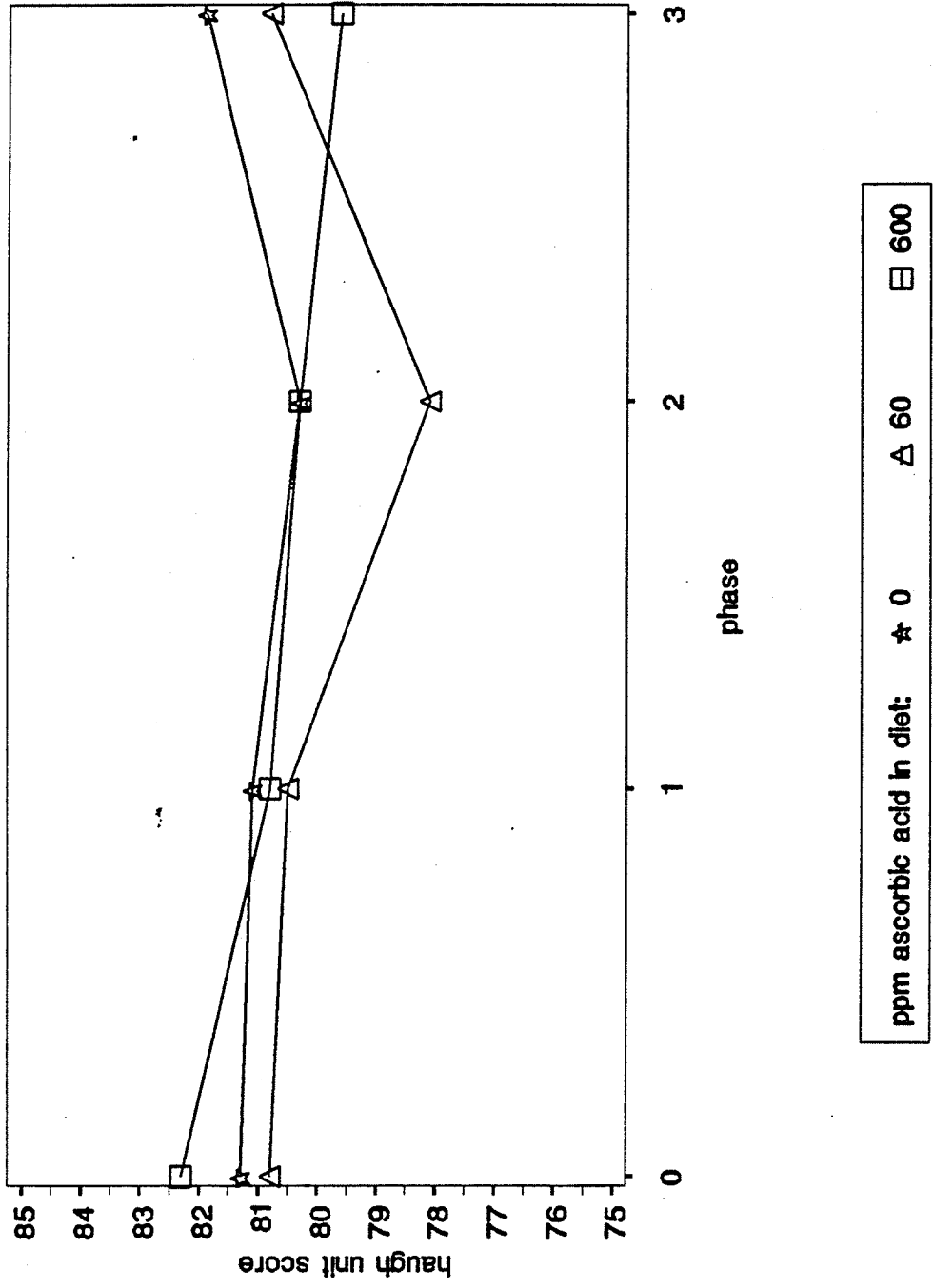


Figure 19. Mean Haugh unit score at 25 C during basal-diet feeding (phase 0), at constant 35 C when treatment groups were fed diets containing different levels of synthetic L-ascorbic acid (phases 1 & 2) and at 25 C during basal-diet feeding post supplementation (phase 3). A phase represents a 7-day period (Experiment 3).

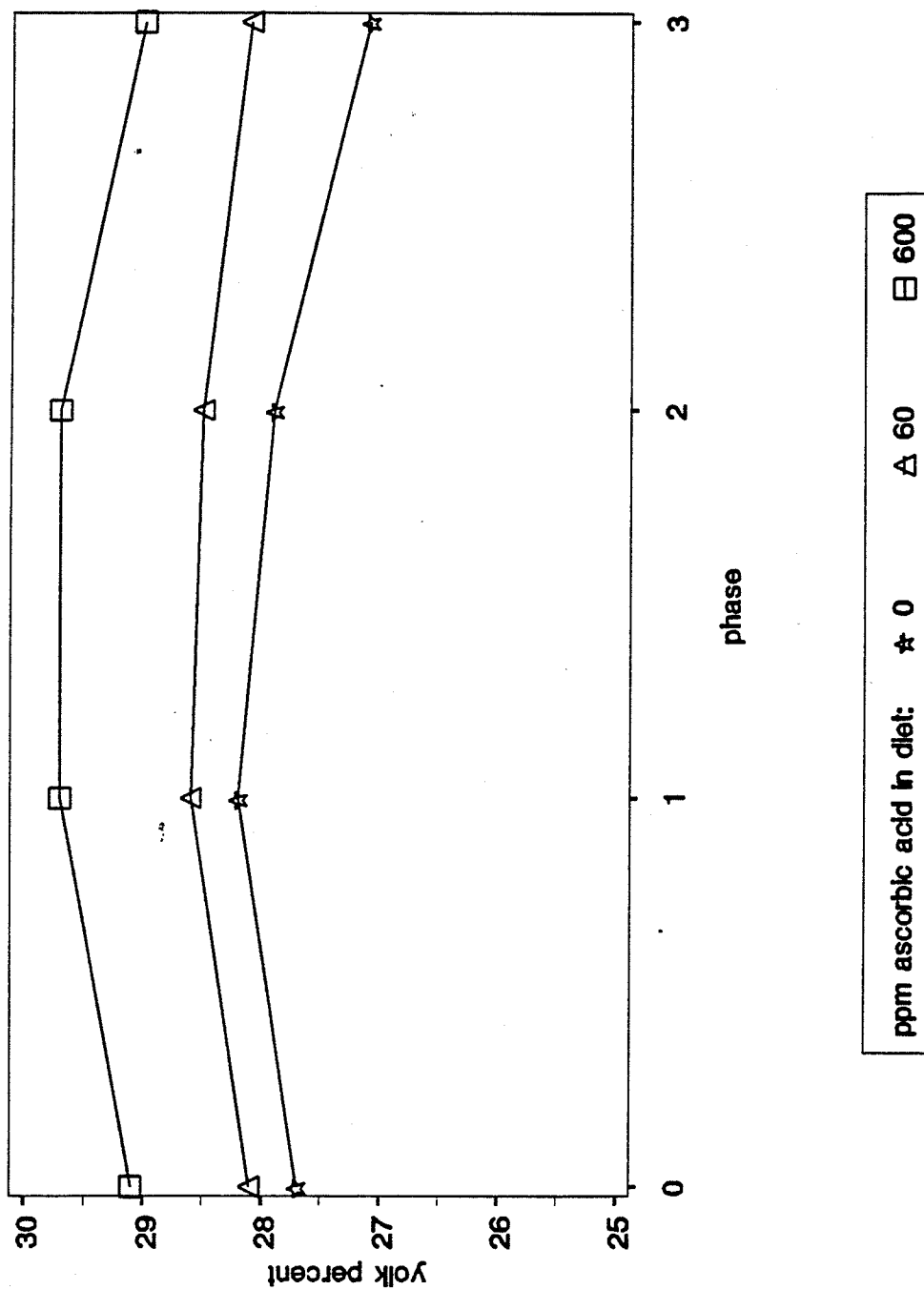


Figure 20. Mean percent fresh yolk to whole egg at 25 C during basal-diet feeding (phase 0), at constant 35 C when treatment groups were fed diets containing different levels of synthetic L-ascorbic acid (phases 1 & 2) and at 25 C during basal-diet feeding post supplementation (phase 3). A phase represents a 7-day period (Experiment 3).

DISCUSSION

Exposing laying hens to a cyclic temperature had less severe detrimental effects than constant temperature even though high temperature coincided with light hours, the period of active feeding. Cyclic temperatures had similar detrimental effects as a constant temperature on feed intake and eggshell quality but egg production, egg weight and interior egg quality were only affected by constant high temperature. El-Boushy and van Albada (1970) found fluctuating temperature (19.4 - 29.4 C) had no effect on shell quality while a constant 29.4 C reduced shell quality. de Andrade *et al.* (1977) reported that cyclic temperatures (26.7 - 35.6 C) had a milder effect than a constant 31 C temperature on egg production and eggshell thickness, although effects on feed intake were similar. Somewhat in agreement with reports above, Deaton *et al.* (1986) reported a 24 - 35 C linear temperature cycle to have no effect on eggshell quality and egg production although it caused reductions in feed intake. From reports cited above and the observation made in the current study, it appears obvious that cyclic temperatures have less detrimental effects in laying hens than constant temperatures and detriments induced by high temperature are not totally due to a reduction in feed intake.

Changes in feed intake and eggshell elasticity under both cyclic and constant temperature in the current study show the sensitivity of the two parameters to elevated temperature. Jones *et al.* (1976) reported reductions in feed intake within a day of exposure to high temperature and Miller and Sunde (1975) reported a reduction in shell quality within hours of exposure to high temperature, observations which further suggest greater sensitivity of the two parameters. In the current study, hens exposed to constant high temperature showed a moderate recovery in eggshell quality in the second week of exposure. This is an indication of partial acclimation, a greater reduction in egg production or combination of the

two. Complete recovery in eggshell quality was achieved within the first week after exposure to high temperature, although recovery in feed intake was not complete. The lack of parallel trends in feed intake and eggshell quality suggests that the reduction in eggshell quality under high temperature is not directly related to the reduction in feed intake.

In agreement with the current finding, Miller and Sunde (1975) reported an immediate reduction in eggshell quality in hens exposed to high temperature and concluded that the change was not directly related to feed intake. They suggested that the cause to poor shells was either unrelated to feed intake or dependent on another factor or factors along with feed intake. The above observations are in agreement with those made by Emery *et al.* (1984). They reported that pair feeding hens at a constant 23.9 C temperature with those at cyclic temperatures of 15.6 to 37.7 C and 21.1 to 37.7 C resulted in heavier eggs with thicker shells. They concluded that reductions in egg weights and eggshell thickness under cyclic temperatures were not simply a result of reduced feed intake but was also due to a direct effect of heat stress.

Although not statistically significant, results in the current study suggest that supplements of AA gave limited benefits in sustaining egg production under constant high temperature. This observation is in agreement with those by Ahmad *et al.* (1967) and Kechik and Sykes (1974) who reported benefits from AA supplements in egg production for hens exposed to high temperature. However, Harms and Waldroup (1961) and Cheng *et al.* (1990) did not achieve this response. The concentration of AA in work by Cheng *et al.* (1990) falls within the range of concentrations at which response was achieved in reports above, but that for Harms and Waldroup (1961) was below the range. While the difference between the reports above and the report by Harms and Waldroup (1961) may be explained by differences in levels of AA, that by Cheng *et al.* (1990) can not. It should however be noted that dietary

need of the vitamin is assumed to be dependent on conditions as it relates to stress such that response and ultimately the optimum level to induce a response may also be condition dependent. In the current study, hens that had no AA supplement had a numerical recovery in egg production in the week following heat exposure while hens which were supplemented during heat exposure had a decline in production. This in a way further suggests a response to AA in egg production, and probably endogenous-exogenous vitamin balance. The exogenous supplies may have suppressed endogenous synthesis and caused in low endogenous synthesis at withdrawal time. Similarly, if AA improves Haugh unit scores as reported by Cheng *et al.* (1990), a response not achieved in the current study, then low AA status could result in poor Haugh unit scores and hence observed recovery pattern in the current study. Therefore, the observed effect would also suggest impairment of endogenous AA synthesis in supplemented hens during the withdrawal period.

Contrary to reports by Ahmad *et al.* (1967), El-Boushy *et al.* (1968), El-Boushy and van Albada (1970) and Kechik and Sykes (1974), supplemental AA did not counteract the decline in eggshell quality induced by high temperature. The ranges of AA concentrations used in the above reports (44 to 500 ppm) fall within the range of concentrations used in the current study (20 to 600 ppm) so that the concentration of AA was not likely to be a factor in this observed difference. Apart from possible differences in conditions in different environments, no explanation can be given for the differences in eggshell quality response between the current study and the reports above. The proportion of fresh yolk to whole egg tended to be greater in hens subjected to high temperature most probably due to greater water loss from albumen at high temperature.

Plasma AA levels in the controls of Experiment 2 appeared to be higher than expected considering the ranges (7.7 to 19.1 μg) that were reported in the literature (Pardue

and Thaxton, 1986). While the effect of dietary supplement was reflected, there was no significant difference between supplemented diets. The observation that a 24 h exposure to 35 C resulted in a maximum possible oxidation of AA of only 14% implies that most of the supplied AA was ingested in a potent form. It was suspected that dehydrated alfalfa in the basal diet contributed considerable amounts of the vitamin and must have contributed to high plasma levels in Experiment 2. This explanation is supported by lower AA values in the plasma of hens in experiment 3 which were fed a basal diet that did not contain dehydrated alfalfa. Assuming that the absorption of AA in the chicken is a passive process, as was reported for the rat (Stevenson and Brush, 1969), the lack of differences in plasma AA in hens supplemented with different amounts of AA probably indicate greater oxidation and/or excretion of the vitamin at high plasma concentration. Fear of causing a disturbance and possibly mortality prevented determination of plasma AA after the feeding of the test diet in Experiment 3 since production performance was intended to be monitored for a further seven days.

Results in the current study show that a cyclic temperature has a less severe effect than a constant temperature on production in laying hens even if feed intake is impaired to the same degree. During exposure to high temperature, changes in feed intake tend not to be parallel to observable changes in production which suggests that feed intake is not the sole factor inducing the effects on production. Feed intake and eggshell quality are most susceptible to exposure to high temperature. Dietary AA supplement offered only limited benefits in counteracting the heat induced decline in egg production and may have resulted in production decline on withdrawal. It is possible that supplemental dietary AA influences endogenous biosynthesis resulting in low AA and, hence, negative effects that occurred on egg production when withdrawn.

**MANUSCRIPT III: EFFECT OF HIGH TEMPERATURE ON LAYING
HENS FED ASCORBIC ACID SUPPLEMENTED DIETS**

ABSTRACT

Twenty-four Shaver 288 hens, housed in a temperature-controlled chamber, were randomly assigned to either a basal diet or a basal diet supplemented with 1,800 ppm ascorbic acid and subjected to a constant temperature of 35 C for two weeks in Experiment 4 and six weeks in Experiment 5. In both experiments, exposure to 35 C followed a 14-day period of test diet feeding at a temperature of 25 C. Water was supplied by automatic watering cups connected to a tank outside the chamber and lights were set to provide 14 h of light. Production performance and egg characteristics in both experiments, body weight changes, cloaca temperatures, plasma ascorbic acid and plasma cholesterol in Experiment 4 were determined at the two temperature settings. Yolk cholesterol concentration in eggs produced at 35 C was determined in Experiment 4.

Exposing hens to 35 C resulted in changes in behaviour patterns and significant ($P \leq .05$) reductions in feed intake, reductions in body weights and increases in body temperatures. Egg production on egg mass basis was only reduced ($P \leq .05$) in Experiment 4 and only in controls on egg number basis. Eggshell quality was reduced ($P \leq .05$) only in the control group in Experiment 4. The effectiveness of adding 1,800 ppm ascorbic acid to diets in boosting body ascorbic acid was evident from an increase ($P \leq .05$) in plasma ascorbic acid concentration. Supplementing hens with 1,800 ppm ascorbic acid had no effect on feed intake, egg weight, eggshell elasticity, body weights and body temperature but prevented ($P \leq .05$) the decline in egg production associated with exposure to 35 C in the first week in Experiment 4. The results suggested that hens exposed to high temperature have a potential to respond to dietary ascorbic acid supplement by sustained egg production.

KEY WORDS: temperature, hens, ascorbic acid, supplement.

INTRODUCTION

Several forms of stress are known to cause reductions in tissue levels of ascorbic acid (AA) in the chicken. Reductions in AA were reported in the adrenal gland after handling (Freeman, 1967), in blood, intestinal tissue, liver and adrenal glands with infection by intestinal coccidia (Kechik and Sykes, 1979) and in blood and adrenal glands upon exposure to high temperature (Cheng *et al.*, 1990). While a number of reports in the literature have shown a relationship between corticosteroid release and tissue AA depletion (Freeman, 1970; Schmeling and Nockels, 1978; Freeman, 1980; Pardue and Thaxton, 1984), the exact nature of the relationship remains unknown. The depletion of tissue AA suggests a change in either utilisation, biosynthesis or transport of AA or any combination of these processes and a potential need for dietary supplement of the vitamin.

Reports in the literature on the ability of laying hens under high temperature to respond to dietary supplements of AA are inconsistent. Responses in either production or egg quality aspects were reported by Ahmad *et al.* (1967), Kechik and Sykes (1974), El-Boushy *et al.* (1968), El-Boushy and van Albada (1970) and Cheng *et al.* (1990) at concentration ranges of 44 to 500 ppm, while other researchers found no response at concentrations of 22 to 1,000 ppm (Harms and Waldroup, 1961; Heywang, *et al.*, 1964; Lyle and Moreng, 1968). The Association of dietary need for AA to stress suggests that the appropriate level of dietary inclusion will depend on prevailing conditions. Overall, including cases that were not considered to subject hens to heat stress, reports in the literature have tested a wide range of concentrations, responses to dietary supplements being reported at concentrations as low as 22 ppm (Thornton and Moreng, 1959) and as high as 2,600 ppm (Herrick and Nockels, 1969).

Our previous experiments (Experiments 2 and 3) confined AA supplementation to a 14-day high temperature exposure period and used a maximum dietary supplementation level of 600 ppm. The current work was conducted to further study the effect of dietary AA supplementation in hens at a higher level of dietary inclusion and under prolonged exposure to high temperature. The ascorbic acid supplement was introduced into the diet before hens were exposed to the high temperature.

MATERIALS AND METHODS

In each experiment, twenty-four Shaver 288 hens in their first year of laying, selected for high laying activity, were placed in individual cages constructed in a temperature-controlled chamber.¹ Water was supplied by automatic watering cups connected to a tank outside the chamber and lights were set to provide 14 hours of light per day. The chamber temperature was maintained at 25 C and hens were allowed a 14-day adaptation period during which they were fed a wheat-soybean meal diet (Table 4). Following the adaptation period, individual hens were randomly assigned to either the basal diet or the basal diet supplemented with 1,800 ppm L-ascorbic acid² (12 replications per treatment) and production performance and egg characteristics were assessed daily over 14-days. The temperature was then raised to 35 C and production performance and egg characteristics further assessed at the high temperature setting. Feed was stored in a room adjacent to the chamber, new feed being added to feeders each morning.

¹*ENCONAIRE systems Ltd. Winnipeg, Canada.*

²*Lot. No. 31902. Colborn-Dawes (Hoffmann-La Roche). Cambridge, Canada.*

TABLE 4. *Composition of the basal diet (Experiments 4 and 5)*

Ingredient	Percent of diet
Ground wheat	74
Soybean meal	13
Tallow	2
Dicalcium phosphate	1.2
Limestone	5.8
Oystershell	2.5
Vitamin premix ¹	1
Mineral premix ²	.5
Calculated analysis:	
ME (Kcal/kg)	2794
Crude protein (%)	16.9
Lysine (%)	.71
Methionine (%)	.28
Calcium (%)	3.49
Available Phosphorus (%)	.33

¹Vitamin premix formulated to supply per kilogram of diet: 8250 IU vitamin A; 1000 IU vitamin D₃; .0112 mg vitamin B₁₂; 5.46 IU vitamin E; 500 mg DL-methionine; 2.2 mg riboflavin; 4.4 mg Ca-pantothenate; 6.6 mg niacin and 95.5 mg choline.

²Mineral premix formulated to supply 99 mg Mn; 40 mg Zn and 4.78 g NaCl(.007% I) per kilogram of diet.

Exposure to the high temperature along with test diet feeding was continued for two weeks in Experiment 4 and six weeks in Experiment 5. Feed intake was determined each morning at feeding time, all eggs produced were collected daily and weighed. All eggs that had no signs of cracks were tested for shell elasticity with a Marius deformation apparatus.³ In Experiment 4, blood samples were drawn by cardiac puncture from eight hens on each diet during exposure to 25 C and on the last day of exposure to 35 C for plasma ascorbic acid and total cholesterol determinations. Yolk total cholesterol concentration in eggs produced during the second week of exposure to 35 C was determined on eight eggs from each dietary treatment. Cloaca temperatures were taken at 2:00 p.m. on five consecutive days of the second week at each temperature setting using a telethermometer,⁴ the average of five readings being taken as the body temperature for each hen. Body weight changes over the high temperature exposure period were also determined. Statistical analysis was done in a General Linear Model procedure for weekly production performance data comparisons, mean separation being done using Tukey's Studentized Range Test and by t-test for treatment comparisons. In all comparisons, tests of significance were made at $P \leq .05$ (SAS, 1986).

Chemical analyses

The concentration of AA in plasma was determined using the procedure described in Manuscript II. Plasma and yolk total cholesterol concentrations were determined using a Sigma kit enzymatic method.⁵ The procedure was applied to a fresh yolk suspension

³*Marius Instruments, Utrecht, Holland.*

⁴*Electronic digital thermometer, (cat. No. 15-0781) Fisher Scientific Company.*

⁵*Procedure No. 352, 1988. Sigma Chemical Company.*

obtained by diluting approximately .5 g of well mixed fresh yolk with 4 ml normal saline. The enzymes and chemical reactions involved in the test are given in Appendix 6.

RESULTS AND DISCUSSION

As in the preceding experiments (Experiments 2 and 3), exposing hens to 35 C resulted in behaviour changes which included reduced activity, spreading of wings, panting and increased water intake. Production parameters during the pre-exposure phase and the weeks of exposure to high temperature are given in Tables 5, and 6. Feed intake was significantly reduced by exposure to 35 C (Tables 5 and 6), an observation consistent with that made by Tanor *et al.* (1984). Feed intake did not significantly improve over a six week test period (Table 6). The lack of improvement under prolonged exposure indicates that hens were not able to acclimatize to the high temperature.

A high temperature resulted in reductions in egg production which were significant for both treatments in the second week of exposure on egg mass basis but only in the controls on egg number basis in Experiment 4 (Table 5). Although hens showed numerical reductions in Experiment 5, these were not significant (Table 6). The current observation partly supports findings by Ahmad *et al.* (1967) and Tanor *et al.* (1984) who reported reductions in egg production in hens exposed to 35 C.

There were only numerical reductions in egg weight with exposure to high temperature in the current study (Tables 5 and 6). This observation contradicts the report by Tanor *et al.* (1984) in which laying hens exposed to 35 C for as little as three days were reported to produce lighter eggs. Considering that egg weight increases with age, the effect of

TABLE 5. Production parameters during pre-exposure (25 C) and high temperature exposure (35 C) phases for Experiment 4.

Parameter/Ration	Pre-exposure	High temperature		SE ¹
	(14 d)	week 1	week 2	
Feed intake (g):				
control	113.3 ^a	80.0 ^b	85.1 ^b	2.9
1,800 ppm ascorbic acid	112.9 ^a	78.0 ^b	87.9 ^b	3.1
Number eggs/hen/week:				
control	6.2 ^a	5.5 ^{ab}	5.2 ^b	.1
1,800 ppm ascorbic acid	5.9 ^a	5.8 ^a	5.3 ^a	.1
Egg weight (g):				
control	60.5 ^a	58.9 ^a	58.1 ^a	.5
1,800 ppm ascorbic acid	59.7 ^a	58.2 ^a	57.6 ^a	.8
Egg mass (g/hen/week):				
control	378.4 ^a	324.2 ^{ab}	300.3 ^b	9.8
1,800 ppm ascorbic acid	352.0 ^a	337.6 ^{ab}	302.4 ^b	7.5
Eggshell elasticity (μm):				
control	22.3 ^b	25.8 ^a	24.2 ^{ab}	.5
1,800 ppm ascorbic acid	23.4 ^a	27.4 ^a	26.4 ^a	.9

^{a,b}Means within a row with different superscripts are significantly different at $P \leq .05$.
¹n=12

TABLE 6. *Production parameters during pre-exposure (25 C) and high temperature exposure (35 C) phases for Experiment 5.*

Parameter/Ration	Pre-exposure (14 d)	Week on high temperature						SE ¹
		1	2	3	4	5	6	
Feed intake (g):								
control	112.7 ^a	80.0 ^b	85.1 ^b	84.2 ^b	84.9 ^b	82.0 ^b	92.9 ^b	1.6
1,800 ppm AA ²	115.7 ^a	78.0 ^b	87.9 ^b	88.1 ^b	90.8 ^b	88.0 ^b	95.9 ^b	2.0
Number eggs/hen/week:								
control	6.1 ^a	5.5 ^a	5.0 ^a	5.2 ^a	5.2 ^a	5.3 ^a	5.3 ^a	.1
1,800 ppm AA	5.7 ^a	5.8 ^a	5.3 ^a	5.4 ^a	4.9 ^a	5.4 ^a	4.5 ^a	.1
Egg weight (g):								
control	60.7 ^a	58.9 ^a	58.1 ^a	57.4 ^a	57.7 ^a	58.1 ^a	57.5 ^a	.4
1,800 ppm AA	59.8 ^a	58.2 ^a	57.6 ^a	57.2 ^a	56.7 ^a	56.8 ^a	56.0 ^a	.6
Egg mass (g/hen/week):								
control	369.1 ^a	324.2 ^a	291.1 ^a	299.3 ^a	298.8 ^a	308.0 ^a	304.1 ^a	6.8
1,800 ppm AA	339.1 ^a	337.6 ^a	302.4 ^a	313.2 ^a	275.0 ^a	305.5 ^a	252.0 ^a	7.1
Eggshell elasticity (μm):								
control	22.4 ^a	25.8 ^a	24.2 ^a	23.7 ^a	23.5 ^a	24.1 ^a	25.0 ^a	.4
1,800 ppm AA	23.0 ^a	27.4 ^a	26.4 ^a	27.2 ^a	27.5 ^a	27.3 ^a	27.3 ^a	.7

^{a,b}Means within a row with different superscripts are significantly different at $P \leq .05$.

¹n=12

²Ascorbic acid.

temperature can be said to have been underestimated in the current study. Egg weight did not increase with time in the current study but showed a decline at high temperature and may have been significantly different from what would have been the weight without exposure to high temperature, especially under prolonged exposure. Exposure to high temperature resulted in increases in eggshell elasticity which tended to be greatest in the first week of exposure but only the controls in Experiment 4 had a significant increase in the first week (Tables 5 and 6).

The reduction in feed intake at high temperature resulted in an improvement in feed efficiency of egg production in the first week of exposure to 35 C in Experiment 4 ($2.1 \pm .1$ and $2.2 \pm .1$ versus $1.8 \pm .1$ and $1.6 \pm .1$ for controls and the supplemented group, respectively). This was significant in the supplemented hens, but the combination of greater reductions in egg production and slight increase in feed intake in the second week resulted in poorer feed efficiency ($2.2 \pm .3$ and $2.1 \pm .1$ for controls and the supplemented group, respectively).

Ascorbic acid in the diet had no effect on body temperature and production at 25 C. Space limitation in the chamber resulted in the use of small numbers of replications which caused inherent differences between treatment groups to be more apparent. To improve the assessment of treatment effect, production parameters at 35 C were expressed as percentages of parameters at 25 C (Tables 7, 8 and 9). There was no significant difference in feed intake between treatment groups in any of the weeks in both experiments (Tables 7 and 8). This suggests lack of effect of supplemental AA in reversing the temperature induced reduction in feed intake, an observation contradictory to that made by Thornton and Moreng (1959) who reported greater feed intake in hens supplemented with 22 ppm AA during exposure to high temperature.

TABLE 7. *Parameters at 35 C as percentages of parameters at 25 C for Experiment 4.*

Parameter/Treatment	<u>week of exposure to 35 C</u>		SE ¹
	1	2	
Feed intake:			
control	70.7 ^a	75.2 ^a	1.9
1,800 ppm ascorbic acid	69.1 ^a	77.8 ^a	2.1
Egg production:			
control	88.3 ^b	83.3 ^a	2.6
1,800 ppm ascorbic acid	99.1 ^a	89.6 ^a	3.0
Egg weight:			
control	97.3 ^a	96.0 ^a	.5
1,800 ppm ascorbic acid	97.4 ^a	96.5 ^a	.6
Egg mass:			
control	85.9 ^b	79.9 ^a	2.4
1,800 ppm ascorbic acid	96.5 ^a	86.3 ^a	2.8
Eggshell elasticity:			
control	116.5 ^a	109.1 ^a	2.4
1,800 ppm ascorbic acid	117.9 ^a	113.2 ^a	2.4

^{a,b}Means with different superscripts within a column for each parameter are significantly different at $P \leq .05$.

¹n=12

TABLE 8. Feed intake and egg production at 35 C as percentages of values at 25 C for Experiment 5

Parameter/Treatment	week of exposure to 35 C						SE ¹
	1	2	3	4	5	6	
Feed intake:							
control	71.0 ^a	75.6 ^a	75.7 ^a	76.3 ^a	73.7 ^a	83.60 ^a	1.2
1,800 ppm ascorbic acid	67.8 ^a	76.1 ^a	75.9 ^a	78.0 ^a	75.8 ^a	82.9 ^a	1.5
Egg production:							
control	90.8 ^a	82.9 ^a	87.0 ^a	86.5 ^a	88.4 ^a	86.8 ^a	2.1
1,800 ppm ascorbic acid	109.1 ^a	96.0 ^a	99.5 ^a	89.8 ^a	101.7 ^a	84.0 ^a	3.5

^aMeans within a column for each parameter followed by similar superscripts are not significantly different at P ≤ .05.
¹n = 12

TABLE 9. Egg parameters at 35 C as percentages of values of parameters at 25 C for Experiment 5

Parameter/Treatment	week of exposure to 35 C						SE ¹
	1	2	3	4	5	6	
Egg weight:							
control	97.1 ^a	95.8 ^a	94.2 ^a	94.8 ^a	95.3 ^a	94.4 ^a	.4
1,800 ppm ascorbic acid	97.1 ^a	96.2 ^a	96.5 ^a	95.6 ^a	95.9 ^a	94.4 ^a	.4
Egg mass:							
control	88.1 ^a	79.3 ^a	82.0 ^a	81.6 ^a	84.2 ^a	81.9 ^a	2.0
1,800 ppm ascorbic acid	106.0 ^a	92.5 ^a	96.0 ^a	85.6 ^a	97.4 ^a	79.0 ^a	3.4
Eggshell elasticity:							
control	116.1 ^a	108.7 ^a	105.1 ^b	104.6 ^a	107.2 ^a	110.6 ^a	2.1
1,800 ppm ascorbic acid	119.2 ^a	114.8 ^a	114.9 ^a	115.0 ^a	115.5 ^a	114.1 ^a	1.7

^{a,b}Means within a column for each parameter with different superscripts are significantly different at $P \leq .05$.

¹n = 12

In Experiment 4, hens fed supplemental AA had significantly higher egg number and egg mass production than the controls during the first week of exposure to 35 C (Table 7). While a similar numerical trend was reflected in Experiment 5 (Tables 8 and 9), there was no significant difference between treatments. However, production in hens supplemented with AA was, over the greater part of the six weeks, numerically greater than in the controls. These results show a response to dietary AA in egg production during the first week of exposure to 35 C in the experiment where high temperature had a significant effect on production. This suggests that AA was effective in counteracting the temperature induced reduction in egg production.

With 25, 75 and 400 ppm dietary vitamin C supplementation, Perek and Kendler (1962) reported beneficial effects in egg production during hot summer months. Ahmad *et al.*, (1967) reported 44 ppm dietary AA to be helpful in maintaining egg production in hens exposed to 29.4 and 35 C. In work by Hunt and Aitken (1962), a response to 44 ppm dietary AA in egg production by hens exposed to a temperature of 35 C was achieved in one of two experiments. Somewhat in agreement with the reports above, Kechik and Sykes (1974) reported a non-significant improvement in changes in egg yield which were associated with exposure to 32.2 and 33.3 C when the diet was supplemented with 100 ppm AA. All these reports suggest a response in egg production from dietary AA supplements in hens under high temperature, although the response appears to be inconsistent and perhaps small in magnitude. Results in the current study suggest that the response is most evident during the first few days of exposure to high temperature. These observations contradict those made by Harms and Waldroup (1961) who did not achieve a response in egg production with 22 ppm AA supplement. This contradiction may have been a result of the lower dietary level of AA tested.

There were no significant differences in egg weights between treatment groups in both experiments (Tables 7 and 9) which suggests that supplemental AA had no effect on egg weight in hens exposed to high temperature. Increases in eggshell elasticity tended to be numerically greater in the first week of exposure to high temperature and greater in AA supplemented hens in both experiments (Tables 7 and 9). However, only the third week of exposure to high temperature in Experiment 5 (Table 9) showed a significantly greater eggshell elasticity in supplemented hens compared to controls. Greater deterioration in eggshell quality in hens supplemented with AA could have resulted from greater egg production under a situation of reduced feed intake and may not necessarily indicate that AA induced a detrimental effect. However, a positive response to AA in eggshell quality is not evident. The observation in the current study contradicts reports by Thornton and Moreng (1959), Ahmad *et al.* (1967), El-Boushy *et al.* (1968), El-Boushy and van Albada (1970) and Kechik and Sykes (1974) who reported improvements in eggshell quality from AA supplements at concentration ranges of 22 to 500 ppm in hens under high temperature. On the contrary, Harms and Waldroup (1961), Heywang *et al.* (1964) and Cheng *et al.*, (1990) did not achieve this response at AA concentrations within and above the 22 to 500 ppm range, observations consistent with the current finding. These contradictions can not be clearly explained but probably relate to some other factors unaccounted for as need for the vitamin is assumed to be influenced by stress which in turn will be influenced by environment.

Although there was a significant improvement in feed efficiency of egg production by the supplemented hens in the first week of exposure to high temperature in relation to the pre-exposure phase, there was no significant difference between treatment groups. Exposing hens to 35 C resulted in significant losses in body weights over the 14-day period and a

significant increase in cloaca temperature but there was no significant difference in either of the two parameters between treatment groups (Table 10). An increase in body temperature in hens which were subjected to a series of temperature increments (29.4 C, 35 C and 43.3 C) was reported by Ahmad *et al.* (1967). Likewise, Darre and Harrison (1987) and van Kampen (1988) reported increases in body temperature when hens were subjected to 35 C. While no response in body temperature was achieved from the AA supplement in the current study, Ahmad *et al.* (1967) and Lyle and Moreng (1968) reported moderation in increases in body temperature with supplements of 44 ppm AA in diets. This is another unexplainable contradiction which may have also resulted from other environmental differences.

Supplementation of AA in the diet resulted in significant increases in plasma AA but temperature had no significant effect on plasma AA concentration (Table 10). Cheng *et al.* (1990) reported reductions in blood AA concentration in hens subjected to 31.1 C. Ascorbic acid concentration in some tissues tends to be greater than in plasma (Pardue and Thaxton, 1986). Furthermore, blood cells are known to accumulate AA, so observations in plasma may not necessarily reflect trends of AA status in whole blood and other tissues. The difference between the current observation and that by Cheng *et al.* (1990) may be due to differences in AA status between whole blood and plasma. There is also a possibility that time of blood sampling in relation to the onset of high temperature was a factor in influencing AA concentration. Detailed knowledge of the pattern of AA depletion process during exposure to high temperature is lacking. Blood samples were drawn after 14 days of exposure to high temperature in the current study which probably failed to reflect AA status during the early days of exposure to high temperature. The increase in plasma AA in supplemented hens verified the effectiveness of the administration method in delivering AA to the circulatory system.

TABLE 10. *Body weight changes, body temperature, plasma ascorbic acid, and plasma and yolk cholesterol for Experiment 4*

Parameter/Treatment	Temperature phase		SE	n
	25 C	35 C		
Body weight change (g):				
control	-	-122.8 ^a	19.0	12
1,800 ppm ascorbic acid	-	-137.2 ^a	17.4	12
Cloaca temperature (C):				
control	40.8 ^a	41.5 ^a	.1	12
1,800 ppm ascorbic acid	40.9 ^a	41.4 ^a	.1	12
Plasma ascorbic acid (μ g/ml):				
control	6.5 ^b	6.5 ^b	.4	8
1,800 ppm ascorbic acid	10.6 ^a	11.9 ^a	.8	8
Plasma cholesterol (mg/dl):				
control	122.2 ^b	108.9 ^b	5.1	8
1,800 ppm ascorbic acid	160.4 ^a	136.7 ^a	7.9	8
Yolk cholesterol (mg/g fresh yolk):				
control	-	14.5 ^a	.6	8
1,800 ppm ascorbic acid	-	14.8 ^a	.4	8

^{a,b}Means within a column for each parameter with different superscripts are significantly different at $P \leq .05$.

In a test for repeatability of the cholesterol assay, 16 repeated runs on one sample gave means of 145 ± 1 mg per decilitre for plasma and $12.59 \pm .15$ mg per gram for fresh yolk. The procedure was considered adequate for the intended purpose although it was not checked against another procedure of known reliability. Determined plasma cholesterol concentration in hens supplemented with AA was significantly higher than controls but temperature had no significant effect on plasma cholesterol. In spite of higher determined total plasma cholesterol in AA supplemented hens, the concentration of total cholesterol in egg yolk was not affected. Ascorbic acid is involved in cholesterol transformation to bile acids (Ginter *et al.*, 1979; Harris *et al.*, 1979) such that AA supplementation should have resulted in a reduction in plasma cholesterol. In work by Pardue *et al.* (1985), 1,000 ppm AA ameliorated a plasma cholesterol increase which occurred in response to a 30 minute exposure to 43 C in chicks.

There is no clear explanation for the lack of effect on plasma cholesterol by temperature in the current study. However, it can be speculated that bird age or severity of temperature to which birds were exposed could be factors. It is also possible that the rise in plasma cholesterol could be transient such that the effect may not show days after the onset of high temperature as was the case in the present study. It was suspected that increased AA concentration in the plasma of AA supplemented hens in the current study caused an overestimation of total plasma cholesterol concentration. The cholesterol determination procedure indicates that 7-dehydrocholesterol, a cholesterol derivative whose production is enhanced by AA (Harris *et al.*, 1979) will also react in the test. Values obtained in AA fed hens could have included greater amounts of 7-dehydrocholesterol. This situation along with accelerated cholesterol production to maintain equilibrium may have resulted in the apparent elevation of cholesterol. An overestimate of plasma cholesterol in this way would explain the

lack of effect of AA on yolk total cholesterol in spite of apparent changes in the plasma.

It was concluded that hens subjected to 35 C may respond to dietary supplements of AA only in sustained egg production, especially during the early days of exposure to the high temperature. The lack of consistency in achieving the response is probably a reflection of differences in environments. The effect of temperature may be modulated by relative humidity and a number of sources of stress occur in nature which can not be completely accounted for in any situation and could contribute to the observed results. The economic feasibility of supplementing laying hens exposed to high temperature with dietary AA in practical situations can not be ascertained with results from the current study. However, the response observed and the possibility of environmental influence on response warrants further investigation of the subject.

MANUSCRIPT IV: OCHRATOXIN A TOXICOSIS AND ASCORBIC ACID
SUPPLEMENT IN DIETS OF HENS HOUSED UNDER NORMAL AND HIGH
TEMPERATURE

ABSTRACT

Three experiments were conducted to study ochratoxin A toxicity and the effect of supplemental ascorbic acid in laying hens housed under two temperature environments. In Experiment 6, 18 hens were divided into 3 groups of 6 hens each and fed diets containing either 0, 1.7 or 3.1 ppm ochratoxin A for 14 days. In Experiments 7 and 8, 24 hens were randomly assigned to four dietary treatments in 6 replications. Treatments consisted of a control and three diets containing either 300 ppm ascorbic acid, 3 ppm ochratoxin A or 300 ppm ascorbic acid plus 3 ppm ochratoxin A. Treatment diets were fed for 14 days following the feeding of the basal diet for 14 days. The test period temperature was 25 C in Experiment 7 and 33 C in Experiment 8.

Feeding ochratoxin A at 1.7 ppm significantly ($P \leq .05$) decreased feed intake and increased liver weights and eggshell elasticity. At 3 ppm ochratoxin A significantly ($P \leq .05$) reduced egg production, body weights, plasma Na^+ , K^+ and total Ca^{++} concentrations, and increased plasma Cl^- concentration and aspartate transaminase activity besides affecting feed intake and eggshell elasticity. Exposing hens to 33 C aggravated the negative effects of ochratoxin A and had the added effect of a significant ($P \leq .05$) reduction in egg weights. All the negative effects of ochratoxin A, apart from body weight changes and reductions in feed intake, and increase in eggshell elasticity at 33 C, were either moderated or significantly ($P \leq .05$) reversed by dietary ascorbic acid supplementation. Results obtained suggest a possibility for counteracting production detriments induced by ochratoxin A toxicity in laying hens through dietary supplements of ascorbic acid.

KEY WORDS: ochratoxin A, toxicity, ascorbic acid, hen, temperature.

INTRODUCTION

Ochratoxin A (OA) toxicity in the laying hen occurs at dietary concentrations of between .3 and 4 ppm. This results in nephropathy (Hamilton *et al.*, 1982; Glahn *et al.*, 1989), depressed egg production (Choudhury *et al.*, 1971; Prior and Sisodia, 1978; Hamilton *et al.*, 1982), poor feed efficiency, damage to the liver, mortality (Choudhury *et al.*, 1971), reduced egg weights (Prior and Sisodia, 1978) and reduced eggshell quality (Hamilton *et al.*, 1982). Ochratoxin A has also been shown to cause many adverse metabolic effects. It is an inhibitor of RNA synthesis (Heller and Roschenthaler, 1978; Meisner *et al.*, 1983), protein synthesis (Heller and Roschenthaler, 1978; Bunge *et al.*, 1978) and mitochondrial ATP synthesis (Meisner and Chan, 1974; Wei *et al.*, 1985), and catalytically enhances lipid peroxidation (Rahimtula *et al.*, 1988; Omar *et al.*, 1990; Aleo *et al.*, 1991).

Ochratoxin A binds to serum albumin in circulation (Chang and Chu, 1977) and is mainly excreted in urine unchanged or as its metabolites (Chang and Chu, 1977; Storen *et al.*, 1982). Identified excretory metabolites of OA include ochratoxin α and 4-hydroxy-ochratoxin A (Storen *et al.*, 1982) which is produced in a hydroxylation process involving cytochrome P-450 (Stormer and Pedersen, 1980). Hutchison *et al.* (1971) reported 4-hydroxy-ochratoxin A to be nontoxic in rats, suggesting that the microsomal hydroxylation represented a detoxification process. The activity of cytochrome P-450 is influenced by ascorbic acid (AA) status. Supplements of AA increase the activity of cytochrome P-450 (Sutton *et al.*, 1983) while a deficiency reduces hepatic cytochrome P-450 levels (Holloway and Rivers, 1984). There is experimental evidence of a sparing effect of vitamin C on vitamin E and that the two vitamins have a protective effect against lipid peroxidation (Kawai-Kobayashi and Yoshida, 1986; Chen and Thacker, 1987; Igarashi *et al.*, 1991).

Under normal circumstances, the chicken does not have a dietary requirement for vitamin C as endogenous biosynthetic capacity is adequate (Hart *et al.*, 1925). However, under stress conditions there is a reduction in tissue AA concentration (Freeman, 1967; Cheng *et al.*, 1990). Attempts to verify a dietary need or the ability of laying hens to respond to dietary vitamin C supplementation have yielded inconsistent results. Responses were reported in eggshell quality with dietary AA supplements at 55 ppm (Sullivan and Kingan, 1962) and Haugh unit score with prolonged dietary supplements at 2,600 ppm (Herrick and Nockels, 1969) under normal temperatures. At temperatures exceeding 29 C, responses were achieved in metabolic activity, body temperature regulation, egg production, eggshell quality at 44 ppm (Ahmad *et al.*, 1967), Haugh unit score at 200 ppm (Cheng *et al.*, 1990) and survivability at 25, 75 or 400 ppm (Perek and Kendler, 1963). In contrast, other investigations did not achieve any response in production or egg parameters to long term dietary supplements of AA at 1,200 or 3,300 ppm at normal temperature (Dorr and Nockels, 1971) or dietary supplements of sodium ascorbate at 22 to 1,000 ppm under hot summer weather (Heywang *et al.*, 1964).

The observations that OA causes lipid peroxidation, that the chance of lipid peroxidation is reduced by vitamins C and E and that a metabolic interaction exists between the two vitamins suggests that vitamin C may protect poultry against the toxic effect of OA by reducing the degree of lipid peroxidation. A further interaction between OA and vitamin C is also possible through cytochrome P₄₅₀ and 4-hydroxy-ochratoxin A formation. On the basis of current information it is hypothesized that OA toxicity can be reduced by AA supplementation and that this effect would occur at a normal, and possibly to a greater extent, at an elevated environmental temperature. Three experiments were conducted to study ochratoxin A toxicity at two different temperatures and at different dietary AA concentration.

As far as the authors are aware this is the first study that reports on the protective effect of AA on OA toxicity.

MATERIALS AND METHODS

Experiment 6

The objective of Experiment 6 was to determine the dose response effect of OA on the performance and internal organ condition of the laying hen. Eighteen Shaver 288 hens in their first year of laying, selected for high laying activity and good shell quality, were divided into three groups of six hens each and placed in individual cages in a typical temperate climate layer house at 25 C. Light was provided over a 14 h period in a day and water was supplied by automatic watering cups. Groups were assigned to a basal diet (Table 11) containing 0, 1.7 or 3.1 ppm OA. Ochratoxin A-containing wheat was used as the source of OA and was substituted for wheat in the diet. The production of OA-containing wheat and analyses of OA content in the final product was achieved by the methods described by Frohlich *et al.* (1988). The diets did not contain supplemental vitamin E or an antioxidant and the sunflower oil that was used was aerated overnight to oxidize the vitamin E contained in the oil. The dietary omissions and aeration treatment reduced the concentration of active vitamin E which may increase the toxic effect of OA.

Hens were fed the test diets for a period of 14 days during which feed intake, egg production, egg weight, eggshell elasticity and changes in body weight were recorded. Feed was added to feeders twice a week with feed intake being determined at the end of each week. All of the eggs were counted and weighed but only those that had no visible cracks

TABLE 11. Basal diet composition (Experiments 6, 7 and 8)

Ingredient	% of diet
Ground wheat	74
Soybean meal	13
Sunflower oil	2
Dicalcium phosphate	1.2
Limestone	8.3
Vitamin premix ¹	1.0
Mineral premix ²	.5
Calculated analysis:	
Metabolizable energy (Kcal/kg)	2794
Crude protein (%)	16.9
Lysine (%)	.71
Methionine (%)	.28
Calcium (%)	3.49
Available Phosphorus (%)	.33

¹Vitamin premix formulated to supply per kilogram of diet: 8250 IU vitamin A; 1000 IU vitamin D₃; 0.0112 mg vitamin B₁₂; 500 mg DL-methionine; 2.2 mg riboflavin; 4.4 mg Ca-pantothenate; 6.6 mg niacin and 95.5 mg choline.

²Mineral premix formulated to supply 99 mg Mn; 40 mg Zn and 4.78 g NaCl (.007% I) per kilogram of diet.

were assessed for shell strength as per shell elasticity using a Marius deformation apparatus.¹ The total weight of eggs produced over a given period was expressed as egg mass. On day 15, all hens were sacrificed by cervical dislocation for liver weight measurements and gross lesion examinations on liver, ovary, kidneys and intestines.

Experiment 7

The objectives of Experiment 7 were to determine the effects that supplemental OA and AA have on laying hens when housed at 25 C. Twenty-four Shaver 288 hens were selected and housed as described for Experiment 6. Individual hens were randomly assigned to one of four diets with six birds per diet. The dietary treatments were wheat-soybean meal control diet (R-C), the basal diet supplemented with 300 ppm AA (R-AA),² the basal diet with OA added at 3 ppm (R-OA) and the basal diet supplemented with 300 ppm AA and 3 ppm OA (R-AAOA).

Ochratoxin A was added to the diets as described under Experiment 6. The basal diet was fed to all chickens during a 14-day pretreatment period followed by a 14-day treatment period during which time all four diets were fed. Feed was added to feeders twice a week during both periods. Production performance was assessed over each 14-day period as described under Experiment 6 with body weights being determined at the end of each period. Blood samples were drawn from each hen by wing vein puncture between 8:00 a.m. and 9:00

¹*Marius Instruments, Utrecht, Holland.*

²*Ascorbic acid crystals (97% purity), Lot. No. 31902. Colborn-Dawes (Hoffmann-La Roche). Cambridge, Canada.*

a.m. on day 12 or 13 of the treatment period for chemical analyses.³ Blood collection was done in heparinized vacutainers and samples were submitted for analyses within two hours of first sample draw. At the end of the experiment, all hens were sacrificed by cervical dislocation for liver weight measurements and examinations for gross lesions on liver, kidneys, ovary and intestines.

Experiment 8

The objectives of Experiment 8 were to determine the effects that supplemental OA and AA have on laying hens when housed at 33 C. Twenty-four Shaver 288 hens, were selected as described in Experiment 6 and placed in individual cages in a temperature-controlled chamber.⁴ The birds were fed the basal diet during a 14-day adaptation period, the same diet for a 14-day pretest period and one of the four diets described in Experiment 7 for an additional 14 days, six replications per diet. The chamber temperature was maintained at 25 C during the adaptation and pretest periods but was increased to 33 ± 1 C (30% relative humidity) during the test period.

New feed was added to feeders every morning during pretest and the test periods with feed intake being determined each morning prior to feeding. Production performance was assessed over each 14-day period as described in Experiment 6. Body weights were determined at the beginning and end of the test period. Blood sampling and examination of internal organs was carried out as described for Experiment 7.

³*Analysed by DART^R methods, by Manitoba Agriculture VSB, Winnipeg, Manitoba*

⁴*ENCONAIRE systems Ltd. Winnipeg, Canada.*

All experiments were conducted in accordance with the principles and guidelines presented in the "Guide to the Care and Use of Experimental Animals".⁵ In all experiments, statistical analysis was conducted using a General Linear Model procedure, mean separation being done in Tukey's Studentized Range Test (SAS, 1986). All comparisons for statistical significance were made at $P \leq .05$.

RESULTS

Experiment 6

The addition of OA to diets at 1.7 and 3.1 ppm appeared to reduce the physical activity of hens but did not cause mortality. There were significant reductions in feed intake at both concentrations of OA over the 14-day period with the effect being most pronounced at the higher level of inclusion (Table 12). Reductions in feed intake were accompanied by a loss in body weight at both levels of OA inclusion with the effect being only significant when the diet contained 3.1 ppm OA. Ochratoxin A at 1.7 ppm significantly increased liver size whereas the 3.1 ppm level resulted in an intermediate response that was not significantly different from either the control or 1.7 ppm. The number of eggs laid per week and weekly egg mass production were only significantly reduced at the 3.1 ppm level of OA inclusion with the effect being greater in the second as compared to the first week. Eggshell strength, as assessed by shell elasticity, was significantly reduced by OA at both levels in the first week but only at the 1.7 ppm level in the second week. Examination of internal organs did

⁵*Canadian Council on Animal Care, vol. 2, 1984.*

TABLE 12. *Effect of ochratoxin A (OA) on feed intake, egg production, egg weight, egg mass, eggshell elasticity, body weight and liver weight (Experiment 6)*

Parameters	Treatments			Pooled SEM ¹
	Control	1.7ppm OA	3.1ppm OA	
Feed intake (g/hen/day) ²	107.0 ^a	90.5 ^b	67.0 ^c	2.5
Body weight change (g) ³	12 ^a	-52 ^a	-143 ^b	23
Liver percent ⁴	2.1 ^b	2.5 ^a	2.3 ^{ab}	.1
Week 1:				
Eggs/hen	5.6 ^a	5.7 ^a	4.3 ^b	.3
Egg weight (g)	57.5 ^a	58.2 ^a	57.6 ^a	.4
Egg mass/hen (g)	320 ^a	333 ^a	239 ^b	16
Shell elasticity (μ m)	19.9 ^b	23.1 ^a	24.3 ^a	.4
Week 2:				
Eggs/hen	5.6 ^a	4.7 ^a	2.7 ^b	.4
Egg weight (g)	58.1 ^a	57.7 ^a	57.3 ^a	.4
Egg mass/hen (g)	316 ^a	280 ^a	155 ^b	23
Shell elasticity (μ m)	19.4 ^b	23.3 ^a	21.2 ^{ab}	.8

^{a,b,c}Means with different superscripts within a row are significantly different at $P \leq .05$.

¹n=6

²Average feed intake over the 14-day period.

³Body weight change over the 14-day period.

⁴Liver as percent of body weight.

not reveal any gross lesions with all tissues appearing normal. An examination of ovaries showed that only one of the six hens on the 3.1 ppm OA diet was out of production.

Experiment 7

The effects of OA and supplemental AA on feed intake and egg parameters at a normal temperature (25 C) are expressed as a percent of the pre-test period (Table 13). Feed intake was significantly less in hens fed R-OA compared to hens on R-C. Hens on R-AA and R-AAOA had intermediate feed intake means which were not significantly different from either R-C or R-OA. The number of eggs produced was significantly less in hens fed R-OA compared to hens on R-C and R-AA. Production in hens on R-AAOA was intermediate and not significantly different from either R-C and R-AA or R-OA. Hens on R-OA also produced significantly less total egg mass than the other groups and had correspondingly a lower mean egg weight but this latter difference was not significant. Eggshell elasticity was significantly greater in hens fed R-OA compared to R-C. In contrast, eggshell elasticity for hens fed R-AA and R-AAOA was intermediate and not significantly different from that of hens fed either R-C or R-OA.

Feeding hens the R-OA or R-AAOA diets resulted in significant body weight losses but did not affect relative liver weights (Table 14). The group fed R-OA had significantly lower plasma concentrations of Na^+ and K^+ than hens on R-AA. Hens on R-C and R-AAOA had intermediate means which were not significantly different from either the R-AA or the R-OA group. Plasma Cl^- concentration was significantly increased in hens fed R-OA compared to hens on R-C. Means for hens fed R-AA and R-AAOA were not significantly different from either R-C or R-OA. Plasma total Ca^{++} was significantly lower in hens fed

TABLE 13. Effect of ochratoxin A (OA) and ascorbic acid (AA) on hen performance during a 14-day test period (Experiment 7)

Parameters ¹	Treatments ²				Pooled SEM ³
	R-C	R-AA	R-OA	R-AAOA	
Feed intake (%)	98 ^a	91 ^{ab}	74 ^b	83 ^{ab}	3.2
Egg production (%)	89 ^a	97 ^a	67 ^b	84 ^{ab}	3.1
Egg weight (%)	101 ^a	103 ^a	89 ^a	101 ^a	2.2
Egg mass (%)	90 ^a	100 ^a	66 ^b	84 ^a	3.3
Shell elasticity (%)	96 ^b	103 ^{ab}	158 ^a	121 ^{ab}	8.6

^{a,b,c}Means with different superscripts within a row are significantly different at $P \leq .05$.

¹Values are expressed as a percent of the pre-test period.

²Diets: R-C=control, R-AA=300 ppm AA, R-OA=3 ppm OA and R-AAOA=300 ppm AA, 3 ppm OA.

³n=6

TABLE 14. Effect of ochratoxin A (OA) and ascorbic acid (AA) on body weight, liver weight, selected plasma mineral concentrations and enzyme activity values in hens housed at 25 C (Experiment 7)

Parameters	Treatments ¹				Pooled SEM ²
	R-C	R-AA	R-OA	R-AAOA	
Wt. change (g) ³	7 ^a	-28 ^a	-137 ^b	-118 ^b	21
Liver percent ⁴	2.5 ^a	2.7 ^a	2.7 ^a	2.8 ^a	.1
Minerals (mmol/L):					
Na	153 ^a	153 ^a	149 ^b	152 ^{ab}	.4
K	4.2 ^{ab}	4.3 ^a	3.8 ^b	4.0 ^{ab}	.1
Cl	95 ^b	100 ^{ab}	116 ^a	103 ^{ab}	2.1
Ca	7.8 ^a	6.7 ^{ab}	4.6 ^c	6.3 ^b	.3
Enzymes (Units/L):					
GGT ⁵	28 ^a	26 ^a	33 ^a	36 ^a	1.6
AST ⁶	150 ^b	165 ^{ab}	215 ^a	161 ^b	8.1

^{a,b,c}Means with different superscripts within a row are significantly different at P ≤ .05.

¹Diets: R-C = control, R-AA = 300 ppm AA, R-OA = 3 ppm OA and R-AAOA = 300 ppm AA, 3 ppm OA.
²n = 6

³Body weight change over 14-day period.

⁴Liver as percent of body weight.

⁵γ-glutamyl transferase.

⁶Aspartate transaminase.

R-OA compared to those fed the other diets including R-AAOA. There was no significant difference in plasma Γ -glutamyl transferase enzyme activity among hen groups, but plasma aspartate transaminase activity was significantly greater in the R-OA group than the R-C and R-AAOA groups. Visual examinations of internal organs did not reveal any gross lesions but one of the six hens on R-OA had a pale kidney and one of the six on R-AAOA had a haematoma on the liver.

Experiment 8

The effects of OA and AA on hen performance at high temperature (33 C) are given in Table 15. Hens on R-OA and R-AAOA consumed significantly less feed and had eggshell elasticity values which were significantly greater than the R-C and R-AA groups. Egg production as numbers or mass and egg weight were significantly less in the R-OA group than the other three groups. Feeding R-OA and R-AAOA resulted in significantly greater body weight losses than that produced by R-C and R-AA (Table 16). Livers and thyroids as a percentage of body weight were not significantly different among the treatment groups, but thyroids for hens on R-OA tended to be larger whereas those for hens on R-AA tended to be smaller. Plasma Cl^- concentration was significantly greater in hens fed R-OA compared to R-C and R-AA, whereas, that for hens on R-AAOA was only greater than that of the R-AA group (Table 16). There were no significant differences between treatments in plasma Na^+ , K^+ and total Ca^{++} concentrations. However, hens fed R-OA and R-AAOA tended to have lower plasma total Ca^{++} values than the other two groups with the R-OA group having the lowest value. Plasma Γ -glutamyl transferase enzyme activity in hens fed R-AAOA was significantly greater than the other three treatment groups, whereas plasma aspartate

TABLE 15. Effect of ochratoxin A (OA) and ascorbic acid (AA) on hen performance when housed at 33 C, during a 14-day test period (Experiment 8)

Parameters ¹	Treatments ²			Pooled SEM ³
	R-C	R-AA	R-AAOA	
Feed intake (%)	85 ^a	89 ^a	64 ^b	4.1
Egg production (%)	84 ^a	93 ^a	72 ^a	5.2
Egg weight (%)	99 ^a	101 ^a	99 ^a	.7
Egg mass (%)	83 ^a	95 ^a	71 ^a	5.3
Shell elasticity (%)	107 ^b	107 ^b	146 ^a	6.7

^{a,b,c}Means with different superscripts within a row are significantly different at $P \leq .05$.

¹Values are expressed as a percent of the pre-test period.

²Diets: R-C=control, R-AA=300 ppm AA, R-OA=3.0 ppm OA and R-AAOA=300 ppm AA, 3.0 ppm OA.

³n=6

TABLE 16. Effect of ochratoxin A (OA) and ascorbic acid (AA) on body weight, liver weight and selected plasma mineral concentrations and enzyme activity values in hens housed at 33 C (Experiment 8)

Parameters	Treatments ¹			Pooled SEM ²
	R-C	R-AA	R-AAOA	
Wt. change (g) ²	-47 ^a	-40 ^a	-229 ^b	27
Liver percent ³	2.5 ^a	2.3 ^a	2.4 ^a	.1
Thyroids percent ³	.012 ^a	.010 ^a	.012 ^a	.001
Minerals (mmol/L):				
Na	153 ^a	153 ^a	151 ^a	.5
K	3.8 ^a	3.9 ^a	3.5 ^a	.1
Cl	104 ^{bc}	99 ^c	113 ^{ab}	2.7
Ca	8.0 ^a	6.8 ^a	5.2 ^a	.4
Enzymes (Units/L):				
GGT ⁴	25 ^b	30 ^b	40 ^a	1.6
AST ⁵	135 ^b	161 ^b	175 ^b	21

^{a,b,c}Means with different superscripts within a row are significantly different at $P \leq .05$.

¹Diets: R-C=control, R-AA=300 ppm AA, R-OA=3.0 ppm OA and R-AAOA=300 ppm AA, 3.0 ppm OA.

²n=6

³Body weight change over 14-day period.

⁴As percent of body weight.

⁵γ-glutamyl transferase.

⁶Aspartate transaminase.

transaminase enzyme activity of the R-OA group was significantly greater than the other three groups. Visual examinations of internal organs did not reveal any gross lesions but two of six hens on R-OA had dark livers and a third hen had a haematoma on the liver.

DISCUSSION

The reduction in feed intake and losses in body weight for hens consuming OA observed in the current study is in agreement with the report by Prior and Sisodia (1978) and the growth depression in chicks reported by Peckham *et al.* (1971). Losses in body weight without reductions in feed intake were also reported by Choudhury *et al.* (1971). In the current study, reductions in body weight induced by OA were aggravated by exposure to high temperature such that hens experienced greater weight loss. The decline in egg production caused by OA, which tended to be greater at high temperature, was consistent with reports by Choudhury *et al.* (1971), Prior and Sisodia (1978), and Hamilton *et al.* (1982). A reduction in eggshell quality reported by Hamilton *et al.* (1982) was evident from increased eggshell elasticity in hens fed OA in the current study which also tended to be greater at high temperature. Hens on the 3.1 ppm OA treatment sustained greater eggshell strength in the second week. This may have been influenced by a greater reduction in laying activity during this period. Ochratoxin A at 3.1 ppm compared to that at 1.7 ppm produced the most pronounced toxic effects except for liver size as a percentage of body weight and shell strength in the second week when egg production was severely depressed.

Hens maintained at 25 C and 33 C tended to be affected by dietary OA in a different manner. Plasma minerals at the higher temperature did not appear to be affected by OA,

whereas at 25 C there were decreases in Na^+ , K^+ and total Ca^{++} concentrations and increases in Cl^- concentrations. Increased water consumption in hens maintained at the higher temperature may have affected clearance of electrolytes from the plasma and therefore could have counteracted or modified the effect of OA. Huff *et al.* (1975) also reported a reduction in plasma K^+ in broiler chicks given OA. These researchers suggested that the lowered K^+ was due to selective damage of proximal tubules by OA, the site of K^+ reabsorption. Sodium and chloride concentrations however were unaffected in their studies presumably due to uniform reabsorption along the proximal and distal tubules. Contrary to the reports of these researchers, plasma Na^+ was decreased and plasma Cl^- was increased by OA in the current study. The degree of reduction in the concentration of total Ca^{++} in the plasma of hens fed OA was most likely influenced by the rate of egg production. Hence an effect was not evident at the high temperature as the marked reduction in rate of lay presumably reduced the total need for calcium. A higher rate of lay would require a greater demand on the use of calcium reserves and therefore would affect blood calcium concentrations to a greater degree. Khan *et al.* (1989) also demonstrated that OA can affect calcium metabolism. They reported that OA induced lipid peroxidation was accompanied by a leakage of calcium from calcium loaded microsomes. They hypothesized that OA induced lipid peroxidation was an early event in OA toxicity which results in structural changes in the cell membrane sufficient to allow influx of cellular calcium. This results in a change in all metabolism and ultimately causes cell necrosis. Plasma aspartate transaminase activity, an indicator of liver damage (Bernard and Divers, 1989), was increased by OA in both environments. The presence of visible liver abnormalities in hens on R-OA suggested extensive liver damage by OA, a condition reported by Choudhury *et al.* (1971) and Peckham *et al.* (1971).

No significant response was achieved in any of the production parameters from AA

supplement to the basal diet in the current study. This finding contradicts that made by Ahmad *et al.* (1967) who reported a response in egg production and eggshell quality to dietary AA supplement at a concentration of 44 ppm in hens exposed to temperatures exceeding 29 C.

The addition of AA to diets containing OA partially protected the laying hen against the feed-intake reducing effects of OA at 25 C. Ochratoxin A in general decreased egg weight and egg mass and increased shell elasticity at both temperatures and AA in most cases tended to counteract these negative effects. All of the effects of OA on plasma electrolyte concentrations and plasma aspartate transaminase activity were moderated by the addition of AA to the diet with the effects being particularly dramatic for total Ca^{++} concentration and plasma aspartate transaminase activity. Moderation and reversal of changes in plasma components induced by OA with AA supplement suggests better tissue integrity which is another indication of a protective effect of AA from OA toxicity. Similar protective effects of AA against OA toxicity have not been reported.

Overall, the data in the current study demonstrates that OA produces dramatic negative effects on the laying hen and that AA addition to the diet helps to counteract these effects. Although the nature of the mechanism or mechanisms involved in this process was not established, these results support the hypothesis that OA toxicity can be ameliorated with AA supplementation. Additional research is required to establish if there is a dose response effect of AA and the nature of the interaction between OA and AA.

GENERAL DISCUSSION

Results in these studies suggest that laying hens have the ability to respond to dietary AA supplements. The response is manifested by a change in yolk proportions under normal conditions, sustained egg production under high temperature and reversal of some of OA induced changes in production, egg quality characteristics and plasma components. Unless the level of AA inclusion in diets or duration of AA feeding were factors, hens under high temperature exposure (Experiment 3) did not show a response in yolk proportions observed under normal temperature (Manuscript 1). This difference suggests a variation in response in yolk proportions under the two temperatures and thus, a potential influence of temperature on the nature of a response. Although added AA levels were higher, the actual levels of AA in diets under high temperature were probably lower than that for diets under normal temperature due to the inclusion of dehydrated alfalfa in the latter.

No work could be found in the literature considering the effect of dietary AA supplement on yolk proportions. Although no clear explanation can be given on this response, lipids, the major component of yolk material, have been reported to be associated with vitamin C through carnitine biosynthesis (Hulse et al., 1978), lipoprotein lipase activity (Tsai et al., 1973) and bile acid synthesis (Ginter, 1973; Ginter et al., 1979; Harris et al., 1979; Holloway and Rivers, 1984; Horio et al., 1989).

Increasing consumer awareness of health risks associated with high cholesterol foods makes the observed effect on yolk a disadvantage if it results in increased total egg

cholesterol. It can not be stated with certainty as to whether this response results in a change in yolk cholesterol content as the exact component involved in the change was not identified. It is unlikely that dietary AA affects egg yolk cholesterol concentration because egg yolk cholesterol concentration was not affected by short-term dietary AA supplements (Experiment 4). However, short-term supplementation did not result in a change in yolk proportion and may have had a different response with respect to cholesterol concentration.

Reported responses in eggshell quality (Thornton and Moreng, 1959; Sullivan and Kingan, 1962) and albumen quality (Herrick and Nockels, 1969) under environments which were considered to be normal were not achieved in the current study. Lack of response, in hens under normal conditions, in egg production, feed efficiency, egg weight, shell quality and albumen quality from AA supplements at 33 ppm was reported by Pepper et al. (1961) and egg weight, shell thickness and albumen quality from long-term supplements at 1,200 or 3,300 ppm by Dorr and Nockels (1971). These observations are in agreement with the current finding. Inconsistencies in achieving responses probably result from factors which are not accounted for but may be different between experiments. The association of vitamin C to stress adds a complication in assessing response because stress is difficult to define and a situation involving stress can be difficult to determine. Hence, two environments may be considered normal and identical while imposing different effects on birds.

Results obtained in the current study show that a reduction in feed intake is not the sole factor responsible for detriments observed under high temperature, an observation in agreement with Miller and Sunde (1975) and Emery et al. (1984), which accommodates the possibility of AA involvement. Furthermore, the response to AA in egg production for hens exposed to high temperature achieved in Manuscripts II and III and responses in egg production, egg quality characteristics and some plasma components in hens fed OA in

Manuscript IV suggest influences of the environment in response to dietary AA supplements. Improvements in egg production in hens under high temperature from AA supplements within the concentration range tested in the current study were also reported by Perek and Kendler (1962, 1963), Hunt and Aitken (1962) and Ahmad et al. (1967). However, an improvement in eggshell quality reported by Thornton and Moreng (1959), Ahmad et al. (1967), El-Boushy et al. (1968), El-Boushy and van Albada (1970) and Kechik and Sykes (1974) was only achieved in hens fed OA at normal temperature. The assessment of shell strength was confounded by rate of lay and probably failed to reflect the true picture in some cases.

Reversal of OA induced changes shows a relationship between OA and AA although it is not possible to establish the exact mechanism(s) involved. What is apparent is that a laying hen can respond to a dietary AA supplement, whether the need is specific for OA and occurs by some chemical interaction between the two, a result of reduced endogenous levels due to stress effect or tissue damage or a combination of any of these can not be established with available information. If the response achieved in hens fed OA is not specific to OA-AA interaction but a result of greater response due to more intense stress, then more beneficial effects can be expected in more stressful environments by way of higher temperature and/or humidity or otherwise. Revealed inconsistencies in response to AA in different experiments make sense if it is true that environment, by way of stress, influences responsiveness to dietary AA. It is very difficult to account for all sources of stress in any situation as possible sources tend to be numerous. The observation that vitamin C affects a variety of aspects must be a reflection of the diversity in vitamin C's biochemical functions.

Overall, results in these studies show that supplementing AA in diets fed to hens offers no benefits under normal production conditions but will help sustain egg production under high temperatures and sustain egg production, improve egg quality and help stabilize

plasma components in hens fed OA. The ability of laying hens to respond to dietary supplements of AA and effect of environment on responsiveness are strongly suggested in these studies. This further suggests that situations arise when the chicken's endogenous biosynthesis of L-ascorbic acid fails to meet metabolic needs for vitamin C. However, the economics of supplementing vitamin C in practical feeding can not be easily evaluated with the current work but the positive responses achieved warrant further investigation of the subject.

SUMMARY AND CONCLUSIONS

Long-term dietary supplements of AA of up to 60 ppm in laying hens kept under normal conditions tended to result in an increase in proportions of fresh yolk to whole egg. A cyclic temperature regime exposing hens to a daily 10 h of 35 C from 25 C had less severe detrimental than a constant 35 C even though the high temperature period coincided with light hours. Feed intake was not the sole factor responsible for production changes observed during exposure to high temperature. Ascorbic acid supplements at concentration ranges of 60 to 1,800 ppm minimized the reduction in egg production associated with exposure to high temperature. Ochratoxin A toxicity in laying hens resulted in severe production changes and changes in blood chemistry which were aggravated by exposure to a constant 33 C. A dietary supplement of AA at 300 ppm was effective in moderating or completely reversing signs of OA toxicity administered through the diet at 3 ppm.

It was concluded that laying hens have the capacity to respond to dietary AA supplements in a number of aspects, a possible reflection of diversity in biochemical functions of the vitamin. There is a potential for counteracting heat induced reductions in egg production and most of the detrimental effects of OA toxicity through AA supplements. Because, response to dietary AA was enhanced by exposure to heat and OA, environmental influences may be factors in determining the value of dietary AA supplements in laying hens. These results provide evidence in support of the idea that environment, perhaps by way of stress, can necessitate provision of dietary supplements of vitamin C in practical feeding.

Further research is suggested to:

1. confirm the response in yolk proportions in hens reared under normal temperature and identify the component of yolk involved as well as the mechanism of the process.
2. further elucidate the response in egg production in hens exposed to high temperature focusing on extent of heat exposure, dietary levels of AA and changes in tissue levels of AA.
3. investigate a potential dose response effect and identify the mechanism involved in AA's counteracting effect of OA toxicity.

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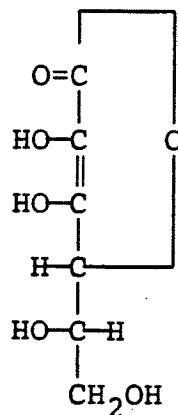
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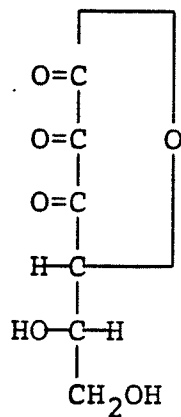
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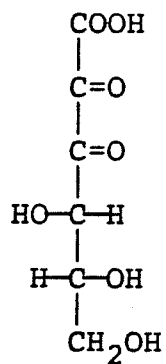
APPENDICES



L-Ascorbic acid
(Reduced form)



Dehydroascorbic acid
(oxidized form)



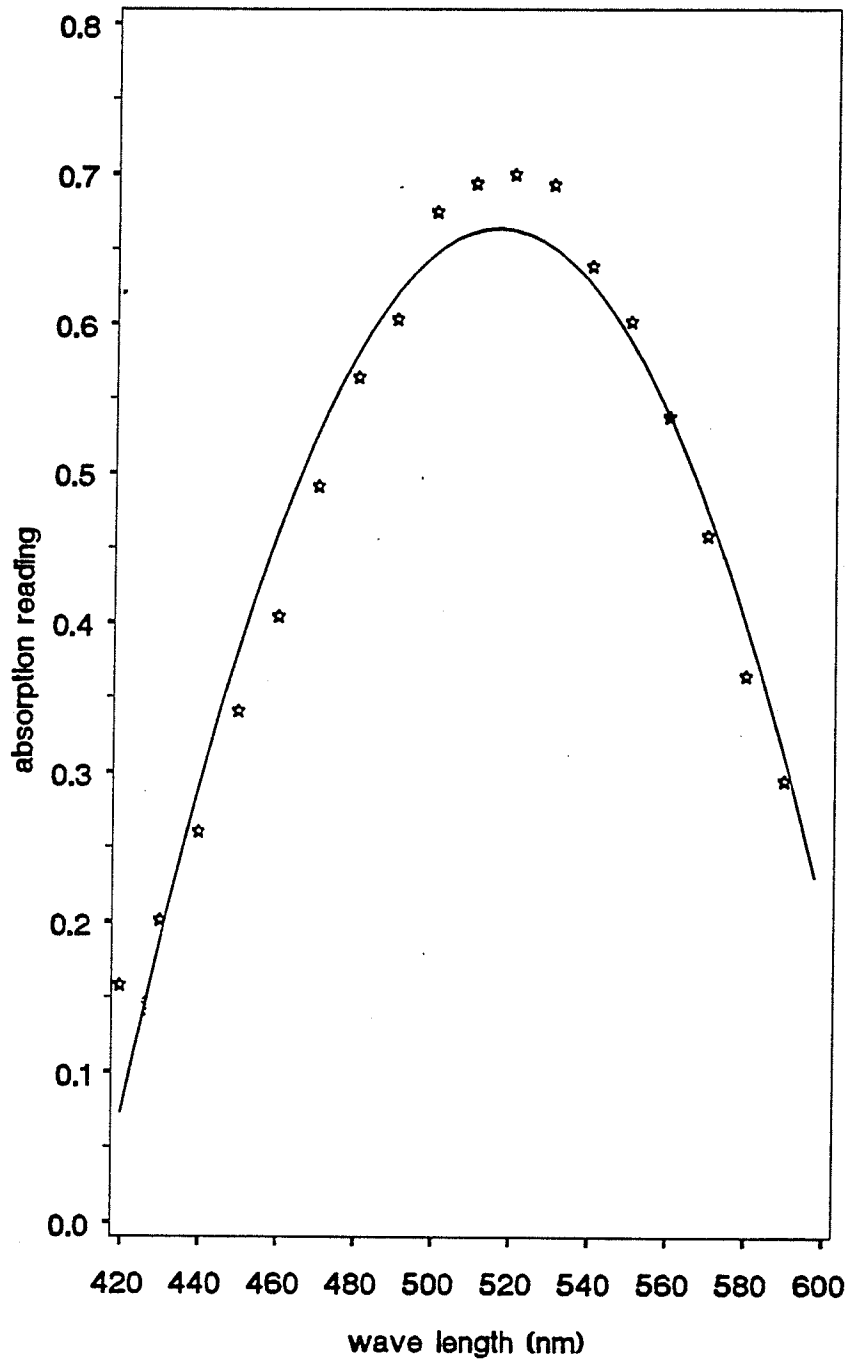
Diketogulonic acid
(further oxidation)

Appendix 1. L-ascorbic acid and the products of two oxidation steps

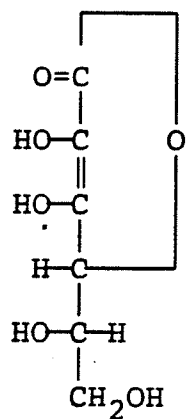
D-glucose 1-phosphate + UTP \longrightarrow UDP-glucose ---(*UDP glucose dehydrogenase*)---

\longrightarrow UDP-D-glucuronate \longrightarrow D-glucuronate + UDP ---(*glucuronate reductase*)---

\longrightarrow L-gulonate \longrightarrow (*gulonolactone oxidase*) \longrightarrow L-ascorbic acid.

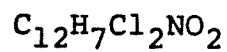


Appendix 3. Absorption spectrum for 2,6-dichloroindophenol dye

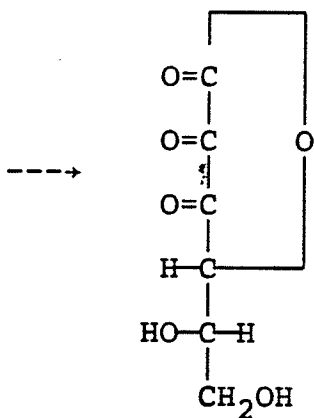


L-Ascorbic acid
(Reduced form)

+

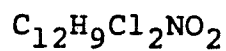


Oxidized
Dichloroindophenol
(Pink at acid pH)



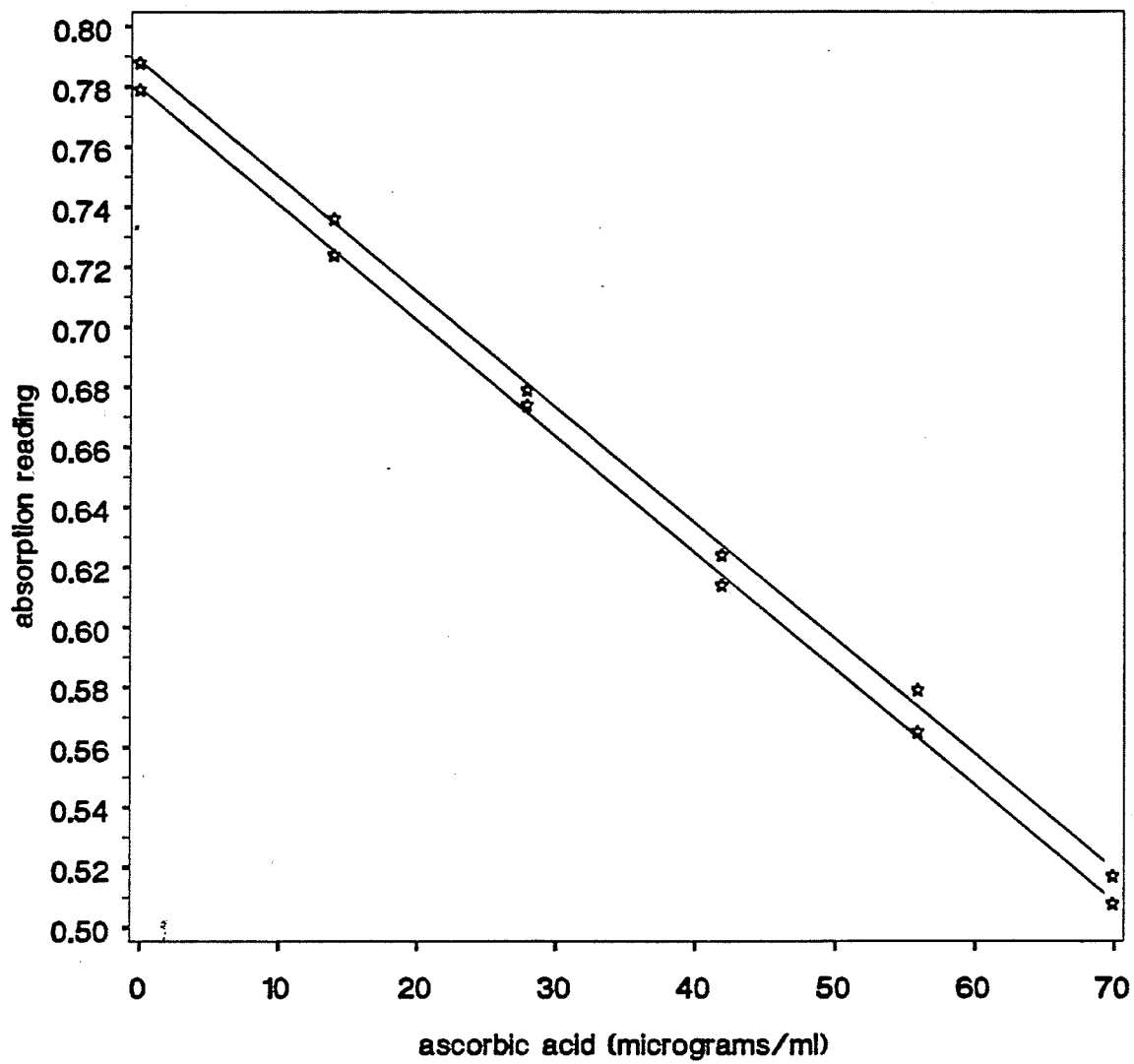
Dehydroascorbic acid
(oxidized form)

+

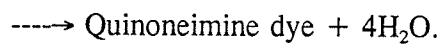
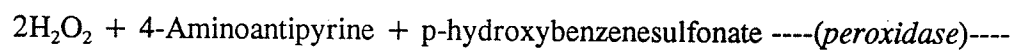
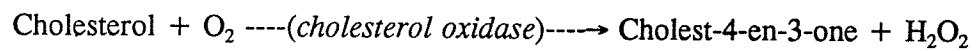
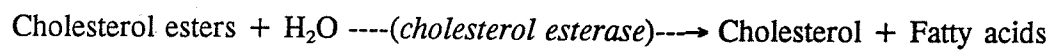


Reduced
Dichloroindophenol
(colorless at acid pH)

Appendix 4. The chemical reaction of L-ascorbic acid determination



Appendix 5. Two sample standard curves for ascorbic acid determination



Appendix 6. Chemical reactions involved in total cholesterol determination