# THE EFFECTS OF ASCORBIC ACID, PROTEIN AND ENERGY LEVELS ON THE PERFORMANCE OF HEAT STRESSED LAYING CHICKENS

BY

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A THESIS

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ADELINE UCHENNA OBIAGELI NWAZOTA

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This thesis, "The effects of ascorbic acid, protein and energy levels on the performance of heat stressed laying chickens", by Adeline Uchenna Obiageli Nwazota meets the regulations governing the award of the degree, Master of Science of Ahmadu Bello University, and is approved for its contribution to scientific knowledge and literary presentation.

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# DEDICATION

DEDICATED

TO

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ABSTRACT:

A study involving three feeding trials was conducted in an attempt to design a hot season feeding proframme for laying birds in the tropics.

The first trial involved 162 29-week old hens in a 3 x 3 factorial design made up of cross-classified combinations of three dietary protein (18, 16 and 14% CP) and three energy levels (2800, 2600 and 2400kcals ME/ Kg feed). The experiment was conducted to determine the optimal protein: energy combinations to be fed during the hot months. Each of the nine treatments was replicated three times. The average maximum indoor temperature was 37.19 C for the study period. Diets 9 *(14%* CP and 2400 Kcal ME/kg feed), 5 (16% CP and 2600 Kcal ME/kg Feed) and 7 (14% CP and 2800 Kcal ME/kg feed) gave better *egg* production with improved feed conversion ratios while *egg* quality was maintained.

For experiments 2 and 3, four isonitrogenous and isocaloric dietary treatments were involved. Each trial had three replicates per treatment and used seventy-two 33-week old hens. In Experiment 2, ascorbic acid (AsA) was supplemented at 0, 200, 400 and 600mg/kg feed to determine if, and the optimal level at which AsA moderated heat stress in laying hens. Ascorbic acid supplementation improved *egg* production feed intake and utilization and cost of feed/Kg *egg,* A supplemental level of 400mg AsA/ Kg diet gave the most efficient response.

In Experiment 3, diet 1 served as control, diet 2 contained 200mg AsA/Kg diet, diet 3 contained 5% palm oil while diet 4 had both 200mg AsA/Kg feed and 5% palm oil supplementation. The objective was to determine the effect of oil alone and in combination with ascorbic acid on heat stressed laying chickens. The diet containing 5% palm oil alone improved *egg* production, *egg* weight, feed intake, daily protein and energy intakes and feed/kg eggs. It also reduced incidence

of cracked eggs and mortality. Supplementation of 200mg AA/Kg diet and 5% palm oil effected a synergistic response which improved hen-day *egg* production, *egg* weight, shell thickness, *feed/Kg eggs,* final body weight while reducing the number of cracked eggs.

It is concluded that heat stressed laying chickens should be fed low energy, low protein diets (14% CP, 2400 Kcal ME/Kg diet, 16% CP, 26C0 Kcals ME/Kg) capable of ensuring adequate energy intake, diets containing 400mg ascorbic acid/Kg or a combination of supplemental ascorbic acid (200mg/Kg diet) plut 5% palm oil.

## INTRODUCTION:

The Nutrient Requirement Tables for livestock give values for rearing birds in the "perfect" environment. For the nutritionist, the perfect environment may be defined as those conditions that maximize gain in weight or egg production with the minimum wastage of nutrients (NRC., 1981). Animals within that environment would be performing near their predicted genetic potentials. In practice, such conditions as the thermo neutral zone are regarded as ideal, and enable the birds to express their expected genetic potentials.

Chickens are reared within environments complicated by a number of factors. These include both the physical and psychological aspects of the birds surrounding. These environmental factors are capable of adversely effecting the productivity of birds (Perek and Kendler, 1963; Njoku, 1986). Temperature, humidity, light (length of day and intensity), altitude as it affects air pressure and partial pressures of oxygen and carbon dioxide, wind velocity (air movement), quality of air and water and density of bird population (NRC, 1981), are such factors some of which may be interdependent. For example, the primary effect of thermal environment on air temperature can quickly be altered by wind, precipitation, humidity or radiation.

Chicken is a homeotherm and maintains a relatively constant core temperature (usually around 40.6 C) by balancing the heat generated from metabolism with that gained from or given up to the environment. However chickens cannot withstand concurrent high temperature and high humidity (El-Boushy and Raterink, 1985). As body temperature rises in response to high ambient temperature, chickens make necessary adjustments to cope with the opndition by reducing feed intake (Dale and Fuller, 1980), slowing down metabolic rate (Emmans, 1974), while heat dissipation increases (Balnave, 1974). These in turn alter the partition of dietary energy. The resulting effect is a change in the energy requirement of the

bird. High environmental temperature provides part of the maintenance energy requirement of the bird (Fuller, 1975), causing a decline in the actual total energy needed by the bird to perform optimally. The reduced feed intake consequent on this decreased energy requirement is accompanied by a drop in the intake of other critical nutrients such as amino acids, vitamins and minerals. Owing therefore to the inadequate level of available critical nutrients within the laying chicken for metabolic processes, there is a decline in the ability of the bird to sustain the production of eggs and the anabolic activity of tissue building (Emmans, 1974). The decline in egg production and growth rate is a common experience of the poultry industry during the hot seasons particularly where no modifications are made in housing and/or management to protect or shelter the birds from inclement ambient conditions.

In Nigeria, the Savannah belt experiences sharp increases in temperature during the months of March to May. The ambient maximum temperature usually ranges from 34 to 38 C (Owonubi, 1986). This temperature range is above the usual thermo neutral zone for the chicken which is in the range of 17.5 to 27 C, and constitutes heat stress which can affect production.

Recognising the adverse metabolic conditions experienced by heat stressed laying birds and the substantial drop in egg and meat production (Babatunde and Fetuga, 1976; Chalwa et al., 1976; Oluyemi and Adebanjo, 1979; Njoku, 1986), the present study was undertaken to design a hot season feeding programme for the laying chickens. The study involves:

(a) a determination of the optimal energy - protein combination in hot season,

(b) assessing the effect of dietary vitamin C on heat stressed

and (c) assessing the effectof addition of oil and vitamin C on laying chickens in hot ambient environment.

# CHAPTER 2:

# REVIEW OF LITERATURE.

REVIEW OF LITERATURE:

## 2. STRESS:

The word 'stress' as pertaining to the living creature is described as "that state within a living creature which results from the interaction of the organism with noxious stimuli or circumstances" (Woolf, 1960). More recently, Gross (1985) described it as "the reallocation of resources in response to perceived environmental changes".

Historically, Bernard in the later part of the 19th century was the first to develope the notion of stress. He proposed the concepts of regulation needed to maintain a constant internal environment. Canon (1929) unravelled the dynamic response of an animal to a potentially dangerous situation and postulated the fight or flight syndrome.

### 2:1:1 THE ADRENAL MEDULLA AND CIRCULATORY REGULATION:

Some of the very initial reactions of an animal to a potentially hostile situation is a dramatic change in its circulatory dynamics. The heart beats more forcibly, the circulation through the skeletal musculature is increased and the circulation to the skin and viscera is decreased (Draper and Lake, 1967).

The adaptive significance of such changes for fight or flight postulated by Canon (1929) shows that the adrenal medulla, which is under the direct control of the central nervous system (CNS), plays a key role in this rapidly responding system (Malmejac, 1964).

Research suggests that any condition that causes an accelerated release of hormones from the pituitary adrenal system is pontentially damaging to the animal (Brown, 1967). Selye (1937, 1952) proposed that the General Adaptation Syndrome (GAS) is the sum of all indefinite responses to a systemic stressor. The major physiological and functional changes in GAS are: (a) enlargement of the adrenals, coupled with an increased output of cortical steroids;

(b) involution of the thymus and other lymphatic organs, with associated blood element changes and (c) the occurrence of gastro-intestinal ulcers in mammals.

The corticotrophin releasing factor (CRF) is the releasing mechanism of the GAS. Every stressor stimulates the anterior pituitary through the hypothalamus to release higher amounts of adreno-corticotrophic hormone (ACTH) (Brown, 1967). ACTH causes an increase, first in the output of glucocorticoids and subsequently in the weight of the adrenals.

The most important symptoms of GAS in poultry are reviewed by Brown (1967). These include enlargement of the anterior pituitary, probably due to the increased production and output of ACTH (Garren and Barbar, 1955; Garren and Shaffner, 1956) adrenal enlargement caused by interrenal hypertrophy and hyperplasia (Brown, 1959; Siegel, 1959 a, 1959 b, 1960; Flickenger, 1961); cholesterol depletion of the adrenals together with an increased output of corticosterone (Siegel, 1960; Flickenger, 1961; Brown, 1961); lymphatic involution, atrophy of the thymus (Garren and Shaffner, 1954), the bursa of Fabricius (Garren and Shaffner 1956; Kalnat, 1958; Siegel, 1961) and the spleen (Garren and Shaffner, 1956). The regression of the bursa of Fabricius is a useful indicator of stress in young birds. As the bird matures, the bursa disappears under the influence of gonadal steroids (Panda, 1960). Other symptoms are, a decrease of lymphocytes and an increase of heterophils (Newcomer, 1958; Schlinder 1962); increase of blood citric acid (Hill, 1961 a, b;); retardation of growth (Garren and Shaffner, 1956; Conner, 1959) or loss of weight (Garren and Barber, 1955); and increased glucogenolysis,, evidenced by an elevated excretion of sodium, potassium, total nitrogen and uric acid (Brown, et al., 1958 a, 1958 b).

These symptoms do not always occur simultaneously (Siegel, 1960 and Schlinder, 1962).

Such stress that will activate the GAS may be environmental or pathological. Wood-Gush (1967) divided stressors into (a) factors in the physical environment for example nutritional deficiencies, abnormal temperatures etc., (b) factors derived from particular genotype-environment interactions and (c) factors operating through the social milleu of the animal. These may include high ambient temperatures, overcrowding in pens or cages, diseases, continuous dissipation of noxious stimuli and fear.

2:1:2 PHYSIOLOGICAL REACTIONS OF BIRDS TO HIGH AMBIENT TEMPERATURE STRESS:

Chickens are homeotherms. They will adjust their activities to maintain a constant body temperature. From all the metabolic processes going on simultaneously within the bird's body, heat is generated. This heat maintains the deep body temperature at about 40.6$^{C}_{}$ (North, 1972; Card and Neshem, 1972).

At an ambient temperature of between 14.0 to 21 C and a relative humidity of 75% some of the heat leaving the bird's body is lost to the environment by the mechanisms ofconduction, convection and radiation. Metabolic heat is transported by the blood from the viscera to the peripheral organs from where heat is lost to the environment. The quality of the bird's performance is best within this zone of thermoneutrality or zone of comfort (El-Boushy and Raterink, 1985). Thorton (1961) found that body temperature was lowest when ambient temperature was in the zone of themoneutrality.

As the ambient temperature and/or the relative humidity rises beyond the bird's zone of comfort, from 24 to 30 C the mechanisms of conduction, convection and radiation become inadequate in relieving the excess body heat. The bird will adjust its voluntary intake of

feed to reduce its metabolic load (North, 1972; Hutchinson, 1954) and will drink more water to cool the internal organs and prevent dehydration from loss of moisture (North, 1972). When the air temperature rises to 27 C, the bird, as it has no sweat glands, starts panting to relieve the excessive body heat (Randall and Hiestard, 1939). The mechanism of panting is not a reflex action Randall (1943) demonstrated that initiation of panting is caused by an increase in the temperature of the blood flowing over the brain. By panting, more, outside air is brought into contact with moist mucosal membranes of the respiratory tract (North, 1972; Hutchinson, 1954). Lee et al., (1945) noted that the increase in respiration at 35 C was associated with an increase in the rate of evaporative water loss. The incoming air absorbs moisture along with its heat content as the outside air has lower humidity. With further increases in air temperature, the bird releases control measures to enhance homeostasis and most physical activities are reduced (North, 1972; Hutchinson, 1954). At an ambient temperature of 35 C the bird is threatened by subsequent death resulting from heat prostration.

The laying hen which is performing an extra production function is worse threatened by a condition of high ambient temperature. The hen, with its additional viscera mass needs a copious and presumably continuous blood supply during the making of an egg. This raises the question as to the consequences of what may happen as a result of a dramatic shunting away of blood from the ovary and oviduct during the operation of a defence reaction such as is envisaged in a high temperature stress situation.

2:1:3. EFFECT OF HEAT STRESS ON BLOOD CONSTITUENT LEVELS:

The manner in which an animal maintains itself in an environment which is always changing, but not necessarily hostile, has received increased awareness and study since the turn of the 20th century. Seyle (1950) developed the concept of 'Stress' for the series of long term metabolic changes which occur as a result of noxious stimuli. Extensive research in this area has revealed the important acute reactions of the body to stress. Such reactions, particularly in the circulation and redistribution of blood, involve the adrenal medullary hormones, adrenaline and nor -adrenaline, both under the control of the hypothalamus (Malmejac, 1964). Carlson (1966) has demonstrated that the liberation of catecholamines not only anticipate adrenal cortical involvement, but is a necessary prelude to corticosteroid actions, at least in some situations.

The general reaction of mammals and birds in response to a condition of "fight or flight" is initiated and coordinated in the hypothalamus. The adrenal medulla prepares the body for an anticipated violent exercise. The basic pattern involves a shift of blood away from those organs not directly involved in the expected exercise to the peripheral skeletal muscles. Hales (1973) demonstrated this shift by exposing his experimental sheep to mild heat stress.

The laying hen is actually performing productive function above and beyond the maintenance function. Often mild increases of ambient temperature have been known to cause a reduction or even a cessation of egg production in hens (Wilham, 1931; Hinds, 1949; Perék and Kendler, 1963, Oluyemi and Adebanjo, 1979). The laying hen with its very large additional egg producing visceral mass, constantly needs abundant and continuous blood supply to sustain the mechanisms involved in the making of an egg.

Sykes (1955), demonstrated that a dose of 1mg or more of adrebakube was completely effective in delaying oviposition sometimes until the following day. He also showed that adrenaline had the effect of inhibiting muscular activity of the uterus, the shell gland. He noted that a drop in egg production occurred for an average of four days, especially at higher doses of adrenaline. The effect was the inhibition of ovulation.

When Draper and Lake (1967) calculated the total demand of blood by the hen's reproductive organs during active egg production, they found it to be 75ml/min. or at least 20% of the resting cardiac output. They had estimated for a 1.6kg Brown Leghorn with an active ovary, to have a blood volume of about 120ml and a cardiac output of 340ml/min. They suggested that in considering the logistics of the blood transport to the forming egg, the transporting of 1.5 to 2.0g of calcium in 16hours is a most demanding process. So any diversion of blood from the reproductive tract might be expected to have its most obvious effect on shell formation.

A. Hematocrit values:

High ambinet temperatures have been reported to have depressing effect on hematocrit values (Olson, 1937; Huston 1960; 1965 Kubena et al., 1972; Deaton et al., 1969a, 1969b; Washburn and Huston, 1968). These workers have demonstrated that generally low values were observed at high temperatures (30 C and higher) while values were higher for birds maintained at lower temperatures (7.2 and 8.0 C). When broilers and layers were reared simultaneously under similar conditions it was found that for the broiler stock, hematocrit values were not responsive to temperature variations as demonstrated by the layers (Huggins and Lewis, 1978).

Working under constant and cyclic high temperature environments,

de Andrade et al., (1976, 1977) reported that birds held at 31 C showed lower hematocrit readings regardless of whether the temperature was constant or cyclic. They reported also that diet had little or no effect on this parameter. The slight improved effect was from the "high nutrient density" (HND) diet which gave slightly higher hematocrit readings. This effect they felt was due to what Soliman and Huston (1974) had reported as significant increase in packed cell volume in birds receiving 5% additional fat and not differences from increasing protein levels. Zimmerman et al., (1973) reported an increase in blood volume of birds maintained at high temperatures of 27 to 35 C as compared to those at lower temperatures (16 - 24 C).

B. Plasma calcium:

High environmental temperature depresses plasma calcium levels (Bragg et al., 1971); de Andrade et al., 1976, 1977). Bragg et al. (1971) had reported a decreased secretion of $^{45}Ca$ into egg shells at high environmental temperature. Miller and Sunde (1975) reported a decrease in shell thickness of eggs produced at a high temperature (32 C). This was confirmed by both Zimmerman et al., (1972) and de Andrade et al., (1976). Mueller (1959) reported that although birds kept under high environmental temperatures consumed less feed and calcium, they retained similar levels of calcium as those kept under normal temperatures. This is contrary to the findings by Charles et al., (1971) who reported an increase in calcium content of egg shell as ambient temperature rose.

The decrease in blood volume (Zimmerman et al., 1972) and hematocrit values at high temperatures (de Andrade et al. 1976), were explained to be related to the reduction in plasma calcium. This explained partially the reduction in plasma calcium at 32 C by hemodilition

de Andrade and workers (1976) also reported that the use of high nutrient density diets significantly increased plasma calcium levels. Mueller (1959) reported a similar observation. de Andrade et al. (1977) stated that although the reduction in blood calcium may be a contributing factor in reduced shell quality at elevated temperature, it seemed unlikely that it is the major cause. They felt a more reasonable explanation might be that under high temperature stress, blood is shunted to the periphery with a concomitant reduction in blood flow to the internal organs especially the uterus (shell gland). Thus, a reduction in blood flow to the uterus, coupled with a decrease in the calcium content of the blood flowing through it would be additive in their effect on shell formation.

C. Thyroxine Levels in the Serum:

Bell and Freeman (1971), and Grandhi and Brown (1975) reported that thyroxine production decreased in laying fowl at high temperatures. Other workers showed in their various studies that warm temperatures decreased thyroid acitivity, while cold temperatures increased it (Gale, 1973, Johnson, 1976 and de Andrade et al., 1977).

Farrington and Mellen (1971), demonstrated that removal of the hypophysis in chickens caused the packed cell volume (PCV) to be significantly reduced, as a result of the absence of thyroid stimulating hormone (TSH) or thyroxine. When TSH was injected into the hypophysectomized birds, normal hematocrit readings were observed. de Andrade et al. (1977) postulated that a decrease in thyroxine levels in response to high temperatures may decrease the production of follicle stimulating hormone (FSH) as the stimuli from the releasing factor of the FSH on the pitiutary or the hypothalamus is not adequate to trigger off effective stimuli for follicular development. As a possible result, egg weight is reduced as a reflection of a lighter ovum (Miller and Sunde, 1975).

It was therefore suggested that the reduced thyroxine levels at high environmental temperature may contribute to lower egg production and shell quality. (de Andrade et al., 1977).

2:2 EFFECT OF HIGH AMBIENT TEMPERATURE ON FEED INTAKE AND CHICKEN PERFORMANCE:

It is generally accepted that environmental conditions affect the level of voluntary feed intake and the utilization of metabolizable energy (ME) (Emmans, 1974; Valencia et al., 1980; Emery et al., 1984). However, most of the reported studies on changes in feed intake with fluctuation in climatic conditions such as temperature, relative humidity and air movement, were usually done under controlled environments of laboratories (Miller and Sunde, 1975; Vohra et al., 1979; Emery et al., 1984; Tanor et al., 1984). The results from these studies demonstrate rather dramatic adjustments in feed intake at both high and low temperatures. However, under the natural conditions of the farm, birds may behave differently as the farms have considerably more variable climatic conditions from those simulated in the laboratories.

Laying hens have been shown to adapt to a wide range of environmental temperature conditions. Davis et al., (1972) showed that laying hens reared at 18 C needed about 2 - 3 weeks to settle at a new level of 160 Kcal ME/kg 0.75/day when moved to an environment of 35 C. When moved from 18 to 7 C, the ME intake was observed to drop for about one week due to behavioral adjustments before the feed intake attained a new level of 102 per cent of the amount consumed before the transfer. Davis et al., (1973), working under commercial production environment, where within house temperatures ranged between 20 - 37 C, reported that feed intake of laying leghorns dropped

by 1.0 to 1.5g per day per 1 C in temperature range of 25 to 34 C, but by 4.2g per day per 1 C in the range of 32 to 36 C.

Various studies indicate that hot climatic environments which result in reduced feed intake do affect egg production and quality (Miller and Sunde, 1975; Wolfenson, 1979; Hurwitz et al., 1980). The drop in egg production is explained to be due to inadequate consumption of energy (Slaghter and Waldroup, 1984; Summers, 1986). In their study, Slaghter and Waldroup (1984) reported a pronounced reduction in rate of egg production and average egg weight, when hens were allowed only 70 and 80 per cent of the calculated energy: amino acid ratios of the diets. Egg mass which is about the most critical measure of amino acid adequacy of a diet was not significantly affected when the hens were restricted in their energy : amino acid intake. These workers concluded that use of temperature - adjusted energy: amino acid ratios would be a valid and feasible means of formulating diets to meet the changing nutritional needs of the laying hen. Summers, (1986) reported in a review that series of trials confirm that energy is a very critical nutrient in ensuring maximum production from the hen, while not overlooking the energy: protein ratio.

Reid et al., (1978) calculated that for each 10g increase in feed intake, egg weight was improved by an average of 1.53g. Similarly, they found that egg production varied linearly with metabolizable energy intakes. The relationship between metabolizable energy consumption and percent egg production indicated that egg production is decreased by 2.5 percent for each 10Kcal/day reduction ME consumption.

Many investigators have worked on the effect of heat stress on feed intake on the laying hen (Table 1). Payne (1967) calculated that the laying hens will reduce feed intake by 1.5percent for every 1 C increase in ambient temperature through 5 - 30 C. Emmans (1974)

Table 1: Effect of Temperature on Feed Intake of laying Hens, Regardless of Value for Metabolizable Energy in Diet. Feed Intake at 18-25°C Serves as Control Value All Assume Chronic Exposure Y = 24.5 - 1.58 T, Where T = Ambient Temperature and Y = Percent of Control:

| Ambient Temperature C | Feed Intake as % of Control | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Vo et al., 1978a | de Andrade et al., 1976a | de Andrade et al., 1977a | Ahmad et al., 1974a | Miller and Sunde 1975a | Stockland and Blaylock 1974a | Van Kampen 1974[a] | Jones and Barnett, 1974a | Jones et al., 1976[b] | Kohne and Jones 1976b | Mean |
| 4-5 | | | +9.4% | | +8.7% | | | +26.8% | +3.3% | | (4) 15.4%[e] |
| 13 | | | | | | | | + 5.3% | | | (1) 5.3% |
| 18 - 25 | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 100% | | - |
| 27 | | | | | | | | -6.7% | | | (1) -6.7% |
| 29.4 | 122% | | | | | -17% | | | | | (2) -19.5% |
| 30 | | | | 8% | | | | | | -18.6% | (2) -13.3% |
| 31 | | -26% | | | | | | | | | (2) -26% |
| 32 | | | | | -25% | | | | | | (1) -25% |
| 35 | -32 | | | | | | -56% | -18.6% | -43% | -32% | (6) -34.6%[d] |

[a] Chickens, adult female (NRC, 1981).

[b] Turkeys, adult female.

[c] Linear regression used to calculate feed intake at 21 C, and then percent calculated.

[d] Includes values of Davis et al., 1972

[e] Includes values of Bray and Gesell, 1961.

calculated a reduction in daily metabolizable energy requirement of 4.3Kcal per 1 C rise in temperature for white and brown egg layers. This represents 1.5g of feed for a diet formulated to provide the hen with 2900kcal ME/kg feed. Bray and Gessell (1961) reported that hens kept in an environment of 5.5 C consumed about 23 percent more feed and laid 18 percent more eggs than did their counterparts housed in a $24.4^{C}$ environment.

Squibb (1959) concluded that high ambient temperatures may be tolerated by the chicken if the daily minimum house temperature was quite low. Studies carried out under cyclic temperature environments show that laying hens seem to compensate for the adverse effects of the hot periods by taking advantage of the cooler phase of the cycle to increase both feed intake and production (Squibb, 1959; Miller and Sunde, 1975). de Andrade et al., (1977) observed that at noon when the temperature in the cyclic environment was at the highest ($35.6^{C}$) in their trial, eating activity was almost nil.

Studies have shown that when nutrient intake is altered by the environment, the adverse effects on the bird's performance could be minimized by appropriate dietary modifications (Payne, 1966). In their study, March and Biely (1972) reared chicks at 20 and $31^{C}$ using diets with differing graded levels of lysine from deficient levels through to diets with excess of lysine. The reported result gave two response lines which slopes were almost parallel for feed intake effect on bodyweight gain at the two temperature environments. The result indicated that the chicks reared in the hot environment ate at 20 percent less feed than those in the 20 C. environment. When the data were plotted on the basis of effect of lysine intake on weight gain, one response line could describe the effect of the two temperature environments. The conclusion was drawn that the environmental effect

on growth was not due to a change in nutrient requirement for growth but due to the effect of the high ambient temperature in depressing feed intake. The birds in the hot environment consumed less lysine daily and gained less weight.

In order to ensure that when heat stress caused a decline in feed intake of the chicken, that adequate energy and other essential nutrients were ingested to maintain production at a comparatively high level, the "high nutrient density" diets have been advocated (de Andrade et al., 1977; 1977; Dale and Fuller, 1980 and Tanor et al., 1984). Valencia et al., (1980) reported that voluntary feed intake appeared to increase slightly with the feeding of added fat (1.0 to 5.0%) in each of the temperature groups (18.3 and 35 C). Dale and Fuller (1980) made similar observations with improved growth rate for their experimental chicks. The use of high nutrient density diets was reported by Tanor et al., (1984) to help maintain normal egg production, egg weight, and egg shell deformation.

Other studies have advocated the inclusion of vitamin C as possible anti-stress agent in the diet of the laying chicken (Roche, 1987). Ascorbic acid has been reported to improve feed intake (Thorton and Moreng, 1959), growth rate (Kafri and Cherry, 1984; Njoku, 1986), egg number (Perek and Kendler, 1963) and egg quality (El-Boushy and Van Albado, 1970).

## 2:3 EFFECT OF DIETARY ASCORBIC ACID (VITAMIN C) ON THE PERFORMANCE OF HEAT STRESSED CHICKENS:

Several studies have been reported on harmful effects of high environmental temperature on the performance of the domestic fowl especially in regard to the growth rate (Joiner and Huston, 1957; Dale and Fuller, 1980, Kafri and Cherry 1984, 1985; Njoku, 1986), egg production (William, 1931; Warren and Schnepel, 1940; Hinds, 1949;

Hutchinson, 1954; Perek and Kendler, 1963), egg quality (Bennion and Warren, 1933; Warren, 1939; Warren and Schnepel, 1940; Wilson, 1949; Warren et al., 1950; Ragab and Assem, 1953; Hutchinson, 1953; Mueller, 1959, 1961; Miller and Sunde, 1975; de Andrade et al., 1976, 1977; Deaton et al., 1981) and feed intake (Mueller, 1961; Payne, 1966; and Dale and Fuller, 1980).

Ascorbic acid has been accepted as a non-essential dietary ingredient for poultry since the 1920's (Hart et al., 1925). It has been demonstrated that fowls possess the enzymes necessary to complete the synthesis of vitamin C from glucose (Roy and Guha, 1958 a and Marks, 1975). The enzyme l-gulonolactone-oxidereductase which catalyzes the last stage of the synthesis is found only in the kidneys of galliformes and most other families of birds. Thus chickens are supposed to have no need for an exogenous supply of vitamin C.

Early work showed that chicks maintained on ascorbic acid-free diets that were otherwise nutritionally adequate, displayed no adverse effects on growth, production and livability (Shorten and Roy, 1921; Emmett and Peacock, 1922, 1923; Mitchell et al., 1923; Plimmer and Rosedale, 1923). Mitchel et al., (1923) and Sifri et al., (1977) fed different amounts of calcium and ascorbic acid or citric acid to adult male chickens, young chickens or young Japanese quail. The study showed that when ascorbic acid was fed at 1%, no adverse effect on body weight, tibia ash or tibia calcium (Ca) of adult males were observed. In growing chickens, body weight, plasma Ca and tibia mineral content differed with the Ca in the diet but were not influenced by ascorbic acid even at 0.65%.

Kolb (1987) in a review report showed that ascorbic acid plays an important role in certain biochemical reactions in the hepatocytes of the liver. These include:-

i) Detoxification of various compounds by hydroxylation and conversion of cytochrome P450. Thus a normal concentration of ascorbic

acid in the hepatocytes promotes excretion of toxic compounds via the bile and urine. On the order hand, an insufficient concentration of ascorbic acid leads to a reduction in the activity of the enzymes involved in the detoxification processes.

ii) Hydroxylation of cholesterol to 7-alpha-hydroxy-cholesterol, a reaction important in the formation of bile acids. Where an ascorbic acid deficiency exists, the speed at which this hydroxylation and other hydroxylation reactions occur is reduced.

iii) Formation of carnitine from lysine and methionine, by hydroxylation reactions. A deficiency of ascorbic acid reduces the formation of carnitine. Carnitine is important in the transport of fatty acids into the mitochondria. The lowered carnitine level in an ascorbic acid deficient situation reduces this trasport capacity into the mitochondria of the various tissues. This leads to a reduction in the rate of utilisation of long chain fatty acids. The effect on the skeletal musculature is to lower the ability to do heavy physical work.

Low levels of ascorbic acid in the chicken's system have also been implicated in the poor performance of chickens faced with such stressful situations as high environmental humidity and temperature, high productive rate and parasiticiinfestation (Bell et al., 1941; Ketchik and Sykes, 1974; Behm et al., 1978; Sykes, 1978). Under such conditions, Njoku (1986) suggests that endogenous ascorbic acid synthesis is inadequate to meet metabolic needs.

Squibb et al., (1955) found lower serum ascorbic acid levels in birds infected with coryza or New castle disease. A decrease in the plasma ascorbic acid of birds infedted with fowl typhoid was reported by Hill and Garren (1958). Stressed birds, especially the young ones had regressed Bursa of Fabricius (Panda, 1960) while both the young and old birds exhibited regressed thymus (lymphatic

involution) (Garren and Shaffer, 1954). In response to stress, birds increased their production and output of adrenocorticotrophic hormone (ACTH) levels (Germuth and Ottinger, 1950; Garren and Shaffner, 1952). Growth is retarded (Conner, 1959, Garren and Shaffner, 1956) and weight loss is observed (Craig and Vargas, 1985).

Schmeling and Nockles (1977) demonstrated that when mature male birds were injected with 0.02 units of ACTH/g body weight and fed 150, 300 or 3000ppm ascorbic acid in the basal diet, there was a highly significant linear decrease in plasma corticosterone with increasing ascorbic acid in the diet. The turnover rate was higher in males than in hens supplemented with ascorbic acid. Birds of either sex which received supplemental dietary ascornic acid had significantly heavier body weights than control between 3 and 17weeks of age for the males than controls. These workers postulated that since one of the effects of stress is increased corticoid hormone production and release with subsequent catabolism of body tissues, any interference in steroidogenesis and or secretion of corticoids would prevent the catabolic effects of stress and result in improved growth rates.

Ascorbic acid stimulates the transformation of B-lymphocytes into plasma cells under the influence of antigens (Kolb, 1977). In their study, McCorkle et al., (1980) reported that feeding 1% dietary ascorbic acid to young and mature birds who had been previously innoculated with 5% suspension of sheep - red - blood - cells (SRBC) and 0.1ml undiluted Brucella abortus (BA) did not influence cell mediated immunity or antibody response to a T-cell dependent antigen. However, in the presence of increased levels of ascorbic acid, the antibody response to T - independent antigen, BA, was lower in neonates and higher in adults. This agrees with the report of Klob (1977) that

the consumption of ascorbic acid was found to increase after exposure to antigens and infectious diseases. Anderson (1981) reported that ascorbic acid increases the migration capability of granulocytes. In their study, Abdel-Wahab et al., (1977) demonstrated that the depressed activity of the thyroid in vitamin C depleted chicks was only slightly affected by addition of ascorbic acid at the level of 50mg/kg diet. However addition of 100mg/kg restored function. Serum radioactive iodine uptake decreased significantly as the amount of vitamin C supplementation increased.

Disease in the bird not withstanding, unfavourable conditions in hot temperature environments may result in significant vitamin losses during feed production and storage to affect the activity of dietary vitamins and their avaliability to the bird.

Reports from studies on the inclusion of dietary ascorbic acid in chicken diet of heat stressed birds have been quite controversial. There is no general agreement that the supplementation of ascorbic acid in chicken feed achieves a well defined ameliorating effect on the productivity of the bird. However, there is a general consensus that some beneficial response, though small, is elicited when ascorbic acid is added at levels of 20 to 500mg/kg diet. Briggs et al., (1944) and March and Biely, (1953), reported growth stimulations when ascorbic acid was added to highly purified chick diet. Using traditional corn-soy based rations, Dorr and Balloun (1976) working with turkeys and Schmeling and Nockels (1978) with chicks observed this improved growth performance.

From the study carried out by Horning and Frigg (1980) a blood picture of the ascorbic acid level and its biosynthesis in chicks kidney was demonstrated. Male and female Hybbard chickens were used from day old to 56days of age. At hatch the neonate's plasma ascorbic acid level was low. The biosynthetic activity in the kidney on the

first day of hatch is 0.3mg. The blood plasma level increased from 0.5mg/100ml on the 5th day to within 1.8 to 2.0mg/100ml on the 36th day of life. By the age of 20 and 30 days the biosynthetic activity reached 1.2mg per gram kidney protein every 90minutes. Subsequently, the level starts dropping up to the 56th day of life to 0.7mg/g kidney protein every 90 minutes. The liver at 56days of age contained 20mg of ascorbic acid. The increased body weight response observed by Kafri and Cherry (1984) in a temperature - ascorbic acid study seemed to follow a similar pattern. The diet fed to sex intermingled group of birds contained ascorbic acid at the level of 0 or 100mg/kg. Three temperature levels used were a constant 23 C, 32 C or a daily temperature regimen consisting of 32 C for 20hrs and 40C for 4hrs (32/40 C). The results shows that the ascorbic acid-fed female birds, regardless of ambient temperature were significantly larger at 36-day old than those on free - ascorbic acid diet. In the males however, the workers reported a significant ascorbic acid by ambient temperature interaction. In the very hot environment of 32/40 C the ascorbic acid had no effect on body weight. Although both sexes had increased body temperature resulting from the elevated environmental temperature, the ascorbic acid had no significant effect on the body temperature. The workers concluded that ascorbic acid was beneficial to heat stressed commercial broilers.

In his contribution, Njoku (1986) evaluated in two studies the effect of dietary ascor acid supplementation of the broiler chickens from day old to 8 and 9 weeks of age during the hot, dry months of the year in Nigeria. He arrived at the same conclusion as Kafri and Cherry (1984) that ascorbic acid was beneficial to heat stressed commercial broilers. In the first experiment 0,200 400 or 600mg ascorbic acid/kg feed were added to a corn-soya bean meal basal diet. In the second study, 100, 200 or 300mg ascorbic acid/kg feed was added. All the diets

were isonitrogenous and isocaloric in each experiment. The result of experiment 1 show that the inclusion of ascorbic acid had a significant linear and quadratic effects on broiler body weight gain. Supplementation at the 200mg/kg diet level gave the highest gain. Feed intake was unaffected by treatment. However, feed gain ratio at 28 and 56 days showed linear responses. In the second experiment, feed gain response was repeatedly better with ascorbic acid level of 200mg/kg diet. This worker recommends an inclusion of dietary ascorbic acid of 200mg/kg feed to broiler diet during the very hot months of the year.

In a review, Sykes (1978) stated that the immediate stimulus for further investigation of the role of ascorbic acid in layer performance was the work of El-Boushy and Van Albado (1970). In that study, El-Boushy and Van Albado (1970), had 7-month old pullets, 24 in number, placed in either a cold room 13$^{C}$ throughout the experimental period or in a hot room, 30 C for 6 months, with relative humidity at 75 to 80% for two months and then raised again for the rest of the experimental period. Ascorbic acid was given for the last two months. These workers found that heat stress significantly reduced all aspects of shell quality. Specific gravity of eggs and shell deformation, breaking strength and percent shell, but not thickness, tended to fall through out the trial. In the cold room, the use of ascorbic acid in the diet did not significantly affect shell quality. In the hot room ascorbic acid increased specific gravity, weight and percent shell but not thickness, breaking strength or deformation. The high temperature and humidity significantly reduced the plasma calcium while ascorbic acid supplementation raised it in the hot room. The work supports earlier work by Thorton and Moreng (1959) who had reported improvement in egg shell quality of hens kept in controlled high temperature environment.

Thorton (1960) reported increased shell thickness using 44ppm ascorbic acid in 13 but not with 17% protein diet. Herrick and Nockels (1969), fed 30pullets of 7-month old, a corn-soyabean meal diet with or without 0.26% ascorbic acid for 25weeks. They reported significant increases in blood ascorbic acid and egg quality measured in Haugh's Unit. Egg production and weight, whell thickness, feed per dozen eggs, body weight gain and mortality were unaffected by ascorbic acid supplementation. Some reports indicate that ascorbic acid has been beneficial in producing eggs with significantly improved shell quality and egg weight (El-Boushy, 1966; El-Boushy et al., 1968; Arscot et al., 1962 and Chen and Nockles, 1973).

In order to investigate breed responses to elevated temperature and ascorbic acid supplementation, Ahmad et al., (1967) used Single Comb White Leghorn (SCWL), NewHampshire and Delaware laying hens during 3 successive phases of production. After acclimatisation to 21 C, the hens had their environmental temperature raised to 29.4, 35 and 43.3 C. Egg quality, body temperature and oxygen consumption were adversely affected by the high temperatures. The workers found that the SCWL birds were better adapted to heat stress than were the NewHampshires or Delawares. Ascorbic acid helped maintain egg quality, body temperature and metabolic activity.

Working in Isreal's Jordan Valley, in extreme desert conditions, Perek and Kendler (1963) fed their experimental birds three levels of ascorbic acid, 25, 75 and 400ppm in a corn-milo-soyabean meal mash. They reported that supplementing the diet with ascorbic acid significantly increased the egg yield in the treated groups by an average of 23.6% in the three experimental groups of the first trial during a 28-week period, and by 11.2% in the 12weeks duration of the second trial.

Egg weight were significantly higher in the ascorbic acid fed birds of experiment 1 while they failed to obtain similar results in experiment 2. The workers also reported higher culls and mortality among the controls than in the ascorbic acid fed birds. No significant difference were observed in egg shell thickness between the treated groups and controls. The results of this experiment is in agreement with the improved heat resistance and reduced mortality associated with ascorbic acid fed chickens reared in elevated ambient temperature in reported by Thorton (1961); Perek and Kendler (1962); Ahmad et al., (1984).

Oluyemi and Adebanjo (1979), in the humid tropics of Nigeria, fed dietary ascorbic acid of 100, 150, and 200mg/kg diet to 30 - and 62 week old Rhode Island Red X Barred Plymonth Rock layers. They reported that laying was highest with 100mg/kg ascorbic acid. The older layers benefited equally on 100, 150 and 200mg/kg ascorbic acid in comparison with the control. They observed that the increased feed intake associated with the treatments appeared to have been used for egg formation rather than for improving egg quality. The subsequent hybrid chicks produced from the above cross had a growth rate significantly higher with dietary supplementation of ascorbic acid at 150 and 200mg/kg. Pardue et al. (1986) and Peebles and Brake (1985) showed also a decreased mortality rate with ascorbic acid. Pardue et al. (1984) reported that the use of ascorbic acid did not improve overall livability significantly in either sex, but mortality associated with heat stress was reduced in supplemented females.

Lazar et al. (1983) recorded the best increase in egg weight and other egg parameters in their study, when ascorbic acid was fed at levels of 75mg/kg feed. They noted that the increase in egg weight was due to an increase in the weight of shell or yolk.

The supplemented ascorbic acid was associated with a decrease in calcium concentration in the yolk and an increase in phosphorus concentration in the shell.

Some studies using dietary ascorbic acid in feeding trials did not show any consistent trend in improvement of egg shell quality or give significant and sometimes even negative responses to egg production and other parameters in layer performance. In the work reported by Ketchik and Sykes (1974), three experiments were involved. Two experiments were conducted to investigate the effects of dietary ascorbic acid on the performance of laying hens maintained under high environmental temperatures (32.2 and 33.3 C, respectively). The third experiment was conducted under ambient conditions (12.8 to 17.3 C). Weekly changes in egg production, shell deformation, percentage of cracked eggs and body weight showed an improvement in the ascorbic acid-supplemented birds but the differences were non-significant. Egg production and percentage of cracked eggs were not affected when the experimental diet was supplemented with ascorbic acid under ambient conditions.

Pepper et al., (1961) while essessing the influence of calcium and ascorbic acid on egg quality, found that when hens which had been in lay for 12months were fed diets containing 1, 2.5 or 4% calcium with ascorbic acid at 0, or 33mg per kg diet and housed for 12weeks under indoor ambient temperature of between 23.9 - 26.7 C that egg production and feed per dozen eggs improved significantly as the calcium level increased. Ascorbic acid had no effect. Ascorbic acid had no effect. Ascorbic acid seemed to increase loss of egg weight on the 1% Ca diet and to decrease the loss with 4% Ca. This trend was significant at 5 and 9weeks. Ascorbic acid had no consistent effect on specific gravity, plasma Ca, and $p^H$ of the intestine while

Ca gave significantly improved values and linear responses to these parameters. The authors concluded that under their condition of study, the requirement for Ca was greater than 2.5% while supplementary ascorbic acid was not required. Herrick and Nockles (1969) fed, 36 pullets, 25 weeks of age, a diet of maize and soyabean meal supplemented with 260mg ascorbic acid for 7 months. They reported that blood ascorbic acid significantly increased while egg production and weight, shell thickness, feed per dozen eggs, body weight gain and mortality were not affected.

Roche (1987) reports that ascorbic acid is an anti-stress agent which assists in the formation of corticosteriods thus maintaining ascorbic acid body reserves for use during stressful conditions. Its involvement in the synthesis of carnithine, an essential metabolite, provides the muscle with sufficient energy to counteract stress e.g. for the panting activity in birds, and in helping to maintain feed intake to minimize depression in performance (growth, egg production, reproduction) throughout stress periods.

## 2:4 EFFECT OF FAT INCLUSION TO LAYER DIET ON THE PERFORMANCE OF HEAT STRESSED EGG LAYING BIRDS:

Throughout the tropical world and also during the hot summer months of the temperate regions of the world, research workers have noted the deleterious effects of high environmental temperatures on livestock performances. For the chicken, feed intake is shown to be inversely related to environmental temperature (Prince et al., 1961; Byerly et al., 1978). This condition was initially taken for granted as birds mainly ate to satisfy their energy requirements. The high ambient temperature provided part of the needed energy and this did reduce the energy requirements for maintenance with temperatures up to 26 to 27 C. The lower energy requirement which is indicated by the

decrease in oxygen consumption (Barrot and Pringle, 1946) and heat production (Farrel and Swain, 1977), are related to the birds ability to regulate its energy intake to meet its specific needs (Hill, 1962; Morris, 1968).

Reduced intake of feed was usually accompanied by a decline in growth rate (Dale and Fuller, 1980; Valentia et al. 1980, Kilgour and Dalton 1983) and such declines should not occur if the bird was actually meeting its energy needs for maintenance, growth and production.

Working with swine, an animal noted for its intolerance of extreme temperatures, Andrews (1957) observed that when the ambient temperature rose beyond the swine's thermo-neutral zone, the animal reduced feed intake in order to lower the burden of heat dissipation. This has mainly been responsible for the reduction in feed intake associated with long, hot, dry seasons. Reduced feed intake creates for the animal a deficiency of usuable energy which contributed to the slow subsequent growth rate.

Scott et al., (1947) were some of the first workers to demonstrate that in chickens high energy rations tended to promote more rapid growth and better feed conversion than rations of lower energy contents. This finding has since been supported by other workers (Robertson et al., 1948; Donaldson et al., 1956; Fuller and Rendon, 1977, Waldroup et al., 1976; Dale and Fuller, 1980); Working with rats, Forbes et al,, (1946a, b,), Carew and Hill (1964) and (Charles, 1980) showed that fats decreased the heat increment of the diet when fat calories are digested.

Biely and March (1954) and Waibel (1955) reported that the response to added fat was influenced by the level of protein in the diet and showed that weight gains of the chicks and turkey poults were more likely, when the level of protein incorporated in the diet was relatively high. In their contribution, Rand et al., (1958) were able to demonstrate quite

clearly that inclusion of maize oil in a diet resulted in improvements in growth, efficiency of protein and energy utilisation and protein retention. They also postulated that the major oil effects was due either to increased efficiency of metabolizable energy utilization, an improvement in utilization of dietary protein, or the presence of an unidentified growth factor.

In what was referred to as the "extra caloric" effect of supplemental fat, Tourchburn and Naber (1966), reported that addition of fat to turkey diets, improved the utilization of the metabolisable energy. This report was later confirmed by Jensen et al. (1970) who were able to show that the calculated metabolizable energy value of fat could substantially exceed its gross energy. Hurwitz et al. (1980) found that feeding low energy, high protein diets to turkeys resulted in higher maintenance requirements, especially at temperatures between 18 and 28 C. The two turkey diets used differed in three major items- metabolizable energy, crude protein, and total lipid. These workers felt anyone of these factors could be responsible for the differences observed in the maintenance requirements in the diets. Hurwitz et al. (1980) opined that there may be a reduced feed efficiency with diets containing excess protein and that, the high fat content of the high - energy diet may have resulted in an extracaloric effect postulated by Jensen et al. (1970), thereby minimizing the apparent energy requirement with the diet. It has been suggested that the extracaloric value of fat was discernible especially at the region of higher environmental temperature (Lipstein and Bornstein, 1975).

Other studies which have enhanced understanding of the more efficient utilization of the various dietary fats were on the synergistic effect of fat on the metabolizable value of the diet. This is related to the absorbability of the fat. Renner and Hill, (1960) demonstrated

that the degree of unsaturation of fat influenced the rate of its absorption through the gut wall. Thus chicks showed a greater rate of absorption of corn oil than tallow or lard. This synergism is also demonstrated when various types of fats are combined. Renner and Hill (1960) and Young (1961) showed that saturated fatty acids like palmitic (16:0) or stearic (18:0) acids were poorly utilised when fed as free fatty acids. But when fed as intact triglycerides or as a mixture of oleic (18:1) or linoleic (18:2) acids, absorbability was greatly improved. The works of Siedler (1955), Shannon (1971) and recently, Alao and Balnave (1985) support these findings. These workers utilised tallow and linoleic acid and other unsaturated fatty acids in their various studies.

Through their various studies, Mateos and Sell (1981) provided a conclusive evidence to show that the "extra caloric" effect of supplemental fat resulted to a large degree from the reduced rate of food passage through the gut. This delayed passage increased the overall digestibility of the diets as well as enhance a synergistic effect between the saturated and unsaturated fats.

Earlier studies by Fuller and Miora (1973), attempted to increase voluntary feed consumption of young chickens subjected to heat stress by manipulating the diet so as to reduce its heat increment. This can be accomplished by replacing carbohydrate calories with fat calories and by reducing total protein level while maintaining appropriate levels of critical amino acids in relation to energy.

This study and others indicated that the heat stressed bird may have a real problem in consuming enough net energy for optimal growth when fed diets containing high heat increment ingredients, (Jackson et al., 1969; Leeson and Summers 1976; Fuller and Rendon, 1977; Horani and Sell 1977; Dale and Fuller, 1979; Grinbergen et al., 1982; Brue and Latshaw 1985).

Dale and Fuller, (1979) cycled temperatures diurnally (cool: 14.1 ± 1 to 22.4 ± 1 C; hot: 22.3 ± 1 to 33.0 ± 1 C) to simulate the natural conditions. From their results they demonstrated that dietary fat was of greater value in promoting chick growth under heat stress conditions than in moderate environments. In another experiment, Dale and Fuller (1979) showed that reduced feed intake did contribute to the growth depression associated with high temperatures. A follow up work by same workers in 1980 showed that broilers held at constant 31 C environment did not benefit from added fat as occasioned by poor feed intake.

More recently research work on dietary fat inclusion has widened to include studies on ameliorating the diet of heat stressed egg producing birds. The advantages claimed for such use of high energy rations are a reduction in the amount of feed consumed per dozen eggs (Hill et al., 1956; Frank and Waibel, 1960; Treat et al., 1966; DeGroote, 1972; Kim et al., 1976) and an increase in egg production performance (Hill et al., 1956; Donaldson and Gordon, 1960; Campos et al., 1960; Wolf et al., 1969; Bolton et al., 1970; Mowbray and Sykes, 1971; de Andrade et al., 1976, 1977). Working under experimentally induced high temperature conditions, Bennion and Warren (1933); Payne, (1966) and Wilson et al. (1973) had reported decreased egg production and weight and shell thickness. These changes were naturally accompanied by decreaded feed intake.

Working with 13-month old laying hens, to evaluate the net energy (NE) of tallow and its energetic efficiency of metabolisable energy (ME) conversion to NE at 18.3 and 35 C, Valencia et al. (1980) found that ME needs were 20.3% less at 35 than 18.3 C (104 Vs 130 Kcal/kg physiological body weight). There was a reduction in voluntary intake of feed at 35 C, resulting in low consumption of ME. The reduced

maintenance requirement increased the amount of energy available for egg production at 35 C.

Working with various levels of palm oil sludge (POS) inclusion rate, in a corn-soy diet, Leong et al., (1982, 1983) found that the optimal amount of POS was 10% with amino acid supplementations of DL-methionine and lysine to give equable performance in feed intake, egg production, feed efficiency and egg quality comparable to that of the control corn-soyabean meal diet.

Reid (1983) reported an evidence to support the feeding of tallow at levels below 4-5% of the diet to produce all the advantages previously noted by Mateos and Sell (1981). This level of fat inclusion in layer diet was found to increase energy balance through increased egg output and increased body weight. It also had the beneficial effect of stimulating ME intake during periods of either high or low temperature.

In their studies, de Andrade et al., (1977) using cyclic temperature (26.7 - 35.6 C) and constant temperatures (31 C) demonstrated the overall ameliorating effect of "High Nutrient Density" (HND - 20 and 25) diet could have on layer performance. In both environments, they recorded significant improvements in egg production, feed consumption, egg weight, body weight and bird livability. The HND-20 and 25 diets were a corn-soy layers formulation with 20 or 25% more of all nutrients except energy which was increased by 10%. Even the thyroxine and hematocrit levels, which levels have been observed by other studies to be depressed by high ambient temperatures, were shown in these studies to be highers in the HND treated groups of birds.

## 2:5 EFFECT OF VARYING THE PROTEIN: ENERGY CONCENTRATIONS ON THE PRODUCTIVE PERFORMANCE OF HEAT STRESSED BIRDS:

High environmental temperature has been demonstrated to have a depressive effect on productive parameters of the laying hen. Many research workers have demonstrated that under a condition of mild heat stress, egg production, size and shell quality depressions occur while feed efficiency is improved (Bennion and Warren 1933; Wilson, 1949; Hutchinson, 1953; Huston, 1958; Mueller, 1959, 1961; Bragg and Stephenson, 1964; Ahmad et al., 1967; El-Boushy et al., 1968; Bragg et al., 1971; Zimmerman et al., 1972; Miller and Sunde, 1975; de Andrade et al., 1976, 1977; Cowan and Michie, 1980).

Most workers agree that feed intake is inversely related to high ambient temperatures (Bennion and Warren, 1933; Mueller, 1961; Prince et al., 1961; Payne, 1966; Lodhi et al., 1975; de Andrade et al., 1976; Wolfenson et al., 1979; Vohra et al., 1979; Emery et al., 1984;). [illegible] et al., 1984. Egg weight has been widely reported also to be depressed (Bennion and Warren, 1933; Warren and Schnepel, 194C; Hutchinson, 1953; Mueller, 1961; Carmon and Huston, 1965; Miller and Sunde, 1975; de Andrade et al., 1976; Vohra et al., 1979; Wolfenson et al., 1979; Cowan and Michie, 1980).

Hurwitz et al., (1980) working with both chicks and turkey poults of both sexes placed in environmental chambers of 12, 19, 28 and 34 C, demonstrated that between 19 and 34 C, weight gain decreased linearly with temperature. Feed intake decreased in both sexes over the entire range of temperatures tested. Feed efficiency (g gain/g feed intake) increased with temperature to reach a maximum at $27^{C}$ and then decreased between 27 and 34 C especially in the females. Payne, (1966) estimated the reduction in feed intake to be 1.6% per degree Celsius temperature

rise between 17 and 29 C and suggested decreased food consumption was mainly due to a decline in energy requirement rather than an effect of increased temperature per se. Hurwitz et al., (1980) also noted that the maintenance requirement in the laying chicken decreased as environmental temperature increased to reach a low at 27 C followed by an increase up to 34 C. They observed that except for the lowest temperature, 12 C, the maintenance requirement appeared to be higher for low energy high protein diets than for the high energy diet.

The intake of feed is influenced by its metabolizable energy (ME) content. It has been suggested that hens tend to eat to satisfy their energy requirements (NRC, 1977). Earlier research by Titus (1929) evaluated the gross daily maintenance requirements of laying hens at 64 to 66g. of the feed mixture employed and found that 37 to 40g of feed was needed to produce an egg. Later, Peterson et al., (1973), showed that the mature hen obtains more ME from several feed ingredients than does the growing chick, indicating that the calculated energy values for laying hen may be considerably lower than actually realized in feeding.

Although hens do eat to meet their energy needs, they do not consume feed to meet their protein requirement (Lodhi et al., 1975). Consequently, a suboptimal level of dietary protein in relation to energy may become the limiting factor for growth and production in heat stressed birds.

It is a widely accepted principle of poultry nutrition now that dietary energy and the essential nutrients must be considered as an entity when the energy content of the diet is increased (Slaghter and Waldroup, 1984). There is also an inverse change in the total amount of feed consumed which will affect the intake of essential nutrients (Slaghter and Waldroup, 1984). As a result, the calorie -to-protein ratio of a diet is important for its influence on growth and feed

conversion. Frapps (1943) determined that when the levels of productive energy were increased in relation to the protein level, live weight and body fat increased. Hill and Dansky (1956) and Leong et al., (1959) showed that as energy levels of diets increased, the percentage of protein in the diets also had to increase to maintain optimum growth in broiler chicks. Hutagalung et al. (1980) found birds grew faster and had better efficiency of feed conversion on low energy diets with increasing dietary protein. The addition of palmoil in the study increased the net energy of the diets.

Hurwitz et al., (1980) in the turkey study, reported that feeding high protein diet in a hot environment did not overcome the growth depression. They also reported that the calculated amino aicd/kilocalorie dietary requirements for 6 weeks old chickens increased with rising temperature. Similar findings were made by Denton et al., (1974). Menge et al., (1979), reported that for turkey hens, best egg production and largest eggs were laid by hens fed 18% protein diet containing 2.3Kcal of energy/kg feed.

Valencia et al., (1980), using isocaloric diets, fed five protein levels (12, 14, 16, 18 and 20%) to hens kept at 21 or 32 C environment and found that estimates of energetic efficiencies at 21 C varied from 60.9% for the 12% protein diets to 72.4% for the 18% protein. At 32 C, similar diets showed lower energetic efficiencies for ME conversion to net energy (50.6% for the 12% protein diet and 70.6% at 20% protein) due to lower feed intakes.

Slaghter and Waldroup (1984) recommended the use of temperature-adjusted energy: amino acid ratio as the only valid and feasible means of formulating diets to meet the changing nutritional needs of laying hens. Reid (1976) had concluded that a level of 14.6% CP and 2.75 ME Kcal/g was adequate to support an egg production of 77% at an average

protein intake of 16.54g/hen/day. Solun and Bunicelu (1968) working in Rumania had reported a dietary level of 14% protein and 2657 ME Kcal/kg feed for the best performing birds during the hot summer months.

In their contribution, Olomu and Offiong (1983) fed three protein levels of 16, 18 and 20% and three ME levels of 2400, 2600 and 2800 Kcal/kg diet were fed to 24week old layers in hot tropical environment and monitored eventual egg production. They observed that dietary protein level had no significant effect on egg Parameters and hen productive capacity. The highest energy level significantly depressed egg production as well as feed and protein intakes. Their study demonstrated that the use of 16% protein and a ME level of 2400 Kcal/kg diet was adequate for brown egg-type pullets under hot tropical conditions. Similar results had been reported by Millar and Smith (1975) who showed that brown egg type birds not stressed by cage crowding will do well on 16% protein and 2800 Kcal ME/kg feed. Also, Anyaehie and Obioha (1986) concluded that the nutritive value of the laying hen's diet is determined mainly by its amino acid composition and that 16% CP of an all vegetable diet containing 2.80 Kcal ME/kg feed when supplemented with 0.148% L-lysine and 0.109% Dl-methionine to supply a total of 0.693% lysine and 0.615% Methione + cystine levels would support a relatively satisfactory laying hen performance.

Desai and Khire (1981) found that production tended to increase as dietary protein increased to 20%. However, Onwudike (1983), reported that beyond 18% protein no significant effect accured by further increases in dietary protein. Pearson and Herron (1981), calculated that a daily protein intake of 19.5g appeared adequate for laying chickens provided that the essential amino acid concentrations were maintained in the diet. Voreck and Kirchgessner (1980) also reported that a ME of 460kJ/kg $^{0.75}$ daily was adequate to support

laying performance in hens while Neumann and Kirchgessner (1983), showed that protein intake of 17.7g or over gave maximum egg production.

The protein level must provide adequate amounts of the essential amino aicds to support production at high level (Hill, 1969). In general, the amino acids most likely to be limiting in practical diets are methionine and lysine. The INSP (1983) recommended standards for Middle East countries are for lysine 8g/kg feed and for methionine 3.5g/kg feed.

Reports on inclusion of lysine and methionine indicate that lysine included at 600 to 840mg/kg feed and methionine at between 330 and 430mg/kg feed or 600mg/kg feed of methionine + cystine was adequate for laying bird performance (Weerden and Schutte, 1980; McNaughton et al., 1980; Schmidthborn and Tanner 1980; Behrends and Waibel, 1980; Nathaniel and Sell, 1980; McDonald, 1980).

## 2:6 EFFECT OF HIGH ENVIRONMENTAL TEMPERATURE ON EGG SHELL QUALITY:

Egg shell quality is of a major economic importance to the egg industry. It has been estimated that 6.5 percent of the total egg produced annually in the United States of America (USA) are lost or downgraded due to poor shell quality (Keshavarz, 1985). When converted to dollars the staggering annual figure of about $60 million is involved. This problem is not just American but in all important egg producing nations. The figure for the 22 of these nations is about $66 million per year (Keshavarz, 1985).

Despite the extensive research done to date in this area, shell breakage still remains a problem of tremendous concern to egg producers.

The site of egg shell deposition in the hen is the uterus which is also called the shell gland. The egg ramains in the uterus for some 18 - 22hours (Scott et al. 1976). Consequently those factors which

compel the egg to leave this site of shell deposition earlier than the natural length will reduce shell quality.

Poor shell quality has been attributed to the hen itself. Bennion and Warren (1933) and Smith and Oliver (1972) observed that the decline in egg weight and shell thickness at high temperatures was partially due to the direct effect of thermal stress on the hen and not solely due to a reduction in feed intake. This was confirmed by Emery et al. (1984). Peterson and H/J (1980) concluded that it is not only the content of calcium in the feed that determines egg shell quality but that the ability of the laying hen to mobilize bicarbonate ions is also an important factor in shell formation.

The ability of the older hen to absorb or mobilize calcium from the bones was assumed to be responsible for the poor shell quality in the later stage of production cycle of the hen. However, it has been shown (Roland, 1979) that the aged hens ability to absorb and mobilize skeletal calcium in the process of shell calcification was just as good as that of the young hen. The amount of shell deposited did not reduce but remained fairly constant or even increased slightly (Roland et al., 1975, 1978, 1979). The decline in shell quality was due to the continued increase in egg size as laying proceeded. The constant level of calcium available for deposition over the egg is forced to spread out judiciously but more thinly over the larger egg.

In a follow-up work (Roland, 1980) tried unsuccessfully to manipulate dietary protein, amino acids, energy and calcium to reduce egg size while maintaining the amount of shell deposited on the egg. Petersen et al.(1983) demonstrated that restriction of dietary methionine effected the required decrease in egg size while increasing shell quality without any associated decrease in egg production,

The deleterious effect on feed intake and subsequently on shell quality of increases in ambient temperature beyond the zone of comfort for the fowl on feed intake and consequently shell quality, has received attention of many workers (Warren and Schnepel, 1940; Heywang, 1946; Wilson, 1949; Hutchinson, 1953; Mueller, 1959, 1961; Miller and Sunde, 1975; de Andrade et al., 1976, 1977; Deaton et al., 1981; Emery et al., Conversely, low temperature is confirmed to yield enhanced feed consumption which improved shell quality (Campos et al., 1980; Mueller, 1966; Miller and Sunde, 1975; de Andrade et al., 1976; de Andrade et al., 1977; Deaton et al., 1981).

In their contribution, Miller and Sunde (1975) used pullet layers of between 29 and 42 weeks of age. The birds were housed under constant or cyclic temperature environments which varied from 10 to 37.8 C. The authors reported that within hours of moving birds from the cold environment to the hot environment recording of a 50% mortality (two out of four) and the production of shell-less eggs for several days were made indicating a major physiolofical change. The birds showed a rapid adaptation to the change by a cessation of mortality and renewed production of sound eggs. These workers felt that the abrupt adverse effect of the change to the high temperature may be due to the development of respiratory alkalosis and subsequent compensatory renal excretion of calcium bases. They reported that both gradual and abrupt increases in temperature gave immediate declines in shell rigidity, egg weight and feed consumption, but the effect on egg production was not markedly noticed over an extended period of time. Constant high temperature (32 C) resulted in lower shell deformation than in cyclic hot temperature (26 - 38 C), but cyclic temperatures gave higher egg production records. During the three months exposure to the experimental treatments, the proportion of the egg component parts remained unchanged for birds in the constant temperatures of 10, 21 and 32 C and in cyclic

temperatures from 26 to 38 C. Bone mineralization remained unaffected by the treatments, thus indicating a maintenance of hormonal balance even if at a different level. Also bone depletion did not occur under the stress of high temperatures and shell formation even though egg production was maintained.

Deaton et al., (1981) suggested that increasing the differences between day and night temperatures in poultry houses during the summer months may be an effective method of reducing the adverse effect of high environmental temperatures on shell quality.

Miller and Sunde (1975), in agreeing with Mueller, (1959) reported that although birds kept in high environmental temperature consumed less feed and calcium, they retained similar levels of calcium under normal temperature. In their study both Meyer et al. (1973) and de Andrade et al. (1976) agreed that the effects of temperature and diet on shell thickness and shell nitrogen appear to be mediated via changes in the palisade layer of the shell. Meyer et al., (1973) supported Carter's (1969) findings that the scanning electron micrographs revealed that most of the changes responsible for difference in shell breaking strength apparently took place in the palisade layer (Spongy layer) while the mammillary layer remained approximately constant. It is known that the mammillary layer contains most of the nitrogen (Simkiss & Tyler, 1957; Tyler and Simkiss, 1959b). Thus, if the nitrogen and mammillary layer are constant then it is the changes in the palisade layer that gives thickness to the shell structure. In order to ensure higher intake of all critical nutrients for egg production during high temperatures, de Andrade et al., (1976, 1977) fed high nutrient density diets (HND - 20 and HND - 25) to test groups. The trial was successful in increasing egg production but had no effect on specific gravity, shell thickness or percent shell.

de Andrade et al., (1976), showed that the high environmental temperature actually decreased the amount of true shell in the egg while increasing the percentage of nitrogen in the shell, but did not have any significant effect on the calcium content of the true shell. The use of "high nutrient density - 20" diet (HND-20) resulted in significant increases in the true shell while significantly reducing the nitrogen in the true shell. In their subsequent work, de Andrade et al. (1977), were able to demonstrate that calcium consumption did not play a role in the effect of high temperature on egg shell. The birds, in that work, which were fed the HND - 25 diet at 31 C constant environment consumed significantly more calcium (2.5g/bird/day than those in the 31 C cyclic environment (2.13 and 2.36g/bird/day for the control and HND - 25 diet respectively but yet showed lower specific gravity. The study confirmed that an average environmental temperature of 31 C, regardless of whether it was constant or cyclic, significantly decreased shell thickness. The use of HND - 25 diet did not improve shell thickness. This agreed with the findings of Miller and Sunde (1975).

Tannor et al. (1984) used 192 SCWL pullets to study the effect of heat stress and diet composition on feed intake and laying performance at 21, 25 and 33weeks of age. For each age group, all birds were fed a control diet (17.1% CP, 2770 Kcal ME/kg) for 7 days. The birds were kept at a house temperature of 18 C. The house temperature was abruptly increased to 35 C, and the various groups of birds offered either the control diet, a high proteindiet (43.5% CP), a high energy diet (3371 Kcal ME/kg), or a diet of high nutrient density (28.3% CP, 2842 Kcal ME/kg, and 6.5% Ca) for a 3-day period. After the 3-day test period, temperature was returned to 18 C. The birds were maintained on

the experimental diets for 4 days prior to returning to the control diet. The authors reported that feed consumption, egg production, egg weight, and egg shell thickness decreased ($P \leq 0.05$) with heat stress. Increases in energy and calcium intake helped partially to maintain normal egg production, egg weight, and egg shell deformation. Egg weight and egg shell deformation returned to pretest levels within 8 - weeks post-experimental period. With the exception of birds receiving the control and high density diets at 21weeks of age, data taken during the test period showed that heat stress caused a significant reduction in the liveweight of the birds.

In conjuction with heat stress, shell quality is affected by many nutritional factors. Among them are calcium, (Scott et al., 1977) phosphorus Scott et al. (1976) sodium (Hill, 1969) potassium chloride (Mongin, 1968) manganese, (Hill, 1969) vitamin D, (Shen et al. 1981) and recently Vitamin C (El-Boushy and Van Albado, 1970). Of these factors calcium is the most important ingredient in the feed to ensure the perfect egg shell.

Calcium, when fed inadequately produce eggs with inferior shell quality (Scott et al., 1971). Research workers agree that a calcium level of 3.0 to 4% in the diet and a total phosphorus level of between 0.55 and 0.8% are adequate to maintain egg weight and maintain shell quality (Pepper et al., 1961; Kovack, 1977; Garlich 1979; Vest et al., 1979; El-Boushy and Papadoponles, 1979; Scott et al., 1982). Proportion of shell increased with increasing calcium in the diet (El-Boushy and Papadoupolus, 1979; Ousterhout, 1980; Mathur et al., 1982. Ousterhout (1980) recommended that calcium level be decreased during the early months of production to maximize early egg weight, then increased as the hen ages to maximize shell quality by increasing shell weight while limiting increases in egg weight. Barton (1979) recommends a 3.25% Ca.

and 0.4% available phosphorus (aP) until 36 to 40weeks and a switch to 3.5% Ca until 55 to 60weeks of age with a final ration of 3.75 to 4% Ca depending on seasonal changes.

Excess phosphorus level give inferior shell quality (Scott et al., 1976; Ousterhout, 1980; Hennig et al., 1982). During daily temperatures of 32 C or more, NRC (1983) recommended 32.5g/kg feed and 5.0g/kg feed for calcium and phosphorus respectively for the Middle Eastern countries.

# CHAPTER 3.

# EXPERIMENTS.

## Experiment 1: Effect if various levels of protein and energy on the performance of heat stressed laying chickens.


### Summary

One hundred and sixty-two, 29 week old layers were used in a 3 x 3 factorial design to determine the optimal protein - energy combination capable of maintaining egg production and egg quality of laying hens during the hot months in a tropical environment. Three protein levels (18, 16 and 14% CP) and three energy levels (2800, 2600 and 2400 Kcal ME/Kg diet), were combined and fed in nine treatments with three replicates per treatment. Feed and water were provided ad libitum. The indoor and outdoor air temperatures were recorded by a maximum and minimum wall thermometer. The study lasted for 16 weeks.

Results showed that egg production was maintained when diets with lower energy and protein levels were fed. Hens on 14% CP combined with 2400 Kcal. ME/Kg diet gave the best production. This was followed by hens fed 16% CP with 2600 Kcal. ME/Kg. diet and 14% CP with 2800Kcal. ME/Kg diet. Hens on 18% CP diet performed poorly under the hot climate.


### INTRODUCTION:

It is recognised that mild heat stress reduces egg production, egg size and egg shell quality while improving feed efficiency (Campos et al., 1960; Mueller, 1961, 1977). Earlier research and the works of Vohra et al. (1979), Tanor et al. (1984) and Emery et al. (1984) have shown that feed consumption is inversely related to environmental temperature. This deleterious influence of high ambient temperatures on layer performance is of particular importance to poultry farmers in the tropics. Decreased feed consumption is also accompanied by

decreased body weight at high temperatures (Wilson, 1949; Huston et al., 1957; Campos et al., 1960; Mueller, 1961 and Miller and Sunde, 1975).

Miller and Sunde (1975), reported an increase in mortality, production of shell-less eggs for several days when birds were moved abruptly into a hot environment. Bray and Gesell (1961) and Payne (1966 a, b) reported that the effects of constant high temperatures on egg production could be minimized by proper nutritional formulation to provide adequate amino acid intake.

Research workers in the tropics have recommended conflicting protein: energy levels for optimum egg production of birds in the tropics. Babatunde and Fetuga (1976), and Olomu (1978) recommended energy level of 2600 Kcal ME/kg feed while the former also found performance superior with 16% CP for laying chickens. A higher level of protein was recommended for the Indian summer with average indoor temperature of 37.32 C. Sungadi et al. (1975) recommends 18% CP while Chawla et al. (1976) opted for 19%. Aguilera et al. (1974) found the optimum protein levels to be between 15 and 18%. Scott (1974) suggested that in a hot climate an ME level of 2400 Kcal/Kg diet was adequate to give satisfactory production of eggs. In their study Olomu and Offiong (1983) recommended the use of 16% CP and energy level of 2400 Kcal/Kg diet for the brown egg type 1 layers.

This study is carried out to determine the best combination of energy and protein levels that will optimise egg production at a constant level of other nutrients during the hot months of the year in a tropical environment.

MATERIALS AND METHODS:

One hundred and sixty-two Shaver Starrcross "X" Hubbard Golden Comet of twenty nine weeks of age were used for this experiment. All the birds were vent examined to ensure that they were in lay. The birds were weighed individually and randomly allocated to cages. Two of such cages (total 6 birds) constituted a replicate. Three replicates were randomly selected and assigned one of the nine dietary treatments. At the end of sixteen weeks, all the surviving birds were weighed and each weight was recorded as final body weight.

The laying house, situated at the Ahmadu Bello University Farm, Shika, was the open-sided tropical type, fitted with two-tier battery cages with feeders and waterers. Each cage had a capacity for three birds and measured 49 x 35 x 42cm. Flat metal partitioning plates were inserted in the feeding troughs to separate replicate feeds. Experimental diets and fresh clean water were provided ad libitum. No supplementary light was provided. Laying chickens were observed daily and egg production and mortality records kept. All dead birds were promptly refered to the Faculty of Veterinary Medicine Diagnostic Laboratory for examination. Feed intake was measured at the end of each twenty-eight day period. The temperature of the layer house was recorded daily through the use of a Maximum Minumum Wall Thermometer (G.H. Zeal Ltd., London), while the outdoor air temperature and percent relative humidity readings were supplied daily from the Samaru weather report of the Institute of Agricultural Research (IAR), Ahmadu Bello University, Zaria (Fig. 1). Appendix 9, shows the summaries of temperature data.

A 3 x 3 factorial design comprising of nine dietary treatments with three protein and three energy levels was used. The protein levels were 18, 16 and 14 percent crude protein (CP) while the energy

Table 2: Percentage Composition and calculated analyses of diets including cost per 100kg diet - Experiment 1

| Ingredient | Diets 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| | 16-2800 | 16-2600 | 16-2400 | 16-2800 | 16-2600 | 16-2400 | 14-280 | 14-2600 | 14-2400 |
| Maize | 61.9 | 54.6 | 47.2 | 65.00 | 57.6 | 50.36 | 68.00 | 60.60 | 53.42 |
| Wheat ofaal | 0.00 | 4.5 | 19.0 | 3.50 | 12.5 | 21.50 | 6.50 | 15.70 | 24.00 |
| Soya bean meal | 28.10 | 26.1 | 24.1 | 21.70 | 19.9 | 18.14 | 15.50 | 13.70 | 12.18 |
| Bone meal | 2.40 | 2.40 | 2.40 | 2.40 | 2.40 | 2.40 | 2.40 | 2.40 | 2.80 |
| Limestone | 7.00 | 6.80 | 6.70 | 6.80 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 |
| Salt | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Premix[1] | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Methionine | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| Total | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| Cost of feed per 100kg (3) | 63.29 | 61.96 | 60.63 | 58.88 | 57.55 | 56.32 | 54.47 | 53.21 | 52.06 |
| Calculated Nutrient Levels | | | | | | | | | |
| Crude Protein, % | 17.98 | 17.95 | 17.95 | 15.98 | 15.97 | 15.99 | 14.00 | 14.00 | 14.02 |
| Metabolizable Energy, Kcal/kg. | 2803.00 | 2602.00 | 2400.00 | 2805.00 | 2600.00 | 2402.00 | 2803.00 | 2600.00 | 2402.00 |
| Calcium, % | 3.61 | 3.50 | 3.37 | 3.38 | 3.41 | 3.56 | 3.44 | 3.46 | 3.58 |
| Phosphorus Total, % | 0.79 | 0.62 | 0.81 | 0.63 | 0.73 | 0.81 | 0.68 | 0.74 | 0.86 |
| Phosphorus (avail.), % | 0.44 | 0.41 | 0.38 | 0.44 | 0.43 | 0.47 | 0.43 | 0.45 | 0.52 |
| Lysine, % | 0.97 | 0.95 | 0.30 | 0.80 | 0.78 | 0.78 | 0.66 | 0.64 | 0.75 |
| Methionine + Cystine, % | 0.69 | 0.68 | 0.68 | 0.64 | 0.67 | 0.61 | 1.17 | 0.56 | 0.68 |

1 The Premix (ZOODRY 301) furnished the following amounts of other ingredients per kilogram of feed: Vitamin A2, 16676 I.U. vitamin $D_3$, 3350 I.U., vitamin $E^2$ 16.675 I.U., menadione sodium bisulfite (vit. K)[2], 3.35mg.; vitamin $B_2$ 7.5mg, vitamin $B_6$, 5.0mg; nicotinic acid, 41.67mg., calcium D-pantothenate, 13.35mg.; vitamin $B_{12}$, 0.02mg.; vitamin C, 33.35mg., choline chloride, 33.50mg, manganese, 166.675mg., iron 83.35mg., zinc 66.675mg., copper 4.00mg., iodine 2.335mg, cobalt 0.335mg and Selenium, 0.085mg,

2 - Stablized

3 - Based on 1987 costs.

levels were 2800, 2600 and 2400 Kcal ME/Kg feed. These energy and protein levels were cross-classified in the study (Table 2).

The diets were corn-soy based and each feed was analyzed for proximate fraction (dry matter, protein (CP), ether extract (EE), crude fibre (CF) and nitrogen free extract (NFE) using appropriate A.O.A.C. (1970) procedures (Table 3).

Eggs were collected once a day and recordings of whole and cracked eggs made. On the fifth day of each week (Deaton et al., 1981) all eggs laid by each replicate were collected, labelled and quality evaluated. After four of such evaluations, the data were summarised to obtain the period averages for egg weight, Haugh unit and shell thickness. Egg weight was measured on a Mettlers H balance (Model P. 163). Each egg was broken out at its equator (Keshavarz, 1985) and the height of its albumen and yolk recorded with caliper No 816. The Haugh Unit Score of the egg was calculated from the equation (Card and Neisham, 1972):

$$H.U. = 100 \log (H + 7.57 - 1.7 W^{.37}) \text{ where}$$

H.U. = Haugh unit score

H = Observed albumen height

W = Observed egg weight in grams.

The eggs were sun-dried for three days (Miller and Sunde, 1975) and shell thickness measured at each egg's point of largest circumference (equator) with a Pocket Thickness Measure Micrometer (Ames, Waltham, Massachusetts).

The parameters studied were intitial body weight, hen-day egg production, feed intake, feed per kg egg, feed per dozen egg, cost of feed per kg egg, cost of kg feed per treatment, egg weight, shell thickness, Haugh unit score, percent cracked eggs, mortality and final body weight.

Data collected in the trial were summarized and subjected to analysis of variance. Means of variables with significant treatment differences were further partitioned using Duncan's Multiple Range Test (Duncan, 1955).

## RESULTS:

### EXPERIMENT I:

The results obtained from this study are summarised in Tables 3 to 6, Figures 1 and 2 and Appendices 1 to 7.

Ambient Environmental Temperature: Table 4, Figures 1 and 2, summarise the results of the four 28-day period environmental temperatures (C) and relative humidity (%) for the period of study. The maximum indoor period temperatures ranged between 39.26 and 34.88 C, while the minimum period indoor temperature fluctuated around 23.94 and 21.43 C. The hottest period was the second period while the 4th period was the coolest. From period 3 rainfall was recorded in Samaru and this increased the average relative humidities for periods 3 and 4 while not affecting the minimum and maximum air temperatures.

Egg Production: The summaries of treatment means for the average egg production (hen-day) is shown in Table 5. From the results hens on Treatment 9, that is 14% CP and 2400 Kcal ME/kg feed (14-2400) and Treatment 5 (16 - 2600) produced significantly ($P<0.05$) more eggs than hens in the other treatment groups except treatment 7 (14-2800). Birds fed diets containing 18% CP (Treatments 1, 2 and 3) produced the least number of eggs as production from treatments 2 and 3 was significantly ($P<0.05$) different from the production from treatments 5, 7 and 9. When the treatment means were further partitioned into protein energy main effects (Table 6), the results showed that the group of hens on 14% CP gave the highest ($P<0.05$) egg production which was however not significantly ($P<0.05$) higher than birds fed 16% CP. The hens on 18% CP

Table 3: Proximate chemical analysis of the various diets - Experiment 1

| Diets | Dry Matter (%) | Crude Protein (%) | Ether Extract (%) | Crude Fibre (%) | Nitrogen Free Extract (%) |
|---|---|---|---|---|---|
| 1 | 90.00 | 18.21 | 2.68 | 3.41 | 75.7 |
| 2 | 90.08 | 18.52 | 2.58 | 4.16 | 74.74 |
| 3 | 90.05 | 18.16 | 2.62 | 4.69 | 74.63 |
| 4 | 90.07 | 16.17 | 2.75 | 3.40 | 77.68 |
| 5 | 90.10 | 16.29 | 2.73 | 3.56 | 77.42 |
| 6 | 90.11 | 16.09 | 3.99 | 4.80 | 75.12 |
| 7 | 90.10 | 14.21 | 2.90 | 4.53 | 78.36 |
| 8 | 90.09 | 13.98 | 3.11 | 4.06 | 78.85 |
| 9 | 90.14 | 14.02 | 2.85 | 4.70 | 78.43 |

Table 4: PERIOD[1] SUMMARIES O AIR TEMPERATURES AND RELATIVE HUMIDITY FOR PERIOD OF EXPERIMENT - EXPERIMENT 1

| | AIR TEMPERATURE[2], C | | | | | RELATIVE HUMIDITY, % | | |
|---|---|---|---|---|---|---|---|---|
| | Out Door | | | In Door | | | | |
| Period (dates) | Max | Min | Mean | Max | Min | Mean | AM | PM |
| 1 (March-April 12, 1987) | 36.43 | 21.64 | 29.04 | 38.07 | 23.09 | 25.58 | 33.25 | 16.93 |
| 2.(April-May 11, 1987) | 36.15 | 22.36 | 29.26 | 39.26 | 23.94 | 31.6 | 24.15 | 9.93 |
| 3 (May 12 - June 9 1987) | 33.21 | 21.82 | 27.52 | 36.52 | 23.45 | 29.99 | 69.25 | 46.50 |
| 4 (June 10 - July 8, 1987) | 30.64 | 21.04 | 25.84 | 34.88 | 21.43 | 28.16 | 75.40 | 61.15 |

[1] Equivelent to 28 days

[2] Air temp ranges for laying birds: 12-24 C-Range of ideal temperature for laying hens

25-30 C-Reduced egg size, thin shells, decreased egg production Panting begins

above 30C-Danger of heat prostration. Increasing heat stress

(Ref): EL-Boushy and Raterink, 1985).

Fig. 1: WEEKLY INDOOR AND OUTDOOR AIR TEMPERATURES (C) FOR PERIOD OF EXPERIMENT 1.

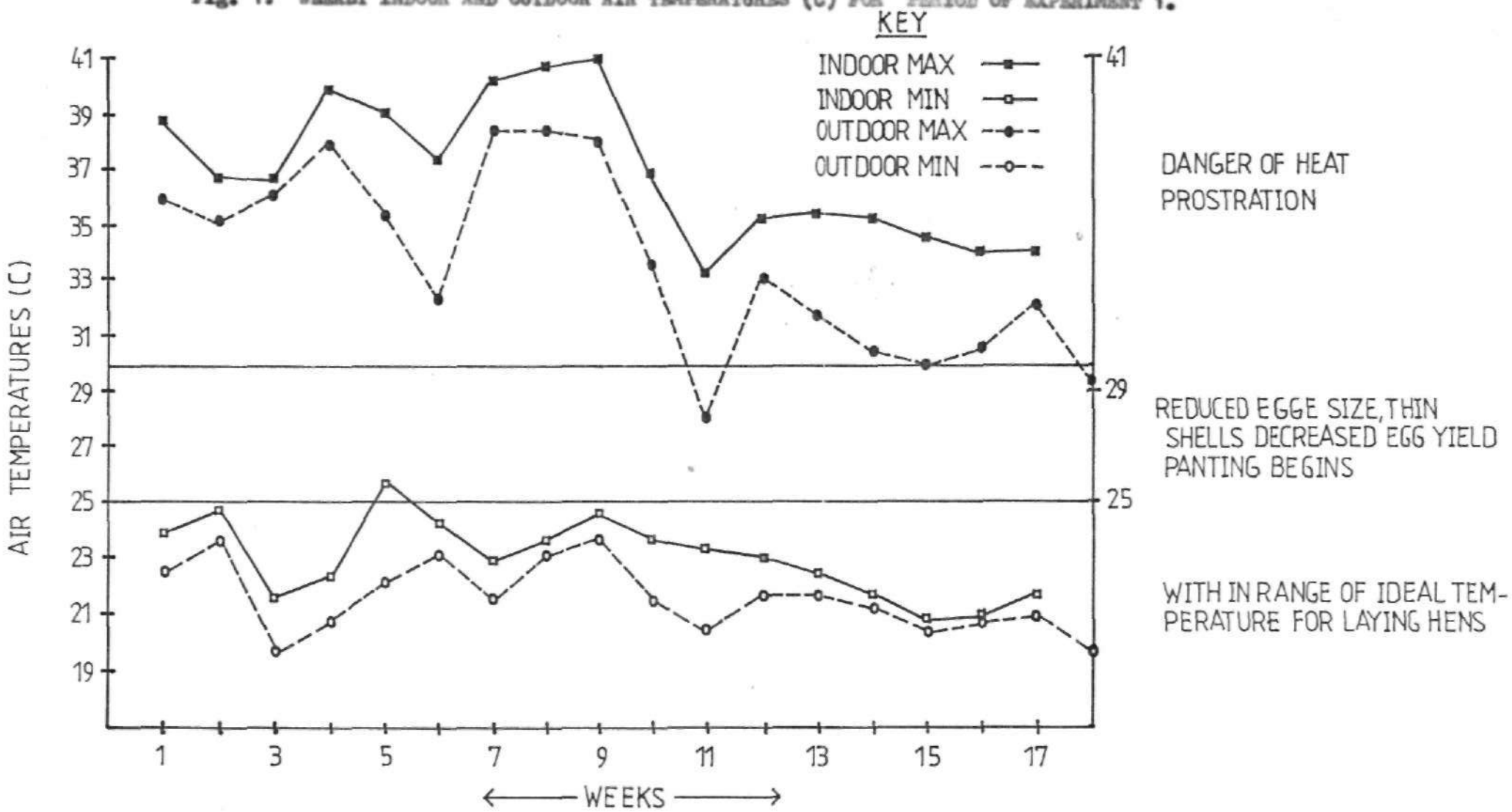


FIG. 2: COMPARISON OF AMBIENT TEMPERATURES (C)
BETWEEN 1938 TO 1986 Vs 1987.

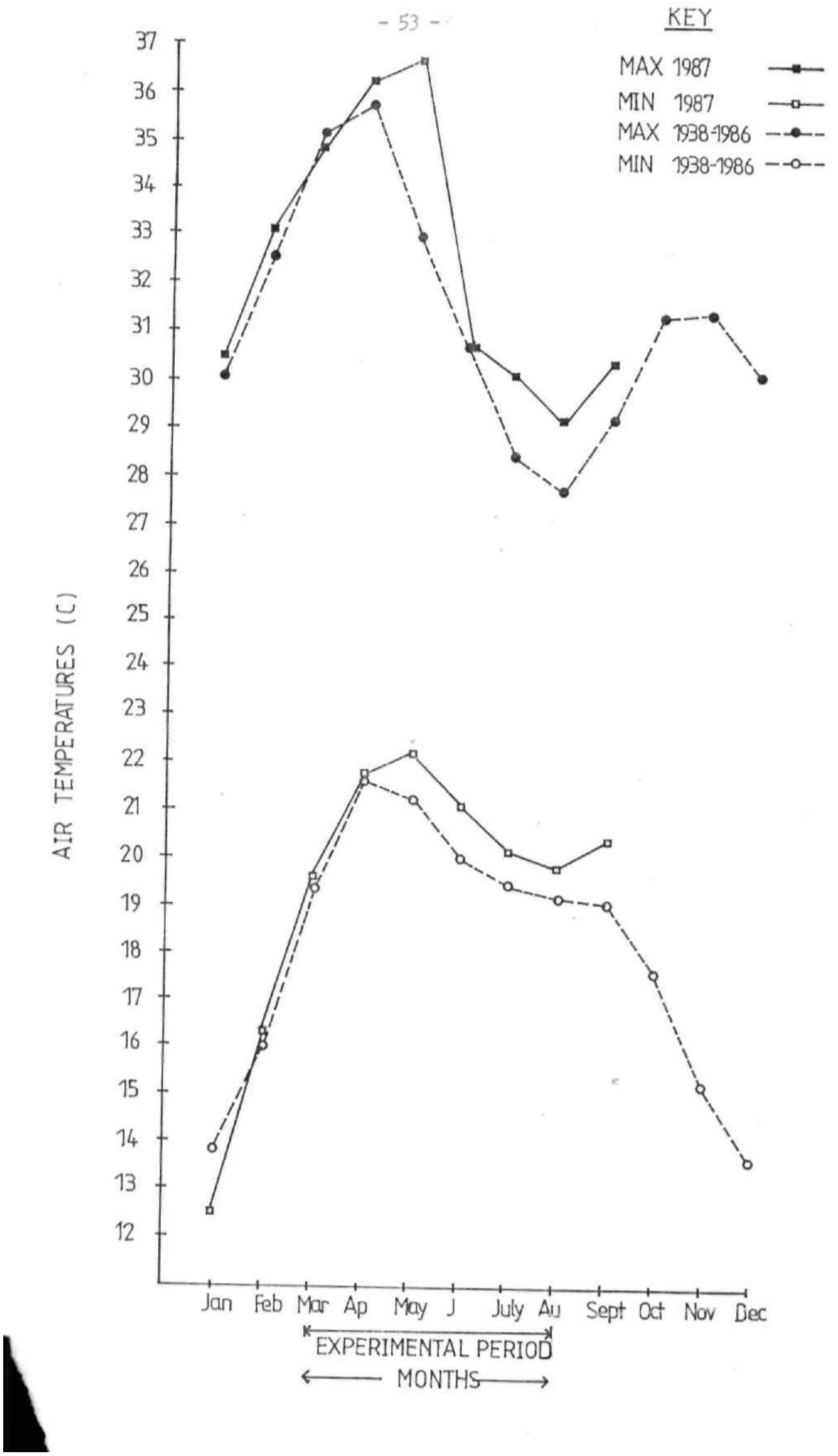

KEY
MAX 1987
MIN 1987
MAX 1938-1986
MIN 1938-1986
AIR TEMPERATURES (C)
37
36
35
34
33
32
31
30
29
28
27
26
25
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Table 5: Treatment means of the effect of feeding different protein : Energy ratios to laying Hens[1] - Epxeriment 1

| Energy, Level, Kcal ME/kg | % Protein level 18% CP | | | 16% CP | | | 14% CP | | | SEM[2] |
|---|---|---|---|---|---|---|---|---|---|---|
| | 2800 | 2600 | 2400 | 2800 | 2600 | 2400 | 2800 | 2600 | 2400 | |
| Treatment | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | |
| Measurement | | | | | | | | | | |
| 1 Hen day Production, % | $57.36^{c}$ | $53.26^{c}$ | $53.3^{c}$ | $57.53^{bc}$ | $66.41^{a}$ | $58.75^{bc}$ | $63.94^{ab}$ | $58.91^{bc}$ | $67.22^{a}$ | 2.22 |
| Average Egg Wt., g | $50.80^{a}$ | $50.60^{a}$ | $49.15^{a}$ | $50.19^{a}$ | $50.09^{a}$ | $51.65^{a}$ | $48.69^{a}$ | $48.97^{a}$ | $49.82^{a}$ | 0.96 |
| Haugh Unit Score, MM | $81.61^{a}$ | $82.30^{a}$ | $83.40^{a}$ | $82.88^{a}$ | $83.58^{a}$ | $79.65^{a}$ | $82.31^{a}$ | $83.28^{a}$ | $83.39^{a}$ | 1.477 |
| Shell Thickness, mm | $0.398^{cd}$ | $0.449^{a}$ | $0.401^{bcd}$ | $0.411^{bc}$ | $0.416^{bc}$ | $0.422^{b}$ | $0.397^{cd}$ | $0.401^{bcd}$ | $0.389^{d}$ | 0.004 |
| Cracked Eggs, % | 2.90 | 0.40 | 2.10 | 0.90 | 1.90 | 1.10 | 3.40 | 3.20 | 3.30 | - |
| Daily Feed intake, g | $98.83^{c}$ | $100.03^{c}$ | $113.83^{a}$ | $91.17^{d}$ | $106.03^{b}$ | $96.90^{c}$ | $100.07^{c}$ | $107.93^{b}$ | $96.97^{c}$ | 1.95 |
| Feed/kg Egg, kg | $3.37^{b}$ | $3.38^{b}$ | $3.42^{b}$ | $3.41^{b}$ | $3.29^{b}$ | $3.19^{b}$ | $3.86^{a}$ | $3.77^{ab}$ | $2.89^{c}$ | 0.0182 |
| Feed/doz. Egg, kg | $2.02^{bc}$ | $2.03^{bc}$ | $2.05^{abc}$ | $2.05^{abc}$ | $1.98^{cd}$ | $1.91^{cd}$ | $2.32^{a}$ | $2.26^{ab}$ | $1.74^{d}$ | 0.0064 |
| Cost of Feed/kg Egg, ₦ | $2.13^{a}$ | $2.09^{a}$ | $2.07^{a}$ | $2.01^{ab}$ | $1.90^{bc}$ | $1.80^{c}$ | $2.11^{a}$ | $2.00^{ab}$ | $1.51^{d}$ | 0.0061 |
| Daily Protein Intake, g | $17.79^{b}$ | $16.67^{bc}$ | $20.47^{a}$ | $15.57^{def}$ | $15.87^{cd}$ | $15.50^{cde}$ | $14.01^{ef}$ | $15.17^{cdef}$ | $13.57^{f}$ | 0.216 |
| Daily Energy intake, Kcal ME/kg | $276.70^{b}$ | $280.1^{b}$ | $318.4^{a}$ | $237.0^{e}$ | $275.70^{b}$ | $251.9^{cd}$ | $240.1^{de}$ | $259.0^{c}$ | $232.7^{e}$ | 13.48 |
| Energy:Protein Intake Ratio | 15.56 | 16.77 | 15.55 | 16.25 | 17.34 | 16.25 | 17.15 | 17.15 | 17.14 | - |
| Final Body Weight, Kg | $1.79^{abc}$ | $1.67^{c}$ | $1.81^{abc}$ | $1.81^{abc}$ | $1.84^{ab}$ | $1.86^{a}$ | $1.74^{abc}$ | $1.71^{bc}$ | $1.68^{c}$ | 0.0014 |
| Mortality, % | 5.56 | 11.11 | - | 11.11 | 5.56 | 5.56 | 5.56 | 22.2 | 11.11 | - |

1. Means within each row with different superscript vary significantly ($P<0.05$)

2. Standard error of means for treatments.

Table 6: Main effect means of feeding different protein : energy combinations to laying hens[1] - Experiment 1

| Measurement | Energy, (Kcal ME/kg Feed) 2800 | 2600 | 2400 | SEM | Level[2] of significance |
|---|---|---|---|---|---|
| Hen-day production, % | 56.61[a] | 59.53[a] | 59.76[a] | 1.284 | NS NS |
| Average Egg weight, g | 49.89[a] | 49.89[a] | 50.21[a] | 0.557 | NS |
| Egg shell thickness, mm | 0.402[a] | 0.422[a] | 0.404[b] | 0.0049 | ** |
| Haugh unit score, mm | 82.27[a] | 83.06[a] | 82.14[a] | 0.853 | NS |
| Cracked Eggs, % | 2.4 | 1.83 | 2.17 | - | - |
| Daily feed intake, g | 96.69[b] | 104.67[a] | 102.57[a] | 2.78 | *** |
| Feed/kg eggs, kg | 3.55[a] | 3.48[a] | 3.17[b] | 0.0149 | * |
| Feed/dozen eggs, kg | 2.13[a] | 2.09[a] | 1.90[b] | 0.0494 | * |
| Cost of Feed/kg egg, ₦ | 2.08[a] | 2.00[a] | 1.79[b] | 0.0494 | ** |
| Daily protein intake, g | 15.46[a] | 15.88[ab] | 16.52[a] | 0.356 | NS |
| Daily energy intake, Kcal/kg | 251.28[b] | 271.60[a] | 267.66[e] | 2.698 | *** |
| Energy : Protein Intake ratio | 16.25 | 17.10 | 16.20 | - | - |
| Final body weight, kg | 1.78[a] | 1.75[a] | 1.79[a] | 0.0253 | NS |
| Mortality, % | 7.4 | 12.96 | 5.56 | - | - |

| | Protein, (%) 18 | 16 | 14 | SEM | Level of significance |
|---|---|---|---|---|---|
| Hen-day production, % | 56.64[b] | 60.90[a] | 63.35[a] | 1.28 | NS |
| Average egg weight, g | 50.18[a] | 50.64[a] | 49.16[a] | 0.557 | NS |
| Egg shell thickness | 0.416[a] | 0.416[a] | 0.396[b] | 0.0049 | * |
| Haugh unit score, mm | 82.44[a] | 82.04[a] | 82.98[a] | 0.853 | NS |
| Cracked eggs, % | 1.8 | 1.3 | 3.3 | - | - |
| Daily feed intake, g | 104.23[a] | 98.03[b] | 101.66[a] | 2.78 | *** |
| Feed/kg eggs, kg | 3.39[a] | 3.30[a] | 3.51[a] | 0.0149 | NS |
| Feed/dozen eggs, kg | 2.03[a] | 1.98[a] | 2.10[a] | 0.0494 | NS |
| Cost of feed/kg egg, ₦ | 2.10[a] | 1.90[b] | 1.87[b] | 0.0494 | ** |
| Daily protein intake, g | 18.31[a] | 15.32[b] | 14.32[b] | 0.356 | NS |
| Daily energy intake, Kcal ME/kg | 291.72[a] | 254.87[bc] | 243.95[c] | 2.698 | *** |
| Energy: Protein intake ratio | 15.94[b] | 16.64 | 17.14 | - | - |
| Final body weight, kg | 1.76[b] | 1.84[a] | 1.71[b] | 0.0253 | ** |
| Mortality, % | 5.56 | 7.4 | 12.96 | - | - |

[1] Means within each row in each main effect with different superscript vary significantly.

[2] * ($P \leq 0.05$)
** ($P \leq 0.01$)
*** ($P \leq 0.001$)
NS = Non-significant

performed the least and the difference was significant (P/0.05) from those on feed containing 14 and 16 CP. The energy effect had no significant (P 0.05) effect among treatments on egg production. The best combinations of protein and energy level were 14% CP and 2400 Kcals ME/kg feed and 16% CP and 2600 Kcals ME/kg feed while the poorest were the diets containing 18% CP, that is 18 - 2600 and 18 - 2400.

Daily Feed Intake: The results for the average daily feed intake are summarized in Table 5. These results show that there were highly significant (P/0.001) differences among the dietary treatments. Hens on Treatment 3 (18 - 2400), consumed the most feed which was highly significant (P/0.05) from that of the other treatment groups. Hens on Treatment 4 (16 - 2800) consumed the least feed which was significantly (P/0.05) different from the feed intake of other treatment groups. Partitioning the results into protein and energy main effects (Table 6) showed that hens on 2600 and 2400 Kcal ME/kg feed levels of energy had the highest intakes which was highly significant (P/0.001) from the intake of birds fed 2800Kcals ME/kg feed. The protein effect showed that hens on 18 and 14% CP consumed more (P/0.001) feed than those on the 16% CP. It must be noted from Table 5 that at the 18% CP level, feed intake increased as energy level decreased and this difference was significant at the 2400 Kcal ME/kg level. For groups on 16% CP diet, feed intake increased (P/0.05) as energy level decreased from 2800 (Treatment 4) to 2600 Kcals ME/kg (Treatment 5). Subsequently as the energy level was further dropped to 2400 Kcal/kg feed (Treatment 6) a significant reduction in feed intake was noted, though this was still significantly (P/0.05) higher than at 2800 Kcal ME/kg feed.

Efficiency of Feed Conversion: Treatment 7 (14-2800) resulted in the poorest efficiency of feed conversion (Kg feed per Kg egg) (Table 5), which was significantly (P$\leq$0.05) different from the other treatments. The feeding of Treatment 9 (14 - 2400) gave the best utilisation of feed for egg production which was significantly (P$\leq$0.05) better than in the other treatment groups. The analysis of treatment means into energy and protein main effects showed a non-significant (P 0.05) Effect all the treatments. Regarding Kg feed per dozen eggs, diets 9, 8 and 5 needed the least feed to produced a dozen eggs.

Egg Weight: Data in Table 5 show that treatment 7 resulted in significantly (P$\leq$0.05) lighter eggs than from the rest of the other treatment groups. There was no significant (P 0.05) difference recorded among the other treatment groups for egg weight. The protein and energy effect on egg weight was not significant (P 0.05) among the treatment groups (Table 6).

Haugh Unit Score: There was no significant (P 0.05) difference among all the treatment groups (Table 4).

The protein and energy effects likewise were non-significant (P 0.05).

Egg Shell Thickness: Treatment 2 (18 - 2600) gave the thickest egg shell for the study which was highly significant (P$\leq$0.01) from the averages recorded for the other treatment groups (Table 5). Treatment 9 (14 - 2800) produced eggs with the thinnest shells. Only a few eggs showed cracks.

Daily Protein and Energy Intake: Hens on Treatment 3 (18 - 2400) consumed significantly (P$\leq$0.05) higher protein than birds in the other treatment groups (Table 5). This was followed by Treatments 1 (18 - 2800) and 2 (18 - 2600). The least protein was consumed by

hens on Treatment 9 (14 - 2400) which consumption was significantly (P/0.05) lower than the other treatment groups. The energy intake was highest for Treatment 3 (18-2400) which was significantly (P/0.05) different from the other treatments. This was followed by Treatments 2, 1 and 5 (16 - 2600) which had a significant (P/0.05) difference from Treatments 8 (14 - 2600) and 6 (16 - 2600). The protein and energy main effects showed that hens fed the 18% CP ate the most protein and energy while those on 14% CP consumed the least (Table 6). For energy effect, hens on 2400 Kcal ME/kg feed consumed more protein and energy than those on 2800 and 2600Kcal ME/kg feed. On the basis of energy: protein ratios it was evident that the 14% CP diets 7, 8, 9) and diet 5 (16 - 2600) gave the highest energy per unit protein consumed. It is also relevant to note that the 14% CP diets produced more eggs than the 18% CP diets (P/0.05) and 16% CP diets (P 0.05).

Initial Body Weight: The randomness with which the hens were allotted to each treatment created an unequal initial body weight in this trial. The average initial weight for the birds was 1.54 kg.

Final Body Weight: Result in Table 5 shows that hens on Treatment 9 had the lightest body weight while Treatment 6 resulted in the heaviest birds. This difference in weight was significant (P/0.05). The protein effect on dietary treatments shows that birds on 16% CP were heavier (P/0.01) than birds on 18 and 14% CP (Table 6). There was no difference (P 0.05) recorded for energy effect on body weight. From these results it could be seen that birds on diet 9 as the lightest birds would have the least maintenance requirement and thus would utilise more of its feed intake for production. Data show this to be true.

Mortality: The highest mortality was effected by Treatment 8 (Table 5). No deaths occurred in Treatment 3. Partitioning mortality into energy

and protein effects show that a combination of 14% CP and 2600 Kcal ME/kg feed resulted in the highest death rate followed by Treatment 5 (16 - 2600), 2 (18 - 2600) and 9(14 - 2400). The causes of deaths were unrelated to the study.

Cost of Feed per Kg Eggs: Treatment 9 produced significantly ($P<0.05$) cheaper feed for egg production than the cost per egg yield of other treatments (Table 5). This was followed by the feed cost for Treatment 6 which was also significantly ($P<0.05$) different from other groups. The most expensive feed for egg production was recorded in Treatment 1 and this was significantly ($P<0.05$) different from Treatments 5, 6 and 9 but not from all the others. The protein and energy main effects in Table 6 show that the most expensive feed is formed from a combination of 18% CP and 2800 Kcal/kg feed while the cheapest feed resulted from combining 14% CP and 2400 Kcal ME/kg feed.

## DISCUSSION:

### EXPERIMENT 1:

The main objective of this study was to compare the combination of various protein and energy levels and determine the best combination that would enable a heat-stressed laying chicken lay optimally in a tropical environment. From the results in Table 3 and Figure 2, it was obvious that Zaria, recorded the hottest and driest month of May in 50years. There was no doubt that the hens were highly stressed by the high abient environmental temperature as panting and wing lifting to dissipate heat were frequently observed despite the supply of adequate cool drinking water. Both of these mechanisms require expenditure of energy resulting in generally higher feed conversion ratio in heat stressed chickens (Dale and Fuller, 1980).

Birds under high heat situations respond by decreasing feed intake (Emmans 1974). As a consequence, there is a consensus that heat-stressed

laying birds should be fed diets with high concentrations of nutrients to provide a high level of dietary protein and energy (de Andrade et al., 1976, 1977; Deaton et al., 1981). This practice ensures the uptake of enough nutrients for optimum metabolic performance. From the results of this study (Table 5) it is evident that the diet containing 14% CP and 2400Kcals ME per kg feed stimulated the most egg production. The heat-stressed hens utilized efficiently the lowest level of protein and energy in the study for the optimum egg production partitioning their ME more effectively into maintenance and productive functions. Thus the superiority in egg production shown by hens on the lowest protein and energy (14 - 2400) diet in the study was due mainly to greater efficiency in the utilization of protein and metabolizable energy since the heat increment levels at this protein-energy combination must have been the lowest.

Chalwa et al. (1976) recommended a protein level of 18.5% and an ME level of 2780 Kcal/kg feed for summer feeding of birds in the tropics. This study does not agree with the findings of Chalwa et al. (1976).

From Table 5, it is also evident that the energy: protein intake ratio for the three dietary treatments that stimulated highest egg production, (Treatments 9, 5 and 7), have higher ratios of 17.14, 17.34 and 17.15 respectively, than did all the other dietary treatment groups except Treatment 8. It does seem therefore that the diets with higher intakes of energy : protein ratio had relatively low protein intakes but appropriate and commensurate energy intake to facilitate egg production.

The partitioning of the results for feed intake into energy and protein main effects (Table 6) showed that there was a highly signific- ($P<0.001$) interaction between dietary energy level and feed intake. At an ME level of 2400 Kcal/kg feed, the hens consumed 102.57g of the feed daily and 104.67g/day at 2600kcal ME/kg feed. However, a further

increase of ME level to 2800 Kcal/kg significantly (P/0.001) depressed feed intake to 98.69g/day. This is in agreement with the findings of Vohra et al., (1979) and Taylor et al. (1984) who also noted that their hens could not eat much of the high nutrient density diet (CP = 28.3%). In this study, the highest protein level was well tolerated by hens as their consumption of the 18% CP diets indicated. There was a linear increase in feed intake as energy level decreased (Table 5).

The efficiency of feed conversion results in Tables 5 and 6 show that the amount of feed required to produce a kilogram of eggs decreased as energy level decreased (P/0.05). This agrees with the findings of McNaughton et al. (1977). The dietary protein however, had little effect on feed efficiency. Similar effects of protein on feed efficiency were also reported by Menge et al. (1979). The hens on Treatment 9 (14-2400) had the lowest feed utilisation efficiency of 1.74 kg feed per dozen eggs. The same hens had the ligtest body weight and produced the most eggs. A relationship could be drawn here that being smaller sized, the maintenance energy requirement of these hens would be much lower than for the heavier hens fed the other dietary treatments.

An increase in dietary protein from 14 to 18% promoted a non-significant (P 0.05) increase in egg weight (Table 5). The protein effect showed the 16% CP gave better egg weights than the 18% CP (Table 6). However, the difference was not significant. This agrees with the findings of Roland (1980). Egg weight decreased when dietary energy was increased irrespective of protein level. This is in agreement with the findings of Menge et al. (1979). Although the weight of eggs for the hens fed 14% CP decreased non significantly as the energy level increased, agreeing with Menge et al. (1979) reports, the egg weight, in this study generally decreased as dietary energy increased from 2400 to 2800 kcal (Table 6). Menge et al. (1979) also

recorded similar decrease in egg weight as the dietary energy level increased from 2.8 to 3.3 Mcal. For the 18% CP diets, (Treatments 1, 2 and 3), egg weights decreased as the energy level decreased from 2800 to 2400 kcal ME/kg feed.

The Haugh Unit Score for albumen quality was relatively constant among the different treatments (Table 5). The energy effect on protein intake increased with decreasing energy level from 2800 to 2400 Kcal ME/kg while the protein effect stimulated a positive linear effect on protein intake (Table 6). Protein intake increased as dietary protein increased from 14 to 18% CP. This agrees with the observation of Keshavarz (1984). Hens on Treatment 3 (18 - 2400) consumed the most protein, had the lowest protein : energy ratio and had one of the lowest levels in egg production in the study.

There was a negative linear effect of level of protein on mortality. Hens on 18% CP generally showed the highest livability Treatment 3 (18 - 2400) had no mortality by the end of the experimental period.

It is evident that in hot months of the year, it is expedient to feed laying birds diets low in energy. From this study diets containing a combination of 14% CP and 2400 Kcals ME/kg feed or 16% CP and 2600 Kcals ME/kg allowed adequate feed, and energy : protein intakes with efficient and high egg production thus making them economically feasible combinations to use under the conditions described.

## EXPERIMENT II: Effect of feeding various levels of vitamin C (Ascorbic acid) to heat stressed hens:

### Summary:

Seventy-two, 33 week old laying hens were used for this 20 weeks experiment to determine the effect of ascorbic acid on the performance of hens during the hot season. Three graded levels of ascorbic acid were added to an isonitrogenous and isocaloric control diet to make

four dietary treatments containing 0, 200, 400 and 600mg ascorbic acid/kg feed. Each treatment was fed to three replicates. Feed and water were provided ad libitum and no artificial light was imposed. Indoor and outdoor temperatures were monitored with maximum and minimum thermometers.

The results of the experiment showed improved ($P<0.05$) hen performance with increases in level of dietary ascorbic acid. However, the study showed that the optimal and most economic level of dietary ascorbic acid inclusion rate was 400mg/kg diet for the period of the study.

## INTRODUCTION:

Emmans (1974) stated that the diminishing energy requirements for maintenance as air temperatures increased was responsible for the reduced intake of feed generally associated with high environmental temperatures. The reduced intake of essential nutrients which accompanied this phenomenon has been found to depress egg production (Tanor et al., 1984), egg weight (Miller and Sunde, 1975); egg shell thickness (ElBoushy and Van Albado 1970) body weight (Valencia et al., 1980) and rate of growth (Dale and Fuller, 1980). Therefore a diet formulated to provide all the necessary nutrients for productive efficiency at a low environmental temperature becomes less adequate as temperature rises.

Ascorbic acid (AA) has been characterized as a non-essential component of poultry ration as it can be intrinsically synthesized by the chicken (Carrick and Hauge, 1925). Recently low blood levels of ascorbic acid has been implicated in the poor performance of heat-stressed birds as the presence of ascorbic acid in the blood serum depresses the level of corticosterone production (Schmeling and

Nockels, 1978) during heat stress (North, 1980).

Perek and Kendler (1963) showed that during conditions of environmental heat stress the capacity of birds to synthesise ascorbic acid is disturbed. Increases in egg production (Peebles and Brake, 1980) shell thickness (El-Boushy and Van Albado, 1970), egg weight (Lazar et al., 1983), feed intake (Thorton and Moreng, 1959) and improved livability (Perek and Kendler; 1963) have been reported with ascorbic acid supplementation in the basal diet.

This study was therefore undertaken to determine the effect of ascorbic acid on the performance of laying hens during the hot, dry season of the Savannah belt of Nigeria.

## MATERIALS AND METHODS:

Seventy - two 33 weeks old Shaver Starrcross 579 x Hubbard Golden Comet laying hens were used for this study. The birds were randomly selected, weighed and placed individually in battery cages. Three of such birds were located in a cage which measured 49 x 35 x 42 cm. Two of such cages constituted a replicate, while three randomly selected replicates were fed one of four dietary treatments. The temperature of the laying tropical half-wall house was monitored daily with a maximum wall thermometer (Casella London 375780) and a minimum wall thermometer (Casella London 37629 C).

Four isonitrogenous and isocaloric corn-soyabean based dietary treatments were formulated (vide Table 7). Diet 1 served as the control and was devoid of supplemental ascorbic acid. Diets 2, 3 and 4 were supplemented with 200, 400 and 600mg of ascorbic acid (Hoffman-La Roche) respectively per kg of feed. Each feed was analyzed for proximate fractions namely dry matter (DM), crude protein (CP), ether extract (EE), crude fibre (CF), and by difference, nitrogen free,

Table 7: Percentage Composition and Calculated analyses of diets including cost per 100kg diet - Experiment 2

| Ingredient | Diet 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Maize | 65.00 | 65.00 | 65.00 | 65.00 |
| Wheat offal | 3.50 | 3.50 | 3.50 | 3.50 |
| Soya beanl meal | 21.70 | 21.70 | 21.70 | 21.70 |
| Dicalcium Phosphate | 1.20 | 1.20 | 1.20 | 1.20 |
| Limestone | 8.00 | 8.00 | 8.00 | 8.00 |
| Salt | 0.25 | 0.25 | 0.25 | 0.25 |
| Premix[1] | 0.25 | 0.25 | 0.25 | 0.25 |
| Methionine | 0.10 | 0.10 | 0.10 | 0.10 |
| Vitamin C | - | 0.02 | 0.04 | 0.06 |
| Total, % | 100.00 | 100.00 | 100.00 | 100.00 |
| Cost of feed/100kg, (¢) | 59.91 | 60.31 | 60.17 | 61.17 |
| Calculated Nutrient Levels: | | | | |
| Crude protein, % | 15.82 | | | |
| Metabolizable Energy, Kcal/kg | 2805 | | | |
| Calcium, % | 3.38 | | | |
| Phosphorus (Total) % | 0.59 | | Ditto | |
| Available Phosphorus, % | 0.36 | | | |
| Lysine, % | 0.91 | | | |
| Methionine + Cystine, % | 0.63 | | | |

1 The Premix (ZOODRY 301) supplied the following per kilogram of diet: Vitamin $A^2$, 16675 I.U., vitamin $D^2_3$, 3350 I.U., vitamin $E^2$, 16.675 I.U., Menadione sodium bisulfite (vit. K)$^2$, 3.35mg, vitamin $B_2$, 7.5mg.; vitamin $B_6$ 5.0mg, nicotinic acid, 41.675mg, calcium D-pantothenate, 13.35mg., vitamin $B_{12}$ 0.02mg., vitamin C, 33.35mg, choline chloride, 333.50mg, manganese, 166.675mg., iron, 83.35mg., zinc 66.675mg, copper, 4.00mg., iodine, 2.335mg., cobalt 0.335mg., and selenium, 0.85mg.

extract (NFE); using the approprate AOAC (1970) procedures (Table 8) Water and feed were provided ad libitum but no supplementary lighting was provided.

Eggs were collected daily and parameters studied and analyzed for are as described in Experiment 1. The study lasted 20weeks of five 28-day periods. At the end of the study, data collected were summarized and analysis of variance as well as Duncan's Multiple Range Test was applied (Duncan, 1955).

4.1.3. EXPERIMENT 2: Results:

The results obtained from this study are summarized in Table 5, 7 and 8 and Appendices 8 to 11.

Ambient Environmental Temperature: A summary of the air temperature (C) for outdoor and indoor environments and the relative humidity (%) for the five 28-day period of this study is listed in Table 8. The range of the indoor ambient maximum air temperatures was between 39.15 and 31.80 C. The outdoor maximum temperatures fluctuated between 37.2 and 29.7 C. The highest indoor maximum temperature occurred during the second period of the study. There was an increase recorded in the percent relative humidity owing to the on-set of the rains in the third period. The lowest maximum indoor air temperature occurred during the 5th period. During most of the study periods the birds were observed to be panting or have their wings spread out in order to dissipate heat.

Egg Production: The mean performance for the entire five 28-day periods for hen-day production (%) is given in Table 8. The result in Table 9 shows that hen-day production improved significantly ($P<0.05$) with ascorbic acid supplementation. Treatment 2 supplemented with 200mg ascorbic acid per kg feed produced significantly more eggs than the control (0mg ascorbic acid). Further supplementation to 400mg

Table 8 : Proximate chemical analysis of the various diets - Experiment 2.

| Diet | Dry Matter (%) | Crude protein (%) | Ether Extract (%) | Crude Fibre (%) | Nitrogen Free Extract (%) |
|---|---|---|---|---|---|
| 1 | 90.25 | 16.82 | 2.72 | 3.40 | 77.06 |
| 2 | 90.08 | 16.78 | 2.80 | 3.57 | 76.85 |
| 3 | 90.20 | 16.91 | 2.75 | 3.39 | 76.93 |
| 4 | 90.18 | 16.74 | 2.67 | 3.63 | 76.96 |

Table 9: Period[2] SUMMARIES OF AIR TEMPERATURES AND RELATIVE HUMIDITY FOR PERIOD OF STUDIES[3] - EXPERIMENTS 2 and 3[1]

| | AIR TEMPERATURE [4][5], C | | | | | | RELATIVE HUMIDITY, % | |
|---|---|---|---|---|---|---|---|---|
| | Out Door | | | In Door | | | | |
| Period | Max | Min | Mean | Max | Min | Mean | AM | PM |
| 1 | 36.40 | 21.46 | 28.93 | 38.07 | 23.09 | 30.58 | 35.16 | 17.97 |
| 2 | 37.18 | 22.93 | 30.06 | 39.15 | 26.22 | 32.69 | 20.82 | 9.50 |
| 3 | 33.57 | 21.15 | 27.36 | 35.95 | 22.85 | 29.4 | 67.14 | 40.18 |
| 4 | 31.04 | 20.39 | 25.72 | 34.34 | 19.11 | 26.73 | 73.57 | 58.72 |
| 5 | 29.69 | 19.96 | 24.83 | 31.80 | 19.72 | 25.76 | 81.15 | 67.62 |

[1] Both experiments conducted under the same environment

[2] Equivalent to 28 days

[3] Measurements commenced 18th March and ended 31st July 1987.

[4] Above the thermo-neutral zone for laying hens

[5] Threat of death by heat prostration

Table 10: Effect of Vitamin C (ascorbic acid) Supplementation on performance of laying hens during the hot season - Experiment 2.

| Trt. No | Dietary Ascorbic Acid (mg/kg feed) | Measurements | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Hen day Production (%) | Egg Wgt. (g) | Haugh Unit (mm) | Shell Thickness (mm) | Cracked Eggs (%) | Feed Intake (g/day) | Feed/kg Egg (kg) | Feed/dozen egg (kg) | Cost of feed/kg Egg (₦) | Daily Protein Intake (g) | Daily Energy Intake (Kcal ME/kg) | Final Body Weight (kg) | Mortality % |
| 1 | - | 54.01$^{c}$ | 49.14$^{b}$ | 91.12$^{a}$ | 0.389$^{a}$ | 21.0 | 103.28$^{b}$ | 3.99$^{a}$ | 2.34$^{a}$ | 2.34$^{a}$ | 16.52$^{c}$ | 368.5$^{c}$ | 1.65$^{a}$ | 11.11 |
| 2 | 200 | 57.93$^{b}$ | 48.92$^{b}$ | 89.69$^{a}$ | 0.399$^{a}$ | 14.3 | 106.08$^{ab}$ | 3.78$^{a}$ | 2.27$^{a}$ | 2.36$^{a}$ | 16.97$^{b}$ | 275.8$^{b}$ | 1.72 | 11.11 |
| 3 | 400 | 61.13$^{ab}$ | 50.38$^{a}$ | 90.30$^{a}$ | 0.403$^{a}$ | 10.7 | 106.02$^{ab}$ | 3.24$^{b}$ | 1.94$^{b}$ | 2.03$^{b}$ | 16.97$^{b}$ | 275.7$^{b}$ | 1.71$^{a}$ | 11.11 |
| 4 | 600 | 64.78$^{a}$ | 49.95$^{ab}$ | 93.80$^{a}$ | 0.390$^{a}$ | 6.7 | 109.80$^{a}$ | 3.35$^{b}$ | 2.01$^{b}$ | 2.11$^{b}$ | 17.57$^{a}$ | 285.6$^{a}$ | 1.72$^{a}$ | 11.11 |
| | SEM[2] | 1.230 | 0.351 | 1.682 | 0.0043 | - | 0.00167 | 0.1096 | 0.0652 | 0.0671 | 0.2688 | 4.376 | 0.0405 | - |

1 Means within each column with different superscript very significantly ($P<0.05$)

2 Standard error of means for treatment.

ascorbic acid (Treatment 3) resulted in significant increase in egg production. Supplementation to 600mg ascorbic acid per kg feed produced a non-significant (P 0.05) increase.

Feed Intake: Average daily feed intake for the experiment are shown in Table 9. Hens on Treatment 4 (600mg ascorbic Acid ) consumed the most feed, 109.8g/day, which was significantly (P/0.05) more than the amount eaten by hens on Treatment 1, the control and birds on Treatments 2 (106.08g) and 3 (106.02g).

Efficiency of Feed Conversion: From Table 9 the results show that the group of hens on Treatments 3 and 4 had similar efficiencies of feed conversion (Kg feed/kg egg = Kg Feed/doz. egg) which were significantly (P/0.05) better than for the hens on either Treatment 2 or 1. The efficiencies of feed conversion for Treatments 1 and 2 did not vary significantly (P 0.05). The efficiency of feed utilisation for Treatment 1 hens which was the lowest for the study was 3.901 while Treatment 3 with the highest conversion ratio was 3.237.

Cost of Feed/Kg Egg: Table 9 shows that Treatment 3 (400mg AsA) had the lowest costing for feed per kg. egg. This was followed by Treatment 4 which had a price that was statistically (P 0.05) at par with the price of Treatment 3. Treatment 1 and 2 at ₦2.34 and ₦2.36/kg eggs respectively were non-significantly (P 0.05) similar but costlier (P/0.05) than Treatments 3 and 4 with their ₦2.03 and ₦2.12/kg Eggs respectively.

Egg Weight: Analysis of data on egg weight (Table 9) show that the feeding of Treatment 3 resulted in eggs with the heaviest weight, 50.38gm in this study. This was followed by Treatments 4 and 1 respectively. Egg weight was the same for Treatments 3 and 4 (P 0.05). Treatment 3 was however, significantly (P/0.05) different from all the other treatments. The other treatments had non-significant difference

among them. It has to be indicated that lower than normal egg weights were obtained because the experiments started right after the hens came into lay and several eggs collected would have depressed the treatment averages.

Shell Thickness: Table 9 indicates that no significant difference (P 0.05) existed among the various treatments for egg shell thickness. The highest average egg shell measurement were obtained from Treatment 3 (0.403mm).

Haugh Unit Score: Treatment 4 had the highest Haugh Unit Score for albumen height and quality of 93.80mm (Table 9). Treatment 2 gave the lowest score of 89.69mm. There was no significant difference (P 0.05) among diets for this parameter.

Cracked Eggs: The results for cracked eggs (Table 9) show a linear trend. Treatment 1, had the highest cracked eggs (21%) in the study. This was followed by Treatment 2 at 14.3%, then Treatments 3 and 4 respectively. Though these figures were not analysed, the trend was consistent over the five 28-day periods.

Daily Protein and Energy Intake: Treatment 4 hens consumed more (P/0.05) protein and energy (17.57g and 285.6 Kcal ME/kg) than any other treatment group (Table 9). This was followed by Treatments 2, and 3 which were similar. Treatment 1 consumed the least protein and energy, 16.5g and 268.5 Kcal ME/kg feed respectively. This was significantly (P/0.05) lower than the consumption rates for the treatments

Final Body Weight: Feeding of Treatment 1 resulted in hens with the lightest body weight, 1.65kg, while Treatment 4 produced the heaviest hens 1.72kg in the study. There was no significant difference (P/0.05) among the treatment groups for body weight.

Mortality: All treatments recorded the same level of deaths.

DISCUSSION:

EXPERIMENT 2:

The results for both outdoor and indoor ambient enviromental temperature establish without doubt that the maximum temperatures which prevailed during the period of this study were well over the thermo neutral zone for the chicken (El-Boushy and Raterink, 1985). There was no doubt of its effect on the experimental hens. They were highly heat-stressed. Panting and wing-lifting to dissipate body heat were observed most of the day among the hens. Such mechanism to relieve body heat occurs at the expense of metabolic energy resulting in generally higher feed conversion ratios for heat-stressed chickens (Dale and Fuller 1980). Sunde and Miller (1975) stated that the high ambient environmental temperature could lead to development of respiratory alkalosis and subsequent compensatory renal excretion of calcium.

Ascorbic acid (AA) at all levels of supplementation stimulated increases in egg production which were statistically ($P \leq 0.05$) different from the performance of the control group (Treatment I). This agrees with the improved egg production reported by Perek and Kendler (1963) and Oluyemi and Adebanjo (1979) when experimental hens were fed ascorbic acid supplemented diets of 25 to 400mg ascorbic acid/kg diet. Increases in egg production were about 7, 13 and 20% over control for diets supplemented with 200, 400 and 600mg ascorbic acid/kg feed respectively. Increasing the ascorbic acid level from 400mg/kg diet (Treatment 3) to 600mg/kg diet (Treatment 4) increased egg production but non-significantly ($P > 0.05$) from 61.13% to 64.78%.

At 400 and 600mg ascorbic acid/kg diet (Treatments 3 and 4 respectively) heavier eggs were recorded which was statistically significant ($P \leq 0.05$) at the 400mg ascorbic acid/kg diet. Perek and

Kendler (1963) and Lazar et al. (1983) got similar responses when up to 400mg ascorbic acid/kg diet was fed in their studies. Haugh Unit Score, shell thickness and final body weight were statistically unaffected (P 0.05) by the use of ascorbic acid in this study. However, there was a tendency for the ascorbic acid treated groups of hen to lay eggs with thicker shells and to exhibit heavier body weight than the control group. Similar observations were reported by El-Boushy and Van Albada (1970), Ketchick and Sykes (1974) and Lazar et al. (1983). The hens in Treatment 3 which laid the heaviest eggs also exhibited the thickest-shelled eggs in the study.

A negative linear effect ($P/0.001$) on number of cracked eggs recorded was elicited in this experiment with increases in ascorbic acid levels. Ascorbic acid supplementation reduced the incidence of egg cracks from 21% in Treatment 1 to 6.7% in Treatment 4, a reduction of over 66%. Ketchick and Sykes (1974) and Pandey et al. (1975) observed that ascorbic acid-treated birds produced eggs with less cracks than did the untreated group.

Ascorbic acid supplementation at all levels significantly improved feed intake, effeciency of feed utilization, daily protein and energy intake of the heat-stressed hens. Although the protein: energy ratio derived from the protein and energy intake were numerically the same for all the treatment groups, the birds fed ascorbic acid supplemented diets utilized the diets more efficiently. This greater efficiency may be attributable to the ability of ascorbic acid to ameliorate the effect of the stress therefore enabling the birds to produce sufficiently well under the hot condition. Feed consumption was significantly ($P/0.05$) increased at the 600mg ascorbic acid/kg diet supplemented diet, though the value was not significantly ($P$ 0.05) different for all the ascorbic acid supplemented diets. In their various

studies, Ketchick and Sykes (1974) and Oluyemi and Adebanjo (1979) observed a similar effect of improved feed consumption when 300mg ascorbic acid was added to one kg of diet fed to hens. Oluyemi and Adebanjo (1979) stated that the increased feed intake associated with the treatments appeared to have been used for egg formation than for improving egg quality. This assertion is corroborated by results presented in Table 9 because though there seemed to be improvement in the Haugh Unit Score and shell thickness, the values recorded were not significantly different among treatments (P 0.05).

The efficiency of feed conversion for the treatments show that the birds whose diets were supplemented with higher levels of ascorbic acid (Treatments 3 and 4) were significantly (P<0.05) more efficient at utilizing feed for productive purposes than either the hens in the control group or those fed diets containing only 200mg ascorbic acid/kg feed. Still the 200mg ascorbic acid/kg hens had a lower (P 0.05) feed efficiency value than the controls.

Mortality and final body weight were unaffected by treatment, though the birds fed ascorbic acid were non-significantly heavier at the end of the experiment.

A consideration of the economics of producing a kilogram of egg shows that despite the additional cost of ascorbic acid supplementation the hens utilised the ascorbic acid-supplemented feed so well that the cost of producing a Kg egg was lower (P<0.05) for the diets containing 400 or 600mg ascorbic acid/kg feed than for the control and 200mg ascorbic acid/kg feed group. From the results of this study, it is evident that supplementation of ascorbic acid at 400mg ascorbic acid level per kg of feed is economical and recommended during the hot months of the year in dry hot tropical environments.

Experiment III: Effect of feeding vitamin C (L-ascorbic acid) and palmoil on the performance of heat stressed laying chickens:

Summary:

Seventy-two, 33 week old layers were used to study the effect of dietary supplementation of ascorbic acid, palmoil or a combination of both in the diet of laying hens during the hot season of the tropical year. The birds were randomly allocated to four dietary treatments with three replicates per treatment. The four dietary treatments each contained 16% CP and 2780Kcal ME/Kg diet. Treatment 1 served as a maize - soyabean meal control diet. Treatment 2 contained 200mg ascorbic acid/Kg diet, while Treatment 3 had 5% palm oil supplementation. Treatment 4 was supplemented with 200mg ascorbic acid/Kg diet and 5% palm oil. Feed and water were offered ad libitum. No artificial light was imposed. Both the indoor and outdoor air temperatures were monitored on maximum and minimum thermometers.

The use of ascorbic acid alone (Treatment 2) improved the hen day egg production, shell thickness and feed utilization. Treatment 3 significantly ($P<0.05$) improved egg production and weight, feed intake and efficiency, cost of feed/kg egg and livability but not shell thickness. Treatment 4 gave synergistic effects ($P<0.05$) in hen day production, egg weight, shell thickness, feed intake, efficiency of feed conversion, cost of feed per Kg egg and final body weight.

INTRODUCTION:

The effects of high seasonal temperature on the egg producing bird affects most of its production parameters. Depression of feed intake which is generally accompaniedby loss of body weight has been reported (Wilson, 1949; Huston et al., 1957; Campos et al., 1960 and

Mueller, 1961). Egg production is decreased (Bennion and Warren, 1933; Robinson and Lee, 1946; Wilson, 1949, Mueller, 1961; Miller and Sunde, 1975; de Andrade et al., 1976, 1977). Egg weight is reported depressed (Bennion and Warren, 1933; Mueller, 1961; Miller and Sunde, 1975; de Andrade et al., 1976, 1977). Birds also lay eggs with thinner shells (Warren and Schnepel, 1940; Mueller, 1961; Harrison and Biellier 1969; de Andrade et al., 1976, 1977; Deaton et al., 1981).

Payne (1966) reported that the adverse effects of a constant high environmental temperature could be minimized when suitable dietary modifications were made. The work of de Andrade et al., (1976, 1977) demonstrated that a high nutrient density diet improved both the weight of eggs as well as increased the rate of egg production during the periods of heat stress when compared to the performance of birds on regular diet.

Perek and Kendler (1963) reported significant increases in egg production when graded levels of ascorbic acid (up to) were added to layers mash. Thorton and Moreng (1953), reported improvement in egg shell quality with ascorbic acid supplementation in the feed. However, a follow up work by Thorton (1960) failed to report any beneficial response with ascorbic acid dietary supplementation, probable due to the low inclusion rate of ascorbic (acid 44mg/kg diet).

Under conditions of heat stress Dale and Fuller (1980) showed that some of the possible benefits of utilizing high levels of fat in poultry diet were increased intake of feed and improved growth. Mateos and Sell (1981) reported that an "extra-caloric" effect in improved layer performance, was stimulated when combinations of animal fat and soy bean oil were supplemented to the basal diet of laying hens. Suggestions have been made that the extracaloric value of fat is discernible, especially at the higher region of environmental temperature (Lipstein and Bornstein, 1975).

In order to investigate the ameliorative effect of dietary supplementation of palm oil and ascorbic acid in the performance of the heat stressed egg laying bird, this study was conducted during the hottest months of the Savannah region of Nigeria.

MATERIALS AND METHODS:

Seventy-two 33 week old Shaver Starrcross 579 x Hubbard Golden Comet laying hens were used for this study. The birds were randomly selected, weighed and placed individually in battery cages. Each cage measured 49 x 35 x 42cm and held three hens. Two of such cages formed a replicate while three randomly selected replicates constituted a treatment and were fed one of the four dietary treatments. The laying house located at the Ahmadu Bello University Farm, was the tropical half-wall type. The indoor temperature of the laying house was monitored daily with a maximum wall thermometer (Casella London 375780) and a minimum wall thermometer (Casella London 37629 C).

Four isonitrogenous and isocaloric corn-soyabean based diets were formulated (Table 9). Diet 1 was the control while diet 2 was supplemented with 200mg Ascorbic acid/Kg feed (Hoffman - La Roche). To diet 3 was added 5.0 percent palm oil while diet 6 was supplemented with 200mg ascorbic acid/kg feed as well as 5% palm oil. Each feed was analyzed for proximate fractions namely, dry matter (DM), crude protein (CP), ether extract (EE), crude fibre (CF), and by difference nitrogen free extract (NFE), using the appropriate AOAC (1970) procedures (Appendix VI). Water and feed were provided ad libitum. No supplementary light was used in the study.

When daily collections of eggs were made, all investigations and production parameters studied were similar to that of Experiments I and II.

Table 11 - Percentage composition and calculated analyses of diets with cost per 100kg diet[3] - Experiment 3.

| Ingredient | Diet 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Maize | 64.00 | 64.00 | 50.30 | 50.30 |
| Wheatoffal | 4.60 | 4.58 | 13.90 | 13.00 |
| Soyabean meal | 21.60 | 21.60 | 12.00 | 21.00 |
| Bone meal | 2.40 | 2.40 | 2.40 | 2.40 |
| Limestone | 6.80 | 6.80 | 6.80 | 6.80 |
| Salt | 0.25 | 0.25 | 0.25 | 0.25 |
| Premix[1] | 0.25 | 0.25 | 0.25 | 0.25 |
| Methionine | 0.10 | 0.10 | 0.10 | 0.10 |
| Palm oil | - | - | 5.00 | 5.00 |
| Vitamin C | - | 0.02 | - | 0.02 |
| Total, % | 100.00 | 100.00 | 100.00 | 100.00 |
| Cost of feed/100kg, (₹) | 59.91 | 62.30 | 67.64 | 68.04 |
| Calculated Nutrient Levels | | | | |
| Crude Protein, % | 16.04 | 16.04 | 16.04 | 16.04 |
| Crude Fibre | 2.85 | 2.87 | 3.50 | 3.50 |
| Metabolizable Energy Kcal/kg | 2780 | 2780 | 2780 | 2780 |
| Calcium, % (Total), | 3.38 | 3.38 | 3.38 | 3.38 |
| Phosphorus (Total), % | 0.59 | 0.59 | 0.59 | 0.59 |
| Available Phosphorus, % | 0.36 | 0.36 | 0.36 | 0.36 |
| Lysine, % | 0.91 | 0.91 | 0.91 | 0.91 |
| Methionine + Cystine, % | 0.63 | 0.63 | 0.63 | 0.63 |

[1]The Premix (ZOODRY 301) supplied the following per kilogram of diet: Vitamin A[2], 16675 I.U.; Vitamin D[2]3, 3350 I.U., vitamin E[2], 16.675 I.U., menadione sodium bisulfite (vit K)[2], 3.35mg; vitamin $B_2$, 7.5mg; vitamin $B_6$, 5.0mg; nicothinic acid, 41.675mg; calcium D-pantothenate, 13.35mg; vitamin $B_{12}$ 0.02mg.; vitamin C, 33.35mg.; choline chloride, 333.50mg.; manganese, 166.675mg; iron 83.35mg.; Zinc 66.675mg; copper, 4.00mg.; iodine 2.335mg.; cobalt, 0.335mg and selenium, 0.85mg.

[2]Stabilized

[3]Based on 1987 costs

The study lasted 20weeks of five 28-day periods. All data collected at the end of the study period were summarized and analysis of variance as well as Duncans Multiple Range Test applied.(Duncan, 1985).

EXPERIMENT 3: RESULTS:

The results derived from this study are shown in Tables 8, 12 and Appendices 8, 12 to 14.

Ambient Environmental Temperature: The ambient environmental temperature recordings are Summarized on Table 8. The summary of the period temperature show that the highest indoor and outdoor maximum temperatures of 39.15 and 37.18 C respectively occurred during the second period of the study. With the onset of the rains in the third period, the percent relative humidity exhibited a sharp rise from 20.82% in the second period to 67.14% in the third period for the morning recordings affecting the air temperatures. The indoor and outdoor environments declined to 31.8 and 29.7 C respectively by the fifth period of the experiment. The birds showed obvious signs of heat stress as panting and wing lifting were observed most of the time.

Hen - day Production: The average egg production data for the study period are indicated in Table 12. These results show that Treatment 4 with 200mg ascorbic acid + 5% oil, produced the most eggs (68.34%). This was statistically ($p$ 0.05) similar to the production performance of Treatment 3 with 5% oil but no ascorbic acid. Both Treatments 3 and 4 had significantly ($P/0.05$) more egg yield than Treatments 1, the control, and 2 with 200mg ascorbic acid only.

Feed Intake: The average feed intake for the various treatment groups are summarised in Table 12. At an average daily feed intake of 107.08g, hens on Treatment 3 consumed the most feed which was statistically ($P$ 0.05) the same as the consumption rate of Treatments 2 and 4 hens

Table 12 : Proximate chemical analysis of the various diets - Experiment 3.

| Diets | Dry Matter (%) | Crude Protein (%) | Ether Extract (%) | Crude Fibre (%) | Nitrogen Free Extract (%) |
|---|---|---|---|---|---|
| 1 | 90.09 | 16.92 | 2.74 | 3.49 | 76.85 |
| 2 | 90.88 | 16.89 | 2.74 | 3.49 | 76.88 |
| 3 | 85.63 | 16.90 | 3.50 | 4.09 | 75.51 |
| 4 | 85.63 | 16.87 | 3.61 | 4.17 | 75.35 |

Table 13. Effect of feeding Palm oil and Vitamin C (ascorbic acid) supplementation on performance of hens during hot seasons[1] - Experiment 3.

| Trt. | Level of Palm oil % | Level of Ascorbic Acid mg/kg | Hen-day Production % | Egg Weight (g) | Haugh Unit (mm) | Shell Thickness (mm) | Cracked Eggs (%) | Feed Intake (g/day) | Feed/ kg Eggs (kg) | Feed/ Doz. Eggs (kg) | Cost of Feed/ kg Egg(kg) | Daily Protien Intake (Kcal ME/kg) | Daily Energy Intake (Kcal ME/kg) | Protein Energy Ratio | Final Body Wg. (kg) | Mo ali (% |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | - | - | 54.01$^{b}$ | 49.14$^{b}$ | 91.12$^{a}$ | 0.389$^{bc}$ | 21.0 | 103.28$^{b}$ | 3.90$^{a}$ | 2.34$^{c}$ | 2.34$^{a}$ | 16.52 | 268.49$^{b}$ | 16.25 | 1.65$^{a}$ | 11. |
| 2 | - | 200 | 57.90$^{b}$ | 48.76$^{b}$ | 90.01$^{a}$ | 0.398$^{ab}$ | 14.0 | 105.78$^{ab}$ | 3.79$^{a}$ | 2.28$^{a}$ | 2.37$^{a}$ | 16.96 | 275.74$^{ab}$ | 16.25 | 1.72$^{a}$ | 11. |
| 3 | 5.0 | - | 64.78$^{a}$ | 52.48$^{a}$ | 87.65$^{a}$ | 0.384$^{c}$ | 4.2 | 107.68$^{a}$ | 3.14$^{b}$ | 1.86$^{b}$ | 2.12$^{a}$ | 17.13 | 278.37$^{a}$ | 16.25 | 1.77$^{a}$ | 0. |
| 4 | 5.0 | 200 | 68.38$^{a}$ | 53.05$^{a}$ | 88.50$^{a}$ | 0.405$^{a}$ | 9.7 | 104.93$^{ab}$ | 2.87$^{c}$ | 1.72$^{b}$ | 1.95$^{b}$ | 16.78 | 272.74$^{ab}$ | 16.25 | 1.80$^{a}$ | 5. |
| | SEM[2] | | 1.47 | 0.41 | 1.94 | 0.005 | - | 0.0019 | 0.127 | 0.065 | 0.078 | 0.31 | 5.053 | - | 0.048 | - |

[1]Means within each column with different superscript vary significantly (P/0.05)

[2]Standard error of means for treatments.

but different (P$\leq$0.05) from Treatment 1.

Efficiency of Feed Conversion: Treatment 4 resulted in the highest feed utilization ratio for the two efficiency parameters measured, Kg feed/kg eggs and Feed/dozen eggs (Table 12). The Kg feed/kg eggs ratio for Treatment 4 was significantly (P$\leq$0.05) higher than for the other treatments. Treatment 3 which followed had a significantly (P$\leq$0.05) lower efficiency than Treatment 4 but was higher (P$\leq$0.05) than those for Treatments 1 and 2. Treatments 1 and 2 did not differ significantly (P $>$ 0.05). Also significantly (P$\leq$0.05) more feed was required by Treatments 1 and 2 to produce a dozen of eggs than Treatments 3 and 4 which did not vary significantly (P $>$ 0.05).

Cost of Feed/Kg Eggs: As shown in Table 12 the feeding of hens with Treatment 4 gave the lowest value in costing of feed per kg eggs (₦1.95) which was significantly (P$\leq$0.05) lower than that from Treatments 1, 2 and 3. The most expensive feed for egg yield came from feeding Treatment 2 diet.

Egg Weight: Eggs from hens fed Treatment 4 and 3 with 53.05 and 52.48g weights respectively produced the heaviest eggs. Both were statistically at par (P $>$ 0.05) between themselves, but were different (P$\leq$0.05) from the average egg weights recorded for Treatments 1 and 2.

Shell Thickness: Results in Table 12 on shell thickness show Treatment 4 to have the highest average egg shell thickness (0.405mm). It was significantly (P $>$ 0.05) at par with the shell thickness measures from Treatment 2 but different (P$\leq$0.05) from Treatment 1 and 3 Treatments 1 and 3 were non-significantly (P $>$ 0.05) different.

Cracked Eggs: It was evident from the results shown in Table 12 that supplementation of the control diet (Treatment 1) with oil or ascorbic acid alone or in combination reduced the incidence of cracked eggs.

The least value of 4.2% was recorded for diet 3 as against 21% for the control.

Daily Protein and Energy Intake: Table 12 shows that the protein intake of the hens was above 16g in each treatment and the values did not vary among themselves. However in regard to daily energy intake the least energy intake was 268.5 Kcal ME/kg diet which was significantly ($P < 0.05$) lower than the intake in Treatment 3 where 5% Palm oil was included in the diet. The values for Treatment 2 and 4 were non-significantly ($P > 0.05$) higher than Treatment 1 and less than Treatment 3. The protein : energy intake ratio was 16.25 for all the treatments.

Final Body Weight: There was no statistically significant ($P > 0.05$) difference among the treatment groups for body weight gain. However, Treatment 3 gave the heaviest hens, 1.8kg while Treatment 1 had the lightest body weights, 1.65kg.

Mortality: Table 12 indicates that hens on Treatment 3 had the best livability, with no deaths. Treatment 4 followed with 5.56% while Treatments 1 and 2 had 11.11% deaths.

EXPERIMENT 3: DISCUSSION:

Both the indoor and the outdoor environmental temperatures recorded during the entire period of this study were beyond the thermo neutral zone for the laying bird. The indoor maximum temperature of 39.15 C was a factor of heat stress for the hens. The panting and wing lifting observed during the period of the experiment was evidence that the hens were undergoing a major physiological stress. Such continuous state of panting could lead to a development of respiratory alkalosis which Miller and Sunde (1975) explained led to subsequent compensatory renal excretion of calcium bases with its subsequent effect on the shell quality of eggs produced by the hen under heat stress.

It is observed from the results of this experiment (Table 12) that the supplementation of 200mg ascorbic acid/kg feed (Treatment 2) or with 5% palm oil (Treatmenr 3) to layers mash during periods of heat stress does stimulate performance in laying hens. Such improved performance during heat stress conditions were earlier reported by Peebles and Brake (1985) on ascorbic acid (AA) effect and by Valencia et al. (1980) on inclusion of 5% oil to hen diet. Although the use of 200mg ascorbic acid/kg diet did not significantly differ from the control diet in egg production, yet it did elicit improved egg numers. The use of 5% oil gave a significant ($P<0.05$) increase in hen-day production which agrees with the findings of de Andrade et al. (1976, 1977) and Cunningham and Morrison (1977).

There was a significant increase in egg weight by the feeding of 5% oil in comparison to the control treatment in this experiment. Treat et al. (1960) and Kondra et al. (1968) reported that the inclusion of fat to layer diet increased egg weight. de Andrade et al. (1976) felt that the increase in egg weight recorded in their study resulted from the addition of soybean oil and not from additional protein.

Ascorbic acid inclusion alone in this experiment effect non-significant improvement in egg shell thickness. El-Boushy and Van Albado (1970) had obtained significant increases in shell thickness in their study. Inclusion of 5% palm oil alone in Treatment 3 did not elicit such an effect. The lack of effect of dietary palm oil supplementation on shell thickness confirms the results of de Andrade et al. (1976, 1977) which showed that the use of high nutrient density diets had no effect on improving egg shell thickness laid by heat-stressed hens. However, the number of cracked eggs from this study were least from the feeding of 5% palm oil. That ascorbic acid also reduced the

number of cracked eggs supports the findings of Thorton and Moreng (1958).

Feed consumption was improved by including either ascorbic acid or palm oil in the diets. Although the effect recorded for feed ingested when ascorbic acid was supplemented was statistically similar to that of the control group, it showed an increment (P 0.05). Significantly improved feed consumption has been reported by Thorton and Moreng (1958). The feeding of palm oil significantly improved feed intake among the test hens. Dale and Fuller (1979) observing same increases in feed intake from their heat-stressed birds felt that added fat may improve the physical characteristics and palatability of the diet to an extent which promotes increased feed consumption. Sunde (1956) felt that the improved palatability of diets containing fat may also have been a contributing factor in the better performance of birds receiving supplemental fat.

Ascorbic acid supplementation at 200mg/kg diet did not stimulate significant improvements in feed utilization (feed/kg eggs or Feed/dozen eggs). The improvements were statistically similar (P 0.05) but slightly better than for the controls. On the other hand, there was a highly significant reduction in the amount of feed necessary to produce a Kg. of eggs with the oil treatment (Treatment 3). This corroborates earlier works by Cunningham and Morrison (1977) and de Andrade et al. (1976, 1977). Cunningham and Morrison (1977) observed this to be due to the lower metabolic cost associated with digestion, reduced requirement and lowered metabolic cost to synthesize fatty acids needed in egg formation while the higher caloric density of dietary fat have been suggested as factors relating to the improved productive efficiency noted in birds fed the fat supplemented laying diets.

The increases recorded in feed intake in the study for both the ascorbic acid and the oil treatments reflect also in the daily

consumption of protein and energy. The birds irrespective of type of treatment had the same energy: protein ratio. This supports the findings of Donaldson and Gordon (1960) and Bragg et al. (1973) that no trend was demonstrated in increased daily caloric intake by laying hens fed diets varying in dietary fat from 1 to 8%.

Despite the very high ambient environmental temperature of this study and the threat of death from heat prostration, mortality was not recorded in this study among the birds fed the diet containing 5% palm oil only (Treatment 3). This supports the findings of de Andrade et al. (1977) that the use of high nutrient density diets significantly improved livability.

When the 200mg ascorbic acid/Kg diet and 5% palm oil were both added to the diet (Treatment 4) and fed, there was an apparent synergism recorded in the performance of the hens. The study recorded significant improvements for nearly all the parameters studied (Table 12). Such synergism was possible as ascorbic acid is involved in the formation of 7-alpha-hydroxy-cholesterol and of carnitine from lysine and methionine (Klob, 1987). The compound 7-alpha-hydroxy-cholesterol is necessary for the formation of bile acids. Bile acids emulsify fat for absorption. Carnitine is important in the transportation of long chain fatty acids, as found in palmoil, into the mitochondria. This effect of ascorbic acid will therefore enhance the utilization of the palmoil. Mateos and Sell (1980) had reported that a synergism occurred also between saturated and unsaturated fatty acids which enhanced the utilisation of the lipid portion of the diet when they fed layer diet containing starch and a combination of animal fat and soybean oil to hens. The superior performance shown by the hens on combined ascorbic acid and oil supplementation (Treatment 4) could be attributed to the slowing down in the rate of passage of the feed through the intestine. This allowed

for better nutrient absorption. Tuckey et al. (1958) reported a reduced rate of passage with yellow grease while Mateos and Bell (1981) showed that this decrease in rate of passage was responsible for the "extra-caloric" effect of supplemental fat. This resulted in ME values for fat near or in excess of its gross energy (Reid, 1983).

The low heat increment effect of fat reported by Fuller and Rendon (1977) was indicated to be responsible for the "extra-caloric" effect of fat (Charles, 1980). Less heat is generated when fat calories are digested (i.e. lower heat increment) resulting in lower body temperature and an increase in productive energy.

Vitamin C is classified as an antistress vitamin (Roche, 1987) useful when birds are subjected to the stress of adverse temperatures (Pardue and Thaxton, 1986). It is also known to reduce the level of corticosterone in the plasma of heat stressed birds (Schmeling and Nockles, 1976). North (1980) had explained that birds with low levels of corticosterone were more docile, grew faster and attained heavier weights, had better feed efficiencies, less carcass fat, lower heart rate, produced more and larger eggs, showed a lower resistance to bacterial disease through decreased antibody production, but showed a higher resistance to many viral infections and responded better to some vaccinations.

It is evident from this experiment that while addition of palmoil or ascorbic acid would actually enhance the performance of heat-stressed laying chickens, a combination of both factors would make for highly significant improvements indeed beyond the effect of either fed alone.

CONCLUSION:

Payne (1966) had stated that the adverse effects of a constant high environmental temperature could be minimized when suitable dietary modifications were made. From the results of the various feeding trials carried out in this study it was shown in Experiment 1 that heat stressed

birds performed best when diets with low levels of crude protein and energy were fed. Diets 9 (14 - 2400) stimulated the highest egg yield as well as improved other productive performance of the heat-stressed hen. This was followed by the feeding of Diet 5, 16% CP and 2600 Kcal ME/kg feed and by feeding Diet 7 (14 - 2800). Earlier, Olomu and Offiong (1983) had stated that under tropical environments, as envisaged here, brown egg laying hens would perform well on a low energy level of 2400 Kcal ME/kg feed and 16% CP. The results of this study agree with this.

Experiment 1 also revealed that under heat stress conditions, high levels of dietary protein of 18% CP and high energy level of 2800 Kcal ME (Table 5) depressed ($P<0.05$) the productive performance of hens.

Experiment 2 confirmed that the supplementation of ascorbic acid at 400mg ascorbic acid/kg feed promoted increased feed intake and efficiency as well as egg production during the hot months of the year in a tropical environment.

Experiment 3 emphasised the ameliorative effect of palm oil on heat-stressed hens. From the experiment, it was obvious that the supplementation of the basal diet with 5% palmoil stimulated significant ($P<0.05$) increases in productive performance in the test hens. This agrees with previous reports of the "extra caloric" effect of dietary oil (Mateos and Sell 1981).

When both 5% palmoil and 200mg ascorbic acid/kg feed were added to the basal diet, a synergistic response resulted. The birds demonstrated highly significant ($P<0.01$) improvements in all parameters measured in the experiment.

It can therefore be concluded that during periods of heat stress in the Savannah belt of Nigeria, the best combinations of protein and energy to attain optimal productive performance from the hen, are 14% CP combined with 2400 Kcal ME/kg feed, 16% CP combined with 2600 Kcal ME/kg

feed and 14% CP combined with 2800 Kcal ME/kg feed. Also the addition of ascorbic acid and palm oil supplements to effect higher increases in all productive measurements in the heat stressed laying hen is highly economical and feasible as revealed by this study.

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# A P P E N D I C E S:

Appendix 1: Weekly and Period[1] Summaries of Air Temperatures and Relative Humidity for Period of Experiment March to July 1987 - Experiment 1.

| Period | Weeks[2] | Air Temperature C(Outdoor) Max | Min | Mean | Relative Humidity % AM | PM | Max | Air Temperature (Indoor) C Min | Mean |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 36.0 | 22.43 | 13.57 | 38.57 | 21.14 | 38.84 | 23.86 | 14.98 |
| | 2 | 35.29 | 23.71 | 11.58 | 51.29 | 28.57 | 36.79 | 24.71 | 12.08 |
| 1 | 3 | 36.57 | 19.71 | 16.86 | 19.0 | 5.29 | 36.71 | 21.50 | 15.21 |
| | 4 | 37.87 | 20.71 | 17.15 | 24.14 | 12.71 | 39.93 | 22.29 | 17.64 |
| | $\bar{X}$ | 36.43 | 21.64 | 14.79 | 33.25 | 16.93 | 38.07 | 23.09 | 14.98 |
| | 5 | 35.43 | 22.00 | 13.43 | 8.43 | 8.14 | 39.14 | 25.64 | 13.5 |
| | 6 | 32.29 | 23.00 | 9.29 | 7.29 | 8.29 | 37.20 | 24.10 | 13.1 |
| 2 | 7 | 38.43 | 21.43 | 17.00 | 35.0 | 11.14 | 40.1 | 22.40 | 17.7 |
| | 8 | 38.43 | 23.00 | 15.43 | 45.86 | 12.14 | 40.6 | 23.60 | 17.0 |
| | $\bar{X}$ | 36.15 | 22.36 | 13.79 | 24.15 | 9.93 | 39.26 | 23.94 | 15.33 |
| | 9 | 38.14 | 23.71 | 14.43 | 51.86 | 21.29 | 40.93 | 24.00 | 16.93 |
| | 10 | 33.71 | 21.43 | 12.28 | 67.14 | 45.86 | 36.93 | 23.57 | 13.36 |
| 3 | 11 | 28.00 | 20.43 | 7.57 | 84.00 | 67.71 | 33.07 | 23.29 | 9.78 |
| | 12 | 33.00 | 21.71 | 11.29 | 74.00 | 51.14 | 35.14 | 22.93 | 12.21 |
| | $\bar{X}$ | 33.21 | 21.82 | 11.39 | 69.25 | 46.50 | 36.52 | 23.45 | 13.07 |
| | 13 | 31.57 | 21.71 | 9.86 | 70.29 | 55.86 | 35.36 | 22.43 | 12.93 |
| | 14 | 30.57 | 21.29 | 9.28 | 75.43 | 60.29 | 35.29 | 21.71 | 13.58 |
| 4 | 15 | 30.00 | 20.43 | 9.57 | 78.90 | 66.29 | 34.71 | 20.71 | 14.00 |
| | 16 | 30.43 | 20.71 | 9.72 | 77.30 | 62.14 | 34.14 | 20.86 | 13.28 |
| | $\bar{X}$ | 30.64 | 21.04 | 9.61 | 75.48 | 61.15 | 34.88 | 21.43 | 13.45 |

1. Period equivalent to 28 days
2. Weekly measurement commenced 15th March and ended 10th of July 1987.

Appendix 2: Average Hen-by Egg Production, (%) - Experiment 1

| Periods | Replicate | Diet 1 | Diet 2 | Diet 3 | Diet 4 | Diet 5 | Diet 6 | Diet 7 | Diet 8 | Diet 9 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 57.43 | 57.43 | 64.28 | 49.72 | 63.43 | 53.15 | 44.57 | 63.43 | 57.43 |
| | 2 | 54.00 | 48.85 | 62.57 | 57.43 | 60.00 | 52.28 | 43.72 | 54.85 | 67.72 |
| | 3 | 36.85 | 53.14 | 52.28 | 61.37 | 53.15 | 52.28 | 53.15 | 59.15 | 58.74 |
| | $\bar{X}$ | 49.43 | 53.14 | 59.71 | 56.28 | 58.86 | 52.57 | 46.86 | 59.15 | 61.14 |
| 2 | 1 | 72.84 | 67.72 | 58.28 | 48.85 | 54.85 | 65.14 | 51.43 | 58.28 | 68.57 |
| | 2 | 61.72 | 60.00 | 66.00 | 54.00 | 78.85 | 67.72 | 64.28 | 63.43 | 69.00 |
| | 3 | 54.00 | 57.43 | 65.32 | 57.43 | 54.00 | 62.57 | 56.57 | 60.85 | 69.43 |
| | $\bar{X}$ | 62.84 | 61.72 | 63.14 | 53.47 | 62.57 | 65.15 | 57.43 | 60.86 | 69.00 |
| 3 | 1 | 65.15 | 66.00 | 67.72 | 49.49 | 67.39 | 58.26 | 47.15 | 62.32 | 63.43 |
| | 2 | 55.72 | 54.65 | 62.57 | 46.93 | 74.57 | 58.26 | 54.85 | 66.00 | 59.36 |
| | 3 | 52.28 | 53.15 | 67.72 | 55.72 | 60.22 | 55.07 | 51.43 | 58.63 | 64.72 |
| | $\bar{X}$ | 57.72 | 56.16 | 66.00 | 50.71 | 67.39 | 57.22 | 51.14 | 62.32 | 62.51 |
| 4 | 1 | 61.72 | 59.15 | 65.15 | 51.43 | 76.82 | 58.28 | 52.28 | 45.36 | 76.08 |
| | 2 | 55.72 | 55.50 | 66.85 | 53.57 | 71.15 | 53.15 | 66.00 | 69.92 | 72,86 |
| | 3 | 60.85 | 57.32 | 68.57 | 53.15 | 82.50 | 70.72 | 51.22 | 54.22 | 79.30 |
| | $\bar{X}$ | 59.42 | 57.32 | 66.86 | 52.72 | 76.82 | 60.72 | 57.50 | 52.70 | 76.08 |
| | $\bar{X}$ | 57.36 | 57.59 | 63.94 | 53.29 | 66.41 | 58.92 | 53.23 | 58.76 | 67.19 |

Appendix 3: Average daily Feed Intake, (g) - Experiment 1.

| Periods | Replicate | Diet 1 | Diet 2 | Diet 3 | Diet 4 | Diet 5 | Diet 6 | Diet 7 | Diet 8 | Diet 9 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 83.6 | 99.11 | 113.5 | 67.7 | 127.2 | 109.2 | 90.7 | 109.4 | 109.3 |
| | 2 | 84.0 | 96.40 | 120.1 | 100.9 | 127.2 | 109.0 | 107.1 | 109.2 | 109.3 |
| | 3 | 89.5 | 99.80 | 117.4 | 72.3 | 137.7 | 109.8 | 88.2 | 109.3 | 109.3 |
| | $\bar{X}$ | 85.7 | 99.10 | 117.0 | 80.3 | 130.7 | 109.3 | 95.3 | 109.3 | 109.3 |
| 2 | 1 | 80.6 | 49.26 | 103.9 | 99.11 | 88.33 | 62.5 | 75.0 | 84.3 | 90.50 |
| | 2 | 80.70 | 79.26 | 109.7 | 100.9 | 88.35 | 62.47 | 80.0 | 84.4 | 90.51 |
| | 3 | 85.0 | 79.56 | 103.9 | 100.27 | 89.98 | 62.7 | 74.8 | 84.3 | 90.49 |
| | $\bar{X}$ | 82.1 | 79.36 | 105.8 | 100.09 | 88.89 | 62.6 | 76.62 | 84.32 | 90.49 |
| 3 | 1 | 116.8 | 99.0 | 118.9 | 88.5 | 77.53 | 98.07 | 101.5 | 120.5 | 68.6 |
| | 2 | 117.0 | 97.6 | 119.3 | 89.7 | 77.53 | 97.94 | 105.9 | 120.5 | 68.3 |
| | 3 | 116.9 | 98.0 | 118.93 | 88.5 | 79.12 | 98.10 | 101.0 | 120.5 | 68.9 |
| | $\bar{X}$ | 116.9 | 98.20 | 119.04 | 88.51 | 78.06 | 98.04 | 102.8 | 120.5 | 68.6 |
| 4 | 1 | 117.6 | 123.5 | 113.5 | 94.1 | 126.5 | 123.5 | 123.5 | 117.6 | 111.8 |
| | 2 | 120.0 | 123.5 | 128.2 | 96.5 | 129.4 | 117.6 | 129.4 | 117.6 | 127.1 |
| | 3 | 94.1 | 123.5 | 98.8 | 95.3 | 123.5 | 111.8 | 123.5 | 117.6 | 119.4 |
| | $\bar{X}$ | 110.6 | 123.5 | 113.5 | 95.3 | 126.5 | 117.6 | 125.5 | 117.6 | 119.4 |

Appendix 4 : Effects of period and diet on feed intake and egg production of laying hens[1] - Experiment 1.

| Measurement | Period | Diet | | | | | | | | | SEM | Period MEAN |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | | |
| Hen-day production, % | 1 | $49.426^{bc}$ | $53.14^{abc}$ | $59.71^{ab}$ | $56.173^{abc}$ | $58.86^{ab}$ | $52.57^{abc}$ | $47.146^{c}$ | $59.149^{ab}$ | $61.296^{a}$ | 5.107 | 55.274 |
| | 2 | $62.853^{abc}$ | $61.716^{abc}$ | $63.2^{abc}$ | $53.426^{c}$ | $62.566^{abc}$ | $65.143^{ab}$ | $57.426^{bc}$ | $60.853^{abc}$ | $69.00^{a}$ | 5.107 | 61.790 |
| | 3 | $57.716^{ab}$ | $57.933^{ab}$ | $66.003^{a}$ | $50.713^{b}$ | $67.393^{a}$ | $57.21^{ab}$ | $51.143^{b}$ | $62.316^{a}$ | $62.503^{a}$ | 5.107 | 59.214 |
| | 4 | $59.43^{bc}$ | $57.323^{bc}$ | $66.856^{ab}$ | $52.716^{c}$ | $76.823^{a}$ | $60.716^{bc}$ | $57.50^{bc}$ | $52.70^{c}$ | $76.08^{a}$ | 5.105 | 62.238 |
| Feed Intake, (g) | 1 | $85.0^{de}$ | $99.0^{c}$ | $115.0^{b}$ | $80.0^{e}$ | $130.0^{a}$ | $109.0^{b}$ | $95.0^{c}$ | $109.0^{b}$ | $109.0^{b}$ | 3.176 | 103.0 |
| | 2 | $82.0^{bcd}$ | $79.0^{cd}$ | $105.0^{a}$ | $100.0^{a}$ | $88.0^{bc}$ | $62.0^{e}$ | $76.0^{d}$ | $84.0^{bcd}$ | $90.0^{b}$ | 3.176 | 85.0 |
| | 3 | $116.0^{a}$ | $98.0^{b}$ | $119.0^{a}$ | $88.0^{c}$ | $78.0^{d}$ | $98.0^{b}$ | $102.0^{b}$ | $120.0^{a}$ | $68.0^{e}$ | 3.176 | 99.0 |
| | 4 | $110.0^{b}$ | $123^{a}$ | $113.0^{b}$ | $95^{c}$ | $126.0^{a}$ | $117.0^{ab}$ | $125.0^{a}$ | $117.0^{ab}$ | $119.0^{a}$ | 3.176 | 116.0 |

[1] Statistical significance at $P \leq 0.05$ is denoted by different letters in a row.

Appendix 5: Egg Weight (g), -- Experiment 1

| Period | Replicates | Diet 1 | Diet 2 | Diet 3 | Diet 4 | Diet 5 | Diet 6 | Diet 7 | Diet 8 | Diet 9 |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 48.08 | 52.27 | 49.40 | 53.29 | 51.76 | 48.94 | 47.90 | 47.25 | 52.96 |
| | 2 | 51.42 | 53.40 | 52.62 | 54.14 | 48.25 | 53.22 | 50.28 | 51.37 | 50.31 |
| 1 | 3 | 50.74 | 49.57 | 49.51 | 49.55 | 49.89 | 53.62 | 46.17 | 50.30 | 48.30 |
| | $\bar{X}$ | 50.08 | 51.75 | 50.51 | 52.33 | 49.97 | 51.93 | 48.12 | 49.64 | 50.51 |
| | 1 | 50.25 | 51.97 | 49.96 | 51.05 | 52.18 | 51.30 | 49.52 | 50.46 | 53.28 |
| | 2 | 49.05 | 53.76 | 50.95 | 44.71 | 47.49 | 50.87 | 49.37 | 45.83 | 53.97 |
| 2 | 3 | 51.74 | 47.45 | 44.49 | 51.78 | 49.89 | 53.52 | 45.93 | 45.25 | 47.49 |
| | $\bar{X}$ | 50.35 | 51.06 | 48.47 | 49.17 | 49.85 | 51.90 | 46.27 | 47.08 | 51.58 |
| | 1 | 52.37 | 46.85 | 49.16 | 49.73 | 49.68 | 52.17 | 50.57 | 51.57 | 52.49 |
| | 2 | 50.36 | 49.61 | 50.35 | 50.45 | 50.02 | 50.66 | 51.41 | 51.26 | 48.19 |
| 3 | 3 | 53.11 | 49.23 | 48.00 | 50.09 | 49.34 | 49.13 | 47.07 | 51.42 | 48.18 |
| | $\bar{X}$ | 51.95 | 49.23 | 49.17 | 50.09 | 49.68 | 50.65 | 49.68 | 51.41 | 50.34 |
| | 1 | 50.00 | 51.06 | 48.47 | 52.33 | 49.85 | 51.90 | 48.69 | 47.08 | 51.58 |
| | 2 | 50.35 | 51.75 | 50.51 | 49.17 | 49.83 | 51.49 | 46.12 | 49.38 | 50.81 |
| 4 | 3 | 50.79 | 50.68 | 49.38 | 50.53 | 49.97 | 51.93 | 48.27 | 49.64 | 50.51 |
| | $\bar{X}$ | 50.79 | 50.68 | 49.38 | 50.53 | 49.83 | 51.49 | 48.69 | 49.38 | 50.81 |
| | $\bar{X}$ | 50.79 | 50.68 | 49.38 | 50.53 | 49.83 | 51.49 | 48.69 | 49.38 | 50.81 |

Appendix 6: Egg Shell Thickness (mm) - Experiment 1

| Period | Replicate | Diet 1 | Diet 2 | Diet 3 | Diet 4 | Diet 5 | Diet 6 | Diet 7 | Diet 8 | Diet 9 |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 0.411 | 0.454 | 0.395 | 0.391 | 0.419 | 0.474 | 0.402 | 0.427 | 0.382 |
| | 2 | 0.390 | 0.468 | 0.417 | 0.431 | 0.402 | 0.448 | 0.414 | 0.424 | 0.385 |
| 1 | 3 | 0.429 | 0.463 | 0.419 | 0.430 | 0.459 | 0.421 | 0.438 | 0.423 | 0.391 |
| | $\bar{X}$ | 0.410 | 0.462 | 0.410 | 0.417 | 0.427 | 0.448 | 0.418 | 0.425 | 0.386 |
| | 1 | 0.433 | 0.425 | 0.420 | 0.391 | 0.400 | 0.414 | 0.422 | 0.379 | 0.383 |
| | 2 | 0.388 | 0.448 | 0.384 | 0.405 | 0.420 | 0.383 | 0.382 | 0.353 | 0.383 |
| 2 | 3 | 0.415 | 0.493 | 0.369 | 0.396 | 0.416 | 0.382 | 0.383 | 0.405 | 0.396 |
| | $\bar{X}$ | 0.412 | 0.454 | 0.390 | 0.397 | 0.412 | 0.393 | 0.396 | 0.379 | 0.387 |
| | 1 | 0.379 | 0.432 | 0.403 | 0.432 | 0.413 | 0.442 | 0.368 | 0.397 | 0.408 |
| | 2 | 0.356 | 0.383 | 0.393 | 0.410 | 0.438 | 0.434 | 0.367 | 0.391 | 0.382 |
| 3 | 3 | 0.382 | 0.407 | 0.412 | 0.421 | 0.388 | 0.426 | 0.403 | 0.394 | 0.395 |
| | $\bar{X}$ | 0.372 | 0.407 | 0.403 | 0.421 | 0.413 | 0.434 | 0.379 | 0.394 | 0.395 |
| | 1 | 0.411 | 0.460 | 0.409 | 0.410 | 0.417 | 0.448 | 0.392 | 0.423 | 0.386 |
| | 2 | 0.410 | 0.451 | 0.391 | 0.404 | 0.422 | 0.390 | 0.383 | 0.381 | 0.386 |
| 4 | 3 | 0.378 | 0.412 | 0.403 | 0.421 | 0.413 | 0.437 | 0.418 | 0.394 | 0.396 |
| | $\bar{X}$ | 0.400 | 0.441 | 0.401 | 0.412 | 0.417 | 0.425 | 0.398 | 0.399 | 0.389 |
| | $\bar{X}$ | 0.399 | 0.441 | 0.401 | 0.412 | 0.417 | 0.425 | 0.398 | 0.399 | 0.389 |

Appendix 7: Mean Unit Score ($\bar{x}_i$) Experiment 1

| Period | Replicate | Diet 1 | Diet 2 | Diet 3 | Diet 4 | Diet 5 | Diet 6 | Diet 7 | Diet 8 | Diet 9 |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 82.22 | 82.85 | 84.76 | 84.91 | 85.02 | 80.66 | 82.02 | 84.94 | 84.94 |
| | 2 | 42.08 | 83.27 | 80.27 | 80.47 | 82.19 | 86.26 | 82.42 | 80.69 | 80.69 |
| 1 | 3 | 76.80 | 77.57 | 69.50 | 82.61 | 81.40 | 76.95 | 82.84 | 86.07 | 86.07 |
| | $\bar{X}$ | 80.37 | 81.23 | 84.85 | 82.66 | 82.87 | 81.29 | 82.47 | 83.90 | 83.90 |
| | 1 | 77.09 | 76.84 | 82.11 | 82.57 | 84.48 | 83.56 | 82.94 | 78.68 | 81.47 |
| | 2 | 85.21 | 83.50 | 77.93 | 82.63 | 84.53 | 76.06 | 85.08 | 84.72 | 85.76 |
| 2 | 3 | 78.92 | 85.96 | 90.36 | 84.89 | 82.91 | 73.20 | 86.38 | 87.09 | 82.49 |
| | $\bar{X}$ | 80.41 | 82.10 | 83.47 | 83.36 | 83.97 | 77.61 | 84.80 | 83.58 | 83.24 |
| | 1 | 80.25 | 82.79 | 81.12 | 85.17 | 83.48 | 81.55 | 86.79 | 80.01 | 86.68 |
| | 2 | 86.39 | 85.18 | 77.84 | 78.90 | 83.6 | 79.52 | 79.99 | 80.40 | 83.70 |
| 3 | 3 | 85.55 | 84.49 | 84.40 | 82.04 | 83.3 | 77.49 | 72.27 | 80.40 | 83.70 |
| | $\bar{X}$ | 82.11 | 82.86 | 83.15 | 82.68 | 83.44 | 86.14 | 84.65 | 82.60 | 83.62 |
| | $\bar{\bar{X}}$ | 82.11 | 82,86 | 83.15 | 82,68 | 83,44 | 86.14 | 84.65 | 82.60 | 83.62 |

Appendix 8: Weekly[1] and Period[2] Sumarries of Air Temperatures and Relative Humidity for Period of Studies: Experiment II

| Period | Weeks | Air Temperature C (Outdoor) | | | Relative Humidity (%) | | Air Temperature C (Indoor) | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Max | Min | Mean A | AM | PM | Max | Min | Mean |
| 1 | 1 | 38 | 21.57 | 16.43 | 44.0 | 19.86 | 38.84 | 23.86 | 14.98 |
| | 2 | 34.29 | 23.14 | 11.15 | 40.29 | 28,71 | 36.79 | 24.71 | 12.08 |
| | 3 | 36.43 | 23.57 | 13.86 | 37.43 | 13.43 | 36.71 | 21.50 | 15.21 |
| | 4 | 36.86 | 18.57 | 18.29 | 19.0 | 9.86 | 39.93 | 22.29 | 17.64 |
| | $\bar{X}$ | 36.40 | 21.46 | 14.93 | 35.18 | 17.97 | 38.07 | 23.09 | 14.98 |
| 2 | 5 | 35.86 | 21.86 | 14.0 | 14.86 | 9.14 | 42.90 | 26.1 | 16.80 |
| | 6 | 35.86 | 26.30 | 9.56 | 6.57 | 7.86 | 38.07 | 24.29 | 13.78 |
| | 7 | 38.14 | 20.86 | 17.28 | 19.14 | 9.43 | 35.13 | 27.71 | 7.42 |
| | 8 | 38.86 | 22.71 | 16.15 | 42.71 | 11.57 | 40.50 | 26.79 | 13.71 |
| | $\bar{X}$ | 37.18 | 22.93 | 14.25 | 20.82 | 9.5 | 39.15 | 26.22 | 12.93 |
| 3 | 9 | 38.57 | 23.29 | 15.28 | 51.43 | 17.57 | 39.70 | 23.57 | 16.13 |
| | 10 | 35.71 | 19.86 | 15.85 | 57.57 | 35.14 | 38.36 | 23.21 | 15.15 |
| | 11 | 30.29 | 20.86 | 9.43 | 79.57 | 58.14 | 34.14 | 22.00 | 12.14 |
| | 12 | 29.71 | 20.57 | 9.14 | 80.00 | 65.86 | 31.60 | 22.60 | 9.0 |
| | $\bar{X}$ | 33.57 | 12.15 | 12.42 | 67.14 | 44.18 | 35.95 | 22.85 | 13.11 |
| 4 | 13 | 32.57 | 18.86 | 13.71 | 65.00 | 49.71 | 35.90 | 21.43 | 14.47 |
| | 14 | 31.71 | 21.71 | 10.0 | 71.43 | 57.29 | 34.40 | 17.20 | 17.20 |
| | 15 | 29.43 | 20.57 | 8.86 | 79.29 | 67.57 | 32.20 | 19.50 | 12.7 |
| | 16 | 30.43 | 20.43 | 10.0 | 78.57 | 60.29 | 34.86 | 18.29 | 16.57 |
| | $\bar{X}$ | 31.04 | 20.39 | 10.65 | 73.57 | 58.72 | 34.34 | 19.11 | 15.24 |
| 5 | 17 | 30.71 | 21.00 | 9.71 | 77.71 | 64.86 | 32.43 | 21.29 | 11.14 |
| | 18 | 30.14 | 19.71 | 10.43 | 78.86 | 64.57 | 32.43 | 21.27 | 11.16 |
| | 19 | 29.57 | 20.14 | 9.43 | 80.71 | 65.71 | 31.35 | 18.30 | 13.05 |
| | 20 | 28.33 | 19.0 | 9.33 | 87.33 | 75.33 | 31.0 | 18.0 | 13.0 |
| | $\bar{X}$ | 29.69 | 12.96 | 9.73 | 81.15 | 67.62 | 31.80 | 19.72 | 12.09 |

1. Weekly measurement commenced 18th March and ended 31sr 1987
2. Period equivalent to 28 days.

Appendix 9: Average Hen-day production (%) - Experiment 2

| Periods | | Diet 1 | Diet 2 | Diet 3 | Diet 4 |
|---|---|---|---|---|---|
| | 1 | 51.42 | 68.92 | 72.0 | 61.72 |
| | 2 | 77.14 | 68.57 | 62.86 | 72.85 |
| 1 | 3 | 68.27 | 61.27 | 65.57 | 75.43 |
| | $\bar{X}$ | 65.27 | 66.40 | 66.80 | 70.0 |
| | 1 | 68.57 | 68.57 | 78.85 | 65.15 |
| | 2 | 34.28 | 51.43 | 61.72 | 57.14 |
| 2 | 3 | 51.43 | 61.72 | 70.28 | 56.58 |
| | $\bar{X}$ | 51.43 | 60.58 | 70.28 | 59.63 |
| | 1 | 51.43 | 61.72 | 51.43 | 72.0 |
| | 2 | 38.57 | 51.46 | 51.43 | 65.14 |
| 3 | 3 | 55.72 | 57.14 | 49.72 | 68.57 |
| | $\bar{X}$ | 48.58 | 56.76 | 50.86 | 68.57 |
| | 1 | 42.85 | 44.57 | 65.15 | 61.72 |
| | 2 | 55.72 | 57.70 | 57.14 | 68.15 |
| 3 | 3 | 45.25 | 52.01 | 62.86 | 65.57 |
| 4 | $\bar{X}$ | 47.94 | 51.43 | 61.72 | 65.15 |
| | 1 | 68.57 | 44.57 | 65.15 | 61.72 |
| | 2 | 47.15 | 58.28 | 61.72 | 68.15 |
| 5 | 3 | 57.85 | 51.42 | 58.28 | 65.57 |
| | $\bar{X}$ | 57.85 | 51.42 | 61.72 | 65.15 |
| | $\bar{X}$ | 54.01 | 57.31 | 62.28 | 65.7 |

Appendix 10: Average Daily Feed Intake, (gram) - Experiment 2

| Periods | | Diet 1 | Diet 2 | Diet 3 | Diet 4 |
|---|---|---|---|---|---|
| | 1 | 76.0 | 83.0 | 95.0 | 81.0 |
| | 2 | 96.7 | 95.0 | 78.0 | 95.0 |
| | 3 | 85.3 | 78.0 | 76.0 | 95.0 |
| Trt. | $\bar{X}$ | 86.7 | 85.0 | 83.0 | 90.3 |
| | 1 | 95.0 | 115.0 | 112.0 | 120.0 |
| | 2 | 95.0 | 108.0 | 106.0 | 112.0 |
| | 3 | 95.0 | 106.5 | 110.0 | 107.0 |
| Trt. | $\bar{X}$ | 95.0 | 109.8 | 109.3 | 113.0 |
| | 1 | 107.0 | 117.2 | 113.0 | 119.0 |
| | 2 | 107.0 | 106.7 | 105.0 | 109.0 |
| | 3 | 95.0 | 105.9 | 110.0 | 114.0 |
| Trt. | $\bar{X}$ | 103.0 | 109.9 | 109.3 | 114.0 |
| | 1 | 117.0 | 112.0 | 119.5 | 120.0 |
| | 2 | 118.9 | 110.00 | 106.0 | 105.4 |
| | 3 | 117.2 | 112.0 | 112.9 | 113.0 |
| Trt. | $\bar{X}$ | 117.2 | 11:3 | 112:8 | 112:8 |
| | 1 | 110.0 | 115.0 | 120.0 | 122.0 |
| | 2 | 120.0 | 112.5 | 114.0 | 115.3 |
| | 3 | 112.0 | 114.5 | 113.0 | 120.0 |
| Trt. | $\bar{X}$ | 114.0 | 114.0 | 115.6 | 119.1 |
| Trt. | $\bar{X}$ | 103.3 | 106.0 | 106.0 | 110.0 |

Appendix 11 : Effects of period and Vitamin C supplementation on feed intake and egg production of laying chicken - Epperiment 2.

| Measurement | Period | Diet 1 | Diet 2 | Diet 3 | Diet 4 | SEM | Period Mean |
|---|---|---|---|---|---|---|---|
| Hen-day production, % | 1 | $64.28^{a}$ | $66.49^{a}$ | $58.28^{a}$ | $60.57^{a}$ | 5.107 | 62.38 |
| | 2 | $51.43^{b}$ | $60.57^{ab}$ | $69.03^{a}$ | $71.14^{a}$ | 5.107 | 63.04 |
| | 3 | $48.57^{a}$ | $57.91^{a}$ | $61.14^{a}$ | $61.91^{a}$ | 5.107 | 57.38 |
| | 4 | $47.94^{b}$ | $53.14^{a}$ | $55.43^{a}$ | $65.14^{a}$ | 5.107 | 55.41 |
| | 5 | 57.86 | $51.62^{a}$ | $61.72^{a}$ | $65.15^{a}$ | 5.107 | 59.09 |
| | $\bar{X}$ | 54.02 | 57.93 | 61.12 | 64.78 | 5.107 | 59.46 |
| Feed Intake, % | 1 | $88.70^{a}$ | $85.33^{a}$ | $83.0^{a}$ | $90.33^{a}$ | 3.176 | 86.84 |
| | 2 | $95.00^{b}$ | $109.83^{a}$ | $109.33^{a}$ | $113.0^{a}$ | 3.176 | 106.79 |
| | 3 | $103.00^{b}$ | $109.93^{a}$ | $109.33^{a}$ | $114.0^{a}$ | 3.176 | 109.07 |
| | 4 | $117.70^{a}$ | $111.33^{a}$ | $112.8^{a}$ | $112.66^{a}$ | 3.176 | 113.62 |
| | 5 | $114.0^{a}$ | $114.00^{a}$ | $115.66^{a}$ | $119.0^{a}$ | 3.176 | 115.67 |
| | $\bar{X}$ | 103.68 | 106.08 | 106.02 | 109.80 | | 106.40 |

[1] Statistical significance at $P \leq 0.05$ is denoted by different letters in a row.

Appendix 12 : Average Hen-day Production, (%) - Epxeriment 3

| Period | | Diet 1 | Diet 2 | Diet 3 | Diet 4 |
|---|---|---|---|---|---|
| | 1 | 51.42 | 68.92 | 75.43 | 90.0 |
| | 2 | 77.14 | 68.57 | 85.72 | 75.43 |
| 1 | 3 | 64.27 | 61.72 | 80.57 | 83.92 |
| | $\bar{X}$ | 64.27 | 66.40 | 80.57 | 83.11 |
| | 1 | 68.57 | 68.57 | 65.15 | 65.15 |
| | 2 | 34.28 | 51.43 | 75.43 | 68.57 |
| 2 | 3 | 51.43 | 61.72 | 70.55 | 72.85 |
| | $\bar{X}$ | 51.43 | 60.58 | 70.38 | 68.86 |
| | 1 | 51.43 | 61.72 | 65.15 | 64.27 |
| | 2 | 38.57 | 51.43 | 64.28 | 82.28 |
| 3 | 3 | 55.72 | 57.15 | 63.43 | 72.0 |
| | $\bar{X}$ | 48.58 | 56.76 | 64.28 | 72.85 |
| | 1 | 42.85 | 44.57 | 51.43 | 58.28 |
| | 2 | 55.72 | 57.70 | 57.71 | 60.0 |
| 4 | 3 | 45.25 | 52.01 | 55.72 | 61.76 |
| | $\bar{X}$ | 47.94 | 51.43 | 54.95 | 60.0 |
| | 1 | 68.57 | 44.57 | 51.43 | 58.28 |
| | 2 | 47.15 | 58.28 | 57.70 | 60.0 |
| 5 | 3 | 57.85 | 51.42 | 55.72 | 61.72 |
| | $\bar{X}$ | 57.85 | 51.42 | 54.95 | 60.0 |
| | $\bar{X}$ | 54.01 | 57.31 | 65.03 | 68.96 |

Appendix 13 : Average daily Feed Intake, (gram) - Experiment 3

| Periods | | Diet 1 | Diet 2 | Diet 3 | Diet 4 |
|---|---|---|---|---|---|
| | 1 | 76.0 | 83.0 | 87.0 | 90.0 |
| | 2 | 98.7 | 95.0 | 101.0 | 95.0 |
| 1 | 3 | 85.4 | 78.0 | 95.0 | 91.0 |
| | $\bar{X}$ | 86.7 | 85.0 | 94.0 | 92.0 |
| | 1 | 95.0 | 115.0 | 100.0 | 93.0 |
| | 2 | 95.0 | 108.0 | 105.0 | 90.0 |
| 2 | 3 | 95.0 | 106.5 | 101.0 | 97.0 |
| | $\bar{X}$ | 95.0 | 109.8 | 102.0 | 90.0 |
| | 1 | 107.0 | 117.2 | 103.0 | 90.0 |
| | 2 | 107.0 | 106.7 | 102.0 | 101.5 |
| 3 | 3 | 95.0 | 105.9 | 102.2 | 94.1 |
| | $\bar{X}$ | 103.0 | 109.9 | 102.4 | 95.2 |
| | 1 | 117.0 | 112.0 | 118.3 | 128.4 |
| | 2 | 118.9 | 110.0 | 120.0 | 129.0 |
| 4 | 3 | 117.2 | 112.0 | 119.0 | 129.9 |
| | $\bar{X}$ | 117.7 | 111.3 | 119.1 | 129.1 |
| | 1 | 110.0 | 115.0 | 117.0 | 117.6 |
| | 2 | 120.0 | 112.5 | 118.0 | 118.0 |
| 5 | 3 | 112.0 | 114.5 | 117.5 | 119.0 |
| | $\bar{X}$ | 114.0 | 114.0 | 117.5 | 118.2 |
| | $\bar{X}$ | 103.63 | 106.0 | 107 | 106.0 |

Appendix 14: Effect of period X palm oil : ascorbic acid supplementation on egg production and feed intake of laying hens - Epxeriment 3

| Measurement | Period Period | Diet 1 | Diet 2 | Diet 3 | Diet 4 | Period Mean | SEM[2] |
|---|---|---|---|---|---|---|---|
| Hen-day production, % | 1 | 64.28[a] | 64.40[a] | 69.72[a] | 67.81[a] | 67.05 | 5.11 |
| | 2 | 51.43[c] | 60.57[bc] | 77.15[a] | 74.83[a] | 66.0 | 5.11 |
| | 3 | 48.57[b] | 57.91[ab] | 70.38[a] | 68.56[a] | 61.35 | 5.11 |
| | 4 | 49.94[b] | 53.14[b] | 59.71[ab] | 70.85[a] | 57.91 | 5.11 |
| | 5 | 57.86[a] | 51.62[a] | 54.95[a] | 60.01[a] | 56.11 | 5.11 |
| | $\bar{X}$ | 54.42 | 57.53 | 66.38 | 68.41 | 61.68 | |
| Feed Intake, g | 1 | 88.7[a] | 85.33[a] | 92.0[a] | 92.0[a] | 89.51 | 3.18 |
| | 2 | 95.0[a] | 109.83[a] | 90.0[a] | 90.0[a] | 96.21 | 3.18 |
| | 3 | 103.0[a] | 109.93[a] | 95.36[a] | 95.36[a] | 100.92 | 3.18 |
| | 4 | 117.7[b] | 111.33[a] | 129.10[a] | 129.10[a] | 121.81 | 3.18 |
| | 5 | 114.0[a] | 114.00[a] | 118.20[a] | 118.20[a] | 116.1 | 3.18 |
| | $\bar{X}$ | 103.68 | 106.88 | 104.93 | 104.93 | 104.91 | |

[1] Statistical significance at P/0.05 is denoted by different superscripts in a row

[2] Standard error of means for treatments.