EFFECTS OF PRODUCTION STRESSES AND ASCORBIC ACID SUPPLEMENTATION ON SOME BIOCHEMICAL PARAMETERS IN THE DOMESTIC FOWL (Gallus domesticus)

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DECLARATION

I, hereby, declare that this thesis is entirely a record of my own research work; and has not been part of any presentation for any other qualification. Writers whose works have been cited are duly acknowledged.



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CERTIFICATION

This thesis entitled "Effects of production stresses and ascorbic acid supplementation on some Biochemical parameters in the domestic fowl (Gallus domesticus)" by **Sunday Ameh IGAH** meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This piece of research work is dedicated to my children and all those who undergo stress situations.

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ABSTRACT

Modern large-scale poultry production is associated with many stress factors. These include exposure to high light and temperature, multiple entries to pens, over-crowding, transportation between farms, etc. It is also known that response to stress can be by way of changes in certain biocheitnical parameters in animals and that ascorbic acid and other micro-nutrient elements supplementation to some extent reduces the effects of stress. This work is aimed at studying the effects of some of these stress factors and the possible role of ascorbic acid in ameliorating the effects. To this end, a total of eight hundred (800) day-old broilers from which a representative sample of five hundred (500) broilers was randomly drawn was experimented with in stages to evaluate the effects of age, sex and stress factors (high / low light exposure, a high / low number of entry to pen and transportation), litis was done with and without ascorbic acid supplementation (300mg / kg diet). Haematocrit (PCV), total serum cholesterol, total serum protein, total! serum creatinine and total available serum sugars were the selected biochemical parameters whose levels were monitored as indices of stress. The results showed that age and sex had similar effects on the levels of these biochemical parameters in the broilers. There were no changes in PCV with age ($P > 0.01$). But levels of other biochemical parameters showed some significant changes ($P < 0.01$), particularly more so in male broilers than in the female ones. High light exposure generally increased the levels of the biochemical parameters especially up to the age of 5 weeks. In some cases, the increase is sex dependent. A high number of entries into the pen significantly increased ($P < 0.01$) the levels of the biochemical parameters in broilers, except for total cholesterol level which dropped ($P < 0.01$) in male broilers at 8 weeks of age. Similarly, total protein and creatinine levels decreased ($P < 0.01$) in male and female broilers at 8 weeks of age; while total sugar levels decreased ($P < 0.01$) in male and female broilers at 3 and 5 weeks of age. But, low light exposure and a low number of entry into the pen were similar in not causing any significant change ($P > 0.01$) to the levels of the biochemical parameters. Transportation significantly increased ($P < 0.01$) only the total cholesterol and sugar levels in broilers at 8 weeks of age. Ascorbic acid supplementation introduced selectivity to the effects of stress factors on the levels of the biochemical parameters. This was shown through either the maintenance of homeostatic levels ($P > 0.01$), significant increase or decrease ($P < 0.01$) of levels. It is observed from this work that change of levels of the biochemical parameters in broilers during production is as a result of the production stresses. The study showed that ascorbic acid supplementation (300mg / kg diet) in broilers, to some extent, ameliorated some of the adverse effects of these production stresses in broilers.

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ABBREVIATIONS

ANOVA	=	Analysis of Variance
BSA	=	Bovine Serum Albumin (5mg / ml) Standard
G.A.S	=	General Adaptation Syndrome
SEM	=	Standard Error of Mean

CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

The problem of malnutrition, particularly protein-energy malnutrition, is a real one in most developing countries of the world. These countries, including Nigeria, are mostly located in the warm humid tropics where the level of animal protein intake is 9g / head / day (F.A.O., 1962; Davidson et. al., 1979) which represents about one tenth of the level of intake in some advanced countries. Poultry keeping provides a method by which rapid transformation in animal protein consumption can be achieved in these developing countries. However, this can take place only after the productive process has been modernized (Ademosun, 1979). Incidentally, poultry keeping, particularly the keeping of the domestic fowl (Gallus domesticus) in Nigeria, like in many other developing countries in the humid tropics generally follows traditional lines without the innovations which modern scientific endeavors have introduced. Thus, the birds under this system are characterized by slow rate of growth, low productivity with low feed efficiency, and heavy parasitic infestation, infection and disease. In the relatively few commercial undertakings, the husbandry methods are based on those developed for the temperate regions (Ademosun, 1979) without taking adequate cognizance of the peculiarities of the local environment. Therefore, the birds in this system are constantly exposed to stress situations. High environmental temperature is one of such stressors and it has been shown that vitamin C can significantly alleviate adverse effect of this stressor (Kechik and Sykes, 1978; Pardue et. al., 1985; Njoku, 1986). Although much attention had been paid to the effect of environmental temperatures on the performance of poultry, hardly has similar consideration been given to assess other aspects of stress which may also affect poultry performance. This is so despite the common knowledge that even under normal ambient temperature, birds are exposed to stresses of disturbance (especially in developing countries with high physical labor input in

the feeding and watering of the birds), vaccination, feed change, housing transfer, transport to slaughter house, etc. Thus, this study was designed specifically with the following objectives:

1. To evaluate the effect(s) of age, sex and treatments on haematocrit (PCV), total serum cholesterol, total serum protein, total serum creatinine and total available serum sugars of broilers during production.

2. To assess the capacity of ascorbic acid supplementation to ameliorate the adverse effect(s) of the production stresses.

In order to achieve the objectives, null hypotheses were formulated as follows:-

Ho_1 = Age of broilers (weeks) has no significant effect on the biochemical parameters of broilers during production. $Ho_1: \mu A = \mu B$ ($P<0.01$).

Ho_2 = Sex of broilers (male or female) has no significant effect on the biochemical parameters of broilers during production. $Ho_2: \mu S = \mu B$ ($P<0.01$).

Ho_3 = Treatments, including ascorbic acid supplementation, have no significant effect on the biochemical parameters of broilers during production. $Ho_3: \mu T = \mu B$ ($P<0.01$).

1.2 LITERATURE REVIEW

1.2.1 DEVELOPMENT OF MODERN POULTRY ENTERPRISE IN NIGERIA

The growth and development of modern poultry enterprise in Nigeria has its roots in the late 1950's when the former Western Regional Government in collaborations with some foreign partners from Israel, Britain and USA introduced the "White man's chickens" - as the improved strains were then called (Nwosu et. al., 1979). Some good breeds of broilers were supplied to Nigeria during that period. The programme involved establishment of multiplication centers and hatcheries from where chickens were supplied to designated farm settlements for further production processes. These settlements played a pioneering role in commercial poultry farming and in popularizing the "Agric. Chickens and Eggs". It was also not surprising that ex-civil

servants who once participated in this programme eventually became the first generation of modern commercial poultry producers in Nigeria.

According to Lamorde (1991) the campaign for the commercial poultry farming gained grounds nation-wide between 1970 and 1980 as a profitable venture in the private sector. Many hatcheries were established for the "Local Sourcing" of commercial day-old chicks. By 1980, there were about 2.5 million total egg incubator capacity in Nigeria; with Lagos and neighboring states contributing about 50 per cent of the total capacity. Domestic poultry meat production which rose from 51,000 metric tonne in the late 1970's to 60,000 metric tonne in early 1980's reached a peak of 67,000 metric tonne in 1986; and then suddenly dropped to 54,000 metric tonne in 1988. The drop in poultry meat production was apparently associated with government fiscal policies which made imported feed inputs very expensive. This forced many modern poultry farmers to close down; leaving cottage poultry keeping to fill the gap.

The total egg output was used to illustrate the effect of government fiscal policies on modern poultry production. It was shown that egg production in Nigeria increased from 314,000 metric tonne in 1980 and reached its peak of 399,000 metric tonne in 1986 before plummeting to 260,000 metric tonne in 1988 during the Structural Adjustment Programme (SAP) (Lamorde, 1991). The same author demonstrated that an interesting aspect of the effect of SAP on modern poultry production had been the enhanced percentage profit to those who decided to remain in business despite the escalating cost of inputs. Poultry farmers achieved the enhanced percentage profit (of approx. 88% in some cases) by not only applying the economy of scale; but also through compounding their own poultry rations, direct maize production to enable reduction of the quantity purchased and by price adjustments of egg and poultry meat such that the prevailing cost of feeds and medicaments were absorbed with allowance for a fair profit margin (Lamorde, 1991).

Despite the promise of huge financial returns on investment for poultry farmers, the fact

remains that poultry production currently fails to meet demand in Nigeria. Unfavorable Government fiscal policies, poor husbandry and / or lack of attention to rural poultry production have often been cited as contributory factors which impede the sustainability of a viable poultry production enterprise in Nigeria (Lamorde, 1991). For example, rural poultry which has been largely neglected represents about 80% of the approximately 150 million total poultry population in Nigeria (F.A.O., 1984).

It seems that, poultry keeping (particularly the keeping of the domestic fowl) in Nigeria at present invariably exposes the birds to a preponderance of production stresses. This may be an additional factor in the current production which fails to meet demand. The birds produced are characterized by slow rate of growth, low productivity with low feed efficiency, heavy parasitic infestation, infection and disease. Therefore, the need to study the effect(s) of production stresses on the biochemical parameters of broilers and the possible role of ascorbic acid (AA) supplementation in ameliorating the adverse effect(s) can not be over-emphasized.

1.2.2 DEFINITION AND TERMINOLOGY OF BIOLOGICAL STRESS

"Stress" is defined as the sum of an animal's defense responses to a specific stimulus (Thaxton and Pardue, 1984). According to this definition, the collective defense responses are "stress" and the "stimulus", which may be one of many internal or external changes including nutritional deficiencies, adverse environmental conditions, and even psychological alterations, is the "stressor". When an animal is subjected to a stressor a series of predictable body responses occur and these responses are termed "stress response". The apparent reason for stress is to insure adaptation to the unexpected change and to re-establish the homeostatic condition.

The adaptive responses are usually sequential; however, the order and timing of the responses depend upon the intensity and duration of the stressor.

1.2.3 THE GENERAL ADAPTATION SYNDROME

Selye (1950) proposed the General Adaptation Syndrome (G. A. S) to account for the time course of stress responses.

GAS consists of three component stages of:- (i) Alarm, (ii) Resistance, and (iii) Fatigue.

During alarm stage the stressor is recognized and instantaneous neuroendocrine responses are mounted. An excellent analogy is the classic fight or flight syndrome, as described originally by Cannon (1929). At resistance stage, sustained effort to adapt to the effects of the stressor is paramount. Normally, the animal re-establishes homeostasis and stress is terminated. If resistance is prolonged and the animal fails to adapt, fatigue is insured. As Selye (1950) aptly pointed out, fatigue generally leads to death.

1.2.4 PRODUCTION STRESSES

In poultry keeping, the quality of husbandry, management, housing, disturbance, light, vaccination and transportation constitute stressors to which the birds will respond. It is such a non-specific response to stressors during the course of poultry production that is collectively termed "Production stresses"; some of which have been selected and articulated for the purposes of the present study.

(i) **Light** : The fowl requires light because prolonged darkness may injure their eyes. Normal light (i.e. 100W incandescent pin bulb per 3.4m^2 floor space per day) is needed for them to see the feed but light that is too bright (i.e. 400W incandescent pin bulb per 3.4m^2 floor space per day) is wasteful and can cause stress for the birds resulting in some reduction in growth rate (Oluyemi and Roberts, 1979). Similarly, a low light exposure (i.e. 60W incandescent pin bulb per 3.4m^2 floor space per day) may be stressful to broilers during production. In general, the benefit of lighting cannot be ignored as even intermittent lighting has been reported to greatly reduce mortality due to ascites in male broiler chicks such that it is being recommended for

consideration as a standard programme in some places (Lewis et. al., 1996; World Poultry, 1998).

(ii) **Number of entries into the pens**: Poultry keeping operations involving physical labor input such as feeding and watering of birds rely heavily on movements as typified by the number of entries into the pens. The number of entries into the pens may be high (i.e. 4 per day) or low (i.e. 1 per day). Depending on stimulus intensity and duration, entry into pen could constitute a production stress as it has the potential of disrupting or breaking the relative quiet, calm, peace or order of broilers.

(iii) **Transportation**: Poultry are transported in many different ways world-wide. Handling in an 'inverted' fashion by some Hawkers is common in Nigeria. There has been a suggestion that handling stress can be reduced in broilers by the use of upright handling (Kannan and Mench, 1996). However, whatever form transportation takes can expose the birds to a plethora of stimuli most of which have been shown to be stressors (see Freeman, 1984).

But, Halliday et. al., (1977) could find no consistent stress response in transported birds. It is possible, however, that this lack of response was related to the shortness of the journey (8 - 16 Km) and the fact that sampling was delayed 1-2 hours after the journey had been completed. In a longer experiment (4hr), where distances of up to 224Km were covered, consistent evidence of increased adrenal cortical activity was obtained (Freeman, 1984).

1.2.5 ASCORBIC ACID, DOMESTIC FOWL AND STRESS

Although, many animals are capable of synthesizing ascorbic acid (AA) and therefore do not require it in their diet, man and other primates and the Guinea pig are unable to do so because they lack the enzyme, l-gulonolactone oxidase, which is required to produce AA out of D-glucoronate (Montgomery et. al., 1983). The vitamin must therefore be added to their diets in order to promote normal connective tissue synthesis (Abalaka et. al., 1990).

Domestic fowl, like most terrestrial vertebrates, possess the innate ability to synthesize ascorbic acid (Pardue and Thaxton, 1986). The ability of the chicken to synthesize AA was demonstrated by Carrick and Hauge (1925), and in the same year Hart et. al., (1925), conclusively showed that chickens do not need vitamin C in their diet.

As a result of such findings, AA is not added to poultry feeds (Pardue and Thaxton, 1986). However, the results reported by Bell et. al.,(1941) indicated that hens are not always able to synthesize enough AA for their needs. Whereas, it is generally accepted (Hart et. al., 1925; Plummer and Rosedale, 1923; and Shorten and Ray, 1921) that chickens do not need AA in their diets; other reports (Holst and Halbrook, 1933; Beaudette, 1923) indicated the possibility that chickens may require AA. Interestingly, the research literature dating from 1933 is full of reports suggesting that particular environmental and pathologic conditions may increase metabolic need for AA beyond the innate synthetic ability of the bird (Pardue and Thaxton, 1986).

Bell et. al., (1941) appear to have demonstrated the need for AA by high producing birds. A major cause of mortality in such broilers is "leg weakness" - a term which has been used to describe a variety of conditions which may be different in their aetiology, clinical signs and severity (Julian, 1990). Briggs et. al., (1944) observed that AA, when fed to chicks receiving various purified rations consistently stimulates the growth rate to a small but definite extent; although, the exact mechanism of action is unknown (Ewing, 1951).

Clearly, therefore, ever since the potential relationship between stress and AA in fowls was first recognized, a record of rather inconsistent and conflicting findings accumulated in the research literature. A review of this subject had been lacking until Pardue and Thaxton (1986) made an attempt to summarize the findings concerning the metabolic and physiologic processes of AA in poultry. Presently, a growing attention has been focused on the potential role of vitamin C supplementation in preventing over-reaction to stressful stimulation and thereby

helping chickens and other animals to cope with such challenges (Jones et. al., 1996). In particular, vitamin C supplementation had been observed to reduce a number of fear-related behavior in broiler chickens (Satterlee et. al., 1994). But an information gap appears to exist in the literature about the effect(s) of age, sex and treatments, including ascorbic acid supplementation, on the levels of some important biochemical parameters of broilers of 3, 5 and 8 weeks of age during production.

1.2.6 THE BIOCHEMICAL PARAMETERS SELECTED FOR STUDY

The biochemical parameters selected for study were as follows:-

(i) Haematocrit or Packed cell volume (PCV): It expresses in percentage terms the portion of total blood volume occupied by red blood cells (Goodale and Widmann, 1969). Green (1982) had reported that a high haematocrit (or PCV) level indicates either an increase in the number of circulating red cells, or a reduction in the circulating plasma volume. Conversely, a low haematocrit (or PCV) level usually indicates a reduction in the number of circulating red cells; but the reduction will also occur if the plasma volume increases. The haematocrit (or PCV) usually ranges between 20 and 40% in birds (see Freeman, 1971). Change of haematocrit (or PCV) levels in broilers could be of physiologic significance (Thaxton, 1978; Wels, 1966; Brake and Thaxton, 1979), probably, as an important index of response to production stresses.

(ii) Total serum cholesterol: It is a white waxy sterol ($C_{27}H_{45}OH$) present in the tissues of the body of animals, in which it performs a number of vital functions.

Its excessive production in man, for instance, is suspected of being a contributory cause to coronary thrombosis (Isaacs, 1971). Total serum cholesterol level is essential to the mechanism for corticosterone synthesis. Corticosterone is an adrenal steroid with stress ameliorative properties (Hart and Newton, 1983). Therefore, total serum cholesterol level could be an

important index of the extent to which cholesterol is mobilized in broilers exposed to production stresses.

(iii) **Total serum protein**: Proteins are a class of complex nitrogenous organic compounds of high molecular weight (18,000 - 10,000,000) which are of great importance to all living matter (Isaacs, 1971). Total serum protein is essential to the mechanisms for maintenance of amino acid pool, colloid osmotic pressure, tissue synthesis and regeneration, growth and maturation. During situations in which carbohydrate and fat are inadequate to meet energy demands, tissue proteins could be drawn on and mobilized to be a veritable source of gluconeogenic energy (Goodale and Widmann, 1969; Green, 1982). Therefore, total serum protein level could be an important index of the extent to which protein is mobilized to perform its various functions in broilers exposed to production stresses.

(iv) **Total serum creatinine**: Creatinine ($C_4H_7N_3O$) is a white crystalline substance, derived from the amino acid creatine ($C_3H_9N_3COOH$), found in urine, blood, etc (Isaacs, 1971). Creatine is synthesized from arginine and glycine by reactions which appear to be irreversible, since the only known endproduct of creatine metabolism is creatinine. Creatinine excretion has long been used as an index of muscle mass on the assumption that a given amount of muscle produces daily a fixed amount of creatinine, approximately 50mg / kg (ARC/MRC Committee, 1974). As creatinine is an index of muscle mass, its serum level could be an important index of the extent to which it is mobilized in broilers exposed to production stresses.

(v) **Total available serum sugars**: Carbohydrates are a large group of organic compounds composed of carbon, hydrogen and oxygen only. With the general formula $C_x(H_20)_y$, it comprises monosaccharides, disaccharides (both sugars), and polysaccharides (starch and cellulose). Carbohydrates play an essential part in the metabolism of all living organisms (Isaacs, 1971). According to Bell (1971) the whole blood of birds carries a much greater amount of reducing sugars than do any mammalian bloods, and there seems to be no reason for this.

Moreover, earlier work had measured the renal threshold for glucose in four 14-month-old hens and found values of 310, 360, 330 and 260mg / 100ml plasma (see Bell, 1971). The same author had reported, also, that glucose concentration in the plasma of normal fowls was relatively higher than in whole blood. For above reasons, and in order for a more representative value of total serum sugar concentration in broilers to emerge, it was proposed by the present study to assay for the total available serum carbohydrate comprising hydrolyzed starches with soluble sugars (see Osborne and Voogt, 1978). The level of such serum sugars could be an important index of the extent to which it is mobilized in broilers exposed to production stresses.

(vi) **Ascorbic acid supplementation (300mg / kg diet)**: The need for ascorbic acid supplementation is underscored by the realization that during increased physical strain through illness, unfavorable environmental conditions or particular demands on capacity, a state can be reached in which the synthesis of vitamin C no longer covers individual requirement. This is suggested by numerous reports of positive effects resulting from administering vitamin C to various species of animals (Marks, 1979). In poultry, better heat resistance, further laying output, increased resistance to breakage of egg-shells, increased seminal production in young cocks, increased resistance to infections, and a reduced requirement for group B vitamins, have been reported (Marks, 1979). A record of the recommended vitamin supplementation levels for poultry feed in hot climates indicates 100 - 150mg / kg diet for broilers (Fenster, 1986). However, a literature survey showing the effect of vitamin C on egg production under conditions of high environmental temperatures had used different dietary levels of ascorbic acid from as low as 25mg / kg diet to as high as 400mg / kg diet, but excluding 300mg / kg diet (Fenster, 1985). As there has been no apparent reason for its exclusion, this work on broilers exposed to production stresses proposes ascorbic acid supplementation at the level of 300mg / kg diet so as to evaluate its role(s).

1.2.7 JUSTIFICATION FOR SELECTION OF THE BIOCHEMICAL PARAMETERS

(i) Literature review had revealed a relative information gap concerning the effect(s) of age, sex, production stresses (i.e. a high / low light exposure, a high / low number of entries into the pens and transportation) with and without ascorbic acid supplementation on some biochemical parameters in domestic fowls as set out in this investigation.

(ii) This work is capable of bridging the relative information gap in this area of research interest that lends itself to sustained further research, in order to extend the frontiers of present day knowledge.

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 EXPERIMENTAL ANIMAL

A total of Eight hundred (800) ANAK day-old broiler chicks were purchased from Kaduna Hatchery. They were group-reared in Ahmadu Bello University poultry farm in Zaria. The broilers first received intraocular vaccination against "Newcastle disease" at one day of age. This consisted of placing a drop of the liquid vaccine in one of their eyes. The second week vaccination against fowl pox employed the 'stick' method of stabbing the wing-web from the under-surface. At six weeks of age, the broilers were immunized against fowl typhoid and boosted against "Newcastle disease" via intramuscular injection into their chest muscle (Oluyemi and Roberts, 1979). Therefore, the broilers undertook the same management protocol of vaccination and acclimatization. They were apparently healthy and synchronized to three weeks of age before the start of the experiment. Five hundred (500) broilers were randomly drawn to constitute a representative sample of the total population and used for the experiments.

2.2 CHEMICALS AND REAGENTS

Unless where stated otherwise, all chemicals and reagents used were of analytical grade. They were obtained from either Sigma Diagnostics (USA) or British Drug House (BDH).

2.3 MATERIALS

Haematocrit (Mikkro - Hamotokrit, Hareus Christ, GMBH. D - 3360 Osterode AM Harz, Postfach 1220). MSE minor centrifuge (ser. No. AA 2254, made in England).

Metler balance (Model H8, Metler Instruments AG Switzerland), Vortex mixer (Type H 2805, Griffin, Acadex Engineering Co. Ltd., Basildon, Essex England), Micro-pipette, Microfuge tubes, Borosilicate culture tubes and test tubes (5ml and 10ml), Un-calibrated capillary tubes,

Aero-freeze refrigerator, Syringes (5ml) and needles (20Gx1½", 19Gx1½").

2.4 PREPARATION OF REAGENTS

1. **Preparation of Biuret reagent:**

This was prepared as described by Wootton and Freeman (1982); in which 9g Sodium Potassium Tartrate ($COOK(CHOH)_2COONa.4H_2O$), was dissolved in 500ml of 0.1M Sodium Hydroxide (NaOH) and 3g Copper Sulphate ($CuSO_4.5H_20$) was added and dissolved by stirring. Then 5g Potassium Iodide (KI) was added and the volume was made up to 1 Litre with 0.1M Sodium Hydroxide. All chemicals except Potassium Iodide (M & Baker Nigeria Ltd) used for preparation of Biuret reagent was supplied by the British Drug House (BDH Chemicals Ltd; Poole, England).

2. **Preparation of Bovine Serum Albumin (BSA; 5mg/ml):**

The method described by Plummer (1978) was used to prepare BSA (5mg/ml; Amour Pharmaceuticals). Defatted BSA (0.5g) was accurately weighed and added to about 80 ml deionized water in a beaker. It was neither stirred nor shaken, but left to stand until the albumin had dissolved. The solution was gently swirled to mixed, then transferred to a 100 ml volumetric flask. The beaker was rinsed out several times with deionized water and the washes were added to the volumetric flask. The flask was carefully inverted several times, without shaking or stirring vigorously, to mix the contents and made up to the mark with deionized water.

For storage of the standard BSA, the 100 ml was dispensed into 5 ml aliquot and stored in the deep freeze until required. Once a tube of BSA solution was thawed for use, any excess remaining was discarded, but not re-frozen.

3. **Preparation of 52% Perchloric Acid**: Add 270 ml of Perchloric acid (sp.gr. 1.70) to 100 ml of water. Cool before use.

4. **Dilution of Concentrated Sulphuric Acid (H_2SO_4)**: H_2SO_4 (sp.gr. 1.84) of 760 ml was

carefully added to 330 ml of deionized water and cooled before use.

5. Preparation of Anthrone reagent: Sufficient 0.1% Anthrone (10-keto-9,10 dihydroanthracene) was made up in the Sulphuric acid for the day's requirements. It was made fresh daily.

6. Preparation of Glucose Standard Solution: D-Glucose anhydrous (BDH Chemicals Ltd, Poole, England; product No. 28450) of 100 mg was dissolved in 100 ml distilled water.

7. Preparation of Dilute Standard Solution: Strong Standard solution of 10 ml diluted to 100 ml with deionized water (ml = 0.1 mg Glucose).

2.5 EXPERIMENTAL DESIGN

The experiments were carried out in stages. The broilers (n = 500) were individually weighed, wing-tagged and randomly allotted to each of 10 experimental deep-litter pens. The pens were located in a dwarf-walled poultry building with each pen providing 3.4 m^2 of floor space (Monsi and Agbogidi, 1991). Classification of the broilers was based on age (3, 5 and 8 weeks) and sex (male or female).

The first stage of the experiment involved evaluation of the effect(s) of age and sex of the broilers on some biochemical parameters during production (Table 1).

Apart from the first stage of the experiment in which there was no treatment (i.e. 10 x 50 = 500), each of the other stages underwent treatments which consisted of two groups, one of which was the control (i.e. 2 x 5 x 50 = 500), in a completely randomized experimental design.

The second stage of the experiment involved evaluation of the effect of light exposure on some biochemical parameters of the broilers during production. Insulation of the pen from natural light by using a suitable tarpaulin material enabled a normal light source of 100W incandescent pin bulb per 3.4m^2 of floor space to be achieved. This normal light exposure also served as CONTROL with which high light exposure of 400W incandescent pin bulb per 3.4m^2 of floor

space was compared in this treatment (Table 2).

The third stage of the experiment involved evaluating the effect of high light exposure on some biochemical parameters of ascorbic acid supplemented (300mg / kg diet) broilers during production. The data (Table 2) of high light exposure without ascorbic acid supplementation served as CONTROL with which ascorbic acid supplemented data was compared in this treatment (Table 3).

The fourth stage of the experiment involved evaluating by using a normal light exposure (Table 2) as CONTROL with which the effects of low light exposure of 60W incandescent pin bulb per $3.4m^2$ of space was compared in this treatment (Table 4).

The fifth stage of the experiment involved evaluating the effect of low light exposure (Table 4) as CONTROL with which ascorbic acid-supplemented data was compared in this treatment (Table 5).

The sixth stage of the experiment involved evaluating the effect of number of entries into the pens on some biochemical parameters of broilers during production. A normal entry into the pens (2 per day) served as CONTROL with which a high number of entries into the pens (4 per day) was compared in this treatment (Table 6).

The seventh stage of the experiment involved evaluating the effect of a high number of entries into the pens (4 per day) on some biochemical parameters of ascorbic-acid supplemented (300mg / kg diet) broilers during production. The data of a high number of entries into the pens (Table 6) served as CONTROL with which the ascorbic acid-supplemented data was compared in this treatment (Table 7).

The eight stage of the experiment involved using a normal entry into the pens (Table 6) as CONTROL with which the effect of a low number of entry into the pens (1 per day) was compared in this treatment (Table 8).

The ninth stage of the experiment involved evaluating the effect of a low number of entry into

the pens (Table 8) as CONTROL with which ascorbic acid-supplemented data was compared in this treatment (Table 9).

The tenth stage of the experiment involved evaluating the effect of transportation (30km / day) on some biochemical parameters of 8 weeks old broilers during production. The data of broilers not transported served as Control with which those transported were compared in this treatment (Table 10).

The eleventh stage of the experiment involved evaluating the effect of transportation (30km / day) on some biochemical parameters of ascorbic acid-supplemented (300mg / kg diet) broilers at 8 weeks of age during production. The data of transported broilers (Table 10) served as CONTROL with which ascorbic acid-supplemented data was compared in this treatment (Table 11).

Finally, treatments were terminated at 8 weeks of age when each of the broilers had achieved a minimum weight of 2 kg.

2.6 COLLECTION OF BLOOD SAMPLES

Blood samples were obtained from the wing vein using 5ml syringe with 20Gx1½" needle for collection from broilers at 3 and 5 weeks of age and 19Gx1½" needle for collection from broilers at 8 weeks of age (Harry and Cooper, 1976). Blood was allowed to stand at room temperature for 1 hr. Sera samples were collected with pasteur pipettes, centrifuged at 3000 rpm for 10 min., pooled and stored at -20°C until assayed.

2.7 BIOCHEMICAL ASSAYS

2.7.1 Determination of Haematocrit (PCV)

This was done by using heparinized capillary and micro centrifuge technique (Green, 1982). Each un-calibrated capillary tube 7.5 cm long, 1 mm in diameter and coated internally

with heparin to prevent blood clotting had one of its ends placed in the drop of blood. The blood entered by capillary attraction. The tube was removed when it was two-thirds full with blood. One end was sealed with plasticine. The tube was then placed with the sealed end outward in the micro-centrifuge (Haematocrit: Mikkro-Hamotokrit, Hareus Christ) and rotated at 10,000 g for 5 mins. With this centrifugal force, the separation of cells from blood was achieved.
The length of the separated cells in the column of the tube was measured to calculate the Haematocrit (Goodale and Widdmann, 1969; Green, 1982).

2.7.2 Estimation of Total serum cholesterol

Total serum cholesterol was determined using Cholesterol Reagent No. 352 (Sigma Diagnostics, 1988). The principle involved the hydrolysis of cholesterol esters by cholesterol esterase (EC 3.1.1.13) to cholesterol. The cholesterol produced was oxidized by cholesterol oxidase (EC 1.1.3.6) to cholest-4-en-3-one and hydrogen peroxide (H_2O_2). The hydrogen peroxide was then coupled with the chromogen, 4-aminoantipyrine and P-hydroxybenzenesulfonate in the presence of peroxidase (EC 1.1.17) to yield a quinoneimine dye which had an absorbance maximum at 500 nm. The intensity of the colour produced was directly proportional to the total cholesterol concentration in the sample.

Cholesterol reagent was prepared according to the Manufacturer's instructions. Series of tubes were set up for blank, standard and samples. The working reagent was warmed to assay temperature (25 °C) and 1 ml was pipetted into each tube. Ten micro-litres (10 μl) each of deionized water (blank), calibrator (standard cholesterol 200mg / dl) and Sample was added to appropriately labeled tubes. The contents of each tube was mixed by gentle inversion. The tubes were incubated in a water bath for 5 mins at 37°C. The absorbance of all tubes at 500 nm was read using spectrophotometer (WPA S105) and recorded within 30 mins after colour development. The total serum cholesterol was calculated as follows:-

Total serum cholesterol (mg/dl) = $\frac{A_{Test} - A_{Blank}}{A_{calibrator} - A_{Blank}} \times [standard]$

Where:-

A_{Test} = Absorbance of Test Sample (e.g. 0.725)

A_{Blank} = " " Blank (e.g. 0.037)

$A_{calibrator}$ = " " Calibrator (e.g. 0.660)

∴ Total serum cholesterol = 220.87 mg/dl.

2.7.3 Estimation of Total Serum Protein

Biuret method (Wootton and Freeman, 1982) was used to estimate total serum protein with BSA (5mg/ml) as standard protein (Plummer, 1978). The biuret method is based on the principle that Copper in alkaline solution reacts with the peptide linkages of amino acids in protein producing a violet colour. The intensity of the color is proportional to the concentration of the protein. To tubes labeled blank, 5 ml of deionized water was added. To tube labeled standard, 3 ml BSA (5 ml) was added. To each of the tubes labeled Test, 0.1 ml serum sample and 2.9 ml of deionized water was added. To all tubes, 0.5 ml of the prepared biuret reagent was added and mixed. The tubes were incubated in a water-bath at 35°C for 5 mins.

Absorbance of standard and test versus blank was read at 540 nm. Total serum protein was calculated as follows:-

Total serum protein (g/dl) = $\frac{A_{Test} - A_{Blank}}{A_{std} - A_{Blank}} \times 5 \times 15 \times 0.01^*$

Where:-

A_{Test} = Absorbance of Test Sample.

A_{Blank} = " " Blank "

A_{std} = " " Standard "

*(Results converted to g/dl by multiplying by factor 0.01).

2.7.4 Estimation of Total serum creatinine

Total serum creatinine was estimated by using Sigma Diagnostics Creatinine Procedure No 555-2. (1989).

The principle involved the manual method in which alkaline picrate reacts with

creatinine to form a red colored complex with other interfering chromogens. Under acidic conditions this color due to creatinine alone is destroyed. The optical density of the red color is proportional to the amount of creatinine in the filtrate. The difference in color intensity measured at or near 500 nm before and after acidification is proportional to creatinine concentration.

To tube labeled blank, 50 μl deionized water was added. To tube labeled standard 50 μl creatinine (3 mg/dl) was added. To tube labeled Test, 50 μl of Serum sample was added. To all tubes, 0.5 ml alkaline picrate solution was added; mixed and allowed to stand at room temperature for 8 - 12 mins. Initial absorbance of standard and test versus blank was read at 500 nm. Subsequently, to all tubes exactly 17.5 μl acid reagent (Catalogue No. 555-2) was added. The contents of the tubes were immediately and thoroughly mixed using a vortex mixer and then allowed to stand for 5 mins at room temperature. Final absorbance of standard and test versus blank was read and recorded at the same wave length used for the initial readings. Sensitivity of the equipment was checked against calibration curve in the manual.

Total serum creatinine (mg/dl) was calculated as follows:-

$$\text{Total serum creatinine (mg/dl)} = \frac{\text{Initial } A_{Test} - \text{Final } A_{Test}}{\text{Initial } A_{std} - \text{Final } A_{Test}} \times 3^{*}$$

Where:-

Initial A_{Test} = Initial Absorbance of Test Sample

Final A_{Test} = Final " " " "

Initial A_{std} = Initial " " Standard "

*(Concentration = 3 mg/dl Creatinine Standard; Catalogue No. 925-3).

2.7.5 Estimation of Total available serum sugars

The method of Osborne and Voogt (1978) was used to estimate total available serum sugars. The principle involved digestion of the serum sample with perchloric acid. Hydrolyzed starches together with soluble sugars were determined colorimetrically.

To tube labeled blank 0.1 ml deionized water was added. To tube labeled standard 0.1

ml glucose standard (0.1mg / ml) was added. To tubes labeled Test 0.1 ml serum sample was added. To all tubes 0.5 ml freshly prepared Anthrone reagent was added. All tubes were stoppered with Aluminum foil and contents were mixed using a vortex mixer. They were incubated in a water-bath at 37°C for 2 - 3 mins then cooled quickly to room temperature and filtered using Whatmann no. 542 filter paper. The filtrate of each tube was transferred to a glass cuvette. Absorbance of standard and test versus blank was read at 630 nm.

Total available serum sugar was calculated as follows:-

$$\text{Total available serum sugars (mg/dl)} = \frac{A_{Test} - A_{Blank}}{A_{std} - A_{Blank}} \times 10 \times 100^{*}$$

*(To correct for dilution of Standard solution).

2.8 STATISTICAL ANALYSIS

The means of the biochemical parameters were subjected to One-way analysis of variance (ANOVA) and the Scheffe' multiple comparison test. In all cases, if the significant probability of any mean and its corresponding control was less than $\propto = 0.01$; then it was concluded that there was a significant difference in the means.

With this statistical analysis, a rejection of the null hypotheses implies that age, sex or treatments did not have significant effect ($P > 0.01$) on the levels of the biochemical parameters of the broilers during production. An acceptance of the null hypotheses indicates that age, sex or treatments had a significant effect ($P < 0.01$) on the levels of the biochemical parameters of the broilers during production (Norusis, 1988).

CHAPTER THREE

3.0 RESULTS AND DISCUSSION

3.1 RESULTS

3.1.1 Effect(s) of age:

The effect(s) of age on some biochemical parameters in broilers at different ages (3, 5 and 8 weeks) are as shown (Table 1).

There was either no significant effect ($P > 0.01$) or a significant effect ($P < 0.01$) on the levels of the biochemical parameters as follows:-

A) No significant effect:

(i) Haematocrit (PCV): Age had no significant effect ($P > 0.01$) on haematocrit (PCV) levels in broilers of 3, 5 and 8 weeks of age (29.63 ± 2.50 to 34.00 ± 3.14 %). The trend showed an insignificant decrease ($P > 0.01$) of levels from base line in broilers of 3 weeks of age (34.00 ± 3.14 %) by about 13% in broilers at 8 weeks of age (29.63 ± 2.50 %).

(ii) Total serum protein: Age had no significant effect ($P > 0.01$) on total serum protein levels in broilers of 3, 5 and 8 weeks of age (1.60 ± 0.09 to 2.03 ± 0.02 g/dl). But, the trend showed a significant increase ($P < 0.01$) of levels from base line in broilers of 3 weeks of age (1.60 ± 0.09 g/dl) by about 27% in broilers at 8 weeks of age (2.03 ± 0.02 g/dl).

(iii) Total serum creatinine: Age had no significant effect ($P > 0.01$) on total serum creatinine levels in broilers of 3, 5 and 8 weeks of age (0.68 ± 0.01 to 0.82 ± 0.01 mg/dl).

But, the trend showed a significant increase ($P < 0.01$) of levels from base line at 3 weeks of age (0.68 ± 0.01) by about 21% in broilers at 8 weeks of age (0.82 ± 0.01 mg/dl).

B) Significant effect:

(i) Total serum cholesterol: Age had a significant effect ($P < 0.01$) on total serum cholesterol levels in broilers of 3, 5 and 8 weeks of age (167.68 ± 1.10 to 192.64 ± 0.03 mg/dl) only. The trend showed a significant increase ($P < 0.01$) of levels from base line in broilers at

TABLE 1: EFFECT OF SEX AND AGE ON SELECTED BIOCHEMICAL PARAMETERS (Mean ± SEM) IN BROILERS

BIOCHEMICAL PARAMETERS	SEX (M/F)	AGE (WEEKS) 3	5	8
HAEMATOCRIT (PCV) %	M	31.38 ± 1.38a	29.88 ± 1.10a	29.63 ± 2.50a
	F	34.00 ± 3.14a,b	31.00 ± 0.92a	32.75 ± 4.84a
TOTAL SERUM CHOLESTEROL (mg/dl)	M	170.47 ± 0.27c,d	180.41 ± 0.37e	192.64 ± 0.03g
	F	167.68 ± 1.10c	170.42 ± 0.02d	183.49 ± 0.08f
TOTAL SERUM PROTEIN (g/dl)	M	1.60 ± 0.09h,i	1.84 ± 0.08i	2.00 ± 0.05i,j
	F	1.70 ± 0.05h,i	1.87 ± 0.02i	2.03 ± 0.02i,j
TOTAL SERUM CREATININE (mg/dl)	M	0.70 ± 0.01k,l	0.70 ± 0.01k,l	0.81 ± 0.01l
	F	0.68 ± 0.01k	0.73 ± 0.01k,l	0.82 ± 0.01l
TOTAL AVAILABLE SERUM SUGARS (mg/dl)	M	268.60 ± 0.01n	284.53 ± 0.03q	348.80 ± 0.02r,s
	F	260.57 ± 0.01m	278.82 ± 0.02p	345.53 ± 0.01r

Sample number is fifty (50) broilers per sex.

a-s Values with different letters differ significantly by ANOVA ($P < 0.01$).

3 weeks of age (167.68 ± 1.10 mg/dl) by about 13% in broilers at 8 weeks of age (192.64 ± 0.03 mg/dl).

(ii) Total available serum sugars: Age had a significant effect ($P < 0.01$) on total available serum sugar levels in broilers of 3, 5 and 8 weeks of age (260.57 ± 0.01 to 348.80 ± 0.02 mg/dl). The trend showed increase of levels from base line in broilers at 3 weeks of age (260.57 ± 0.01 mg/dl) by about 34% in broilers at 8 weeks of age (348.80 ± 0.02 mg/dl).

It might be from these results that the main effect of age was that the levels of some of the biochemical parameters significantly increased ($P < 0.01$) with advancing age of the broilers.

3.1.2 Effect(s) of sex:

The effect(s) of sex on some biochemical parameters in broilers of different sexes (male or female) are also as shown (Table 1). There was either no significant effect ($P > 0.01$) or a significant effect ($P < 0.01$) on the levels of the biochemical parameters.

A) No significant effect:

(i) Haematocrit (PCV): Sex had no significant effect ($P > 0.01$) on haematocrit (PCV) levels (29.63 ± 2.50 to 31.38 ± 1.38 %) in male broilers or (31.00 ± 0.92 to 34.00 ± 3.14 %) in female broilers of 3, 5 and 8 weeks of age. The trend showed an insignificant decrease ($P > 0.01$) of levels from base line in male broilers at 3 weeks of age (31.38 ± 1.38 %) by about 6% in male broilers at 8 weeks of age (29.63 ± 2.50 %). The insignificant decrease ($P > 0.01$) of levels from base line in female broilers at 3 weeks of age (34.00 ± 3.14%) was about 9% when the female broilers were 5 weeks old (31.00 ± 0.92 %).

(ii) Total serum cholesterol: Sex had no significant effect ($P > 0.01$) on total serum cholesterol levels in male broilers (170.47 ± 0.27 mg/dl) or in female ones (167.68 ± 1.10 mg/dl) at 3 weeks of age only. There was no significant sex difference ($P > 0.01$) in the levels.

(iii) Total serum protein: Sex had no significant effect ($P > 0.01$) on total serum protein

levels in male broilers (1.60 ± 0.09 to 2.00 ± 0.05 g/dl) or in female ones (1.70 ± 0.05 to 2.03 ± 0.02 g/dl) at 3, 5 and 8 weeks of age. There was no significant sex difference ($P > 0.01$) in the levels.

(iv) Total serum creatinine: Sex had no significant effect ($P > 0.01$) on total serum creatinine levels in male broilers (0.70 ± 0.01 to 0.81 ± 0.01 mg/dl) or in female ones (0.68 ± 0.01 to 0.82 ± 0.01 mg/dl) of 3, 5 and 8 weeks of age. But, the trend showed a significant increase ($P < 0.01$) only in the levels in female broilers at 3 weeks of age (0.68 ± 0.01 mg/dl) by about 21% at 8 weeks of age (0.82 ± 0.01 mg/dl).

(v) Total available serum sugars: Sex had no significant effect ($P > 0.01$) on total available serum sugar levels in male broilers (348.80 ± 0.02 mg/dl) or in female ones (345.53 ± 0.01 mg/dl) at 8 weeks of age only. There was no significant sex difference ($P > 0.01$) in the levels of 8 weeks old broilers.

B) Significant effect:

(i) Total serum cholesterol: Sex had a significant effect ($P < 0.01$) on total serum cholesterol levels in male broilers (170.42 ± 0.02 to 180.41 ± 0.37 mg/dl) and in female ones (183.49 ± 0.08 to 192.64 ± 0.03 mg/dl) at 5 and 8 weeks of age only. Using the corresponding levels in female broilers as base line (170.42 ± 0.02 mg/dl and 183.49 ± 0.08 mg/dl), there was a significant increase ($P < 0.01$) by about 6% in male broilers at 5 weeks of age (180.41 ± 0.37 mg/dl) and by 5% at 8 weeks of age (192.64 ± 0.03 mg/dl). There was a proportional decrease of levels when the corresponding levels in male broilers were used as base line.

(ii) Total available serum sugars: Sex had a significant effect ($P < 0.01$) on total available serum sugar levels in broilers of 3 and 5 weeks of age only (260.57 ± 0.01 to 268.60 ± 0.01 mg/dl; 278.82 ± 0.02 to 284.53 ± 0.03 mg/dl). Using the corresponding levels in female broilers as base line (260.57 ± 0.01 mg/dl and 278.82 ± 0.02 mg/dl), there was a significant increase ($P < 0.01$) by about 3% in male broilers at 3 weeks of age (268.60 ± 0.01 mg/dl) and by 2% at 5

weeks of age (284.53 ± 0.03 mg/dl). There was a proportional decrease of levels when the corresponding levels in male broilers were used as base line.

These significant effects ($P < 0.01$) of sex on total serum cholesterol and total available serum sugars showed sex dependence of the levels of the biochemical parameters in broilers.

3.1.3 Effect(s) of light exposure:

The effect(s) of light exposure on selected biochemical parameters in broilers of different ages (3, 5 and 8 weeks) are as shown (Table 2). Using normal light exposure of 100W incandescent pin bulb / 3.4m^2 of floor space as control, a high light exposure of 400W incandescent pin bulb / 3.4m^2 of floor space had either no significant effect ($P > 0.01$) or a significant effect ($P < 0.01$) on the levels of the biochemical parameters as follows:-

A) **No significant effect:**

(i) **Haematocrit:** High light exposure had no significant effect ($P > 0.01$) on haematocrit levels in broilers of 3, 5 and 8 weeks of age (29.50 ± 1.58 to 34.00 ± 3.62 %).

(ii) **Total serum cholesterol:** High light exposure had no significant effect ($P > 0.01$) on total serum cholesterol levels in female broilers of 8 weeks of age only (179.74 ± 0.33 to 180.59 ± 0.10 mg/dl).

(iii) **Total serum protein:** High light exposure had no significant effect ($P > 0.01$) on total serum protein levels in male broilers (1.84 ± 0.08 to 1.97 ± 0.10 g/dl) and in female ones (1.87 ± 0.02 to 1.88 ± 0.01 g/dl) at 5 weeks of age . The effect was also insignificant ($P > 0.01$) in female broilers at 8 weeks of age (1.98 ± 0.03 to 1.99 ± 0.01 g/dl).

(iv) **Total serum creatinine:** High light exposure had no significant effect ($P > 0.01$) on total serum creatinine levels in male and female broilers at 3 and 8 weeks of age (0.64 ± 0.01 to 0.71 ± 0.01 mg/dl ; 0.77 ± 0.02 to 0.83 ± 0.01 mg/dl) and in female broilers at 5 weeks of age only (0.71 ± 0.03 to 0.73 ± 0.01 mg/dl).

TABLE 2: EFFECT OF LIGHT EXPOSURE ON SOME BIOCHEMICAL PARAMETERS (Mean ± SEM) IN BROILERS OF DIFFERENT AGES.

BIOCHEMICAL PARAMETERS	SEX (M/F)	AGE (WEEKS) 3 CTRL	3 LIGHT	5 CTRL	5 LIGHT	8 CTRL	8 LIGHT
HAEMATOCRIT (PCV) %	M	31.38 ± 1.38a	34.00 ± 3.62a,b	29.88 ± 1.10a	31.25 ± 0.96a	30.88 ± 2.28a	33.35 ± 2.10a,b
	F	34.00 ± 3.14a,b	30.25 ± 2.62a	31.00 ± 0.92a	29.50 ± 1.58a	32.63 ± 1.04a	29.62 ± 1.50a
TOTAL SERUM CHOLESTEROL (mg/dl)	M	170.47 ± 0.27d	177.12 ± 0.54f	180.41 ± 0.37f	184.27 ± 0.51g	185.66 ± 0.01g	189.62 ± 0.03h
	F	167.68 ± 1.10c	174.92 ± 0.43e	170.42 ± 0.02d	174.99 ± 0.12e	180.59 ± 0.10f	179.74 ± 0.33f
TOTAL SERUM PROTEIN (g/dl)	M	1.60 ± 0.09i	1.93 ± 0.09k	1.84 ± 0.08k	1.97 ± 0.10kl	1.95 ± 0.01k	2.05 ± 0.06l
	F	1.70 ± 0.05i,j	1.84 ± 0.02k	1.87 ± 0.02k	1.88 ± 0.01k	1.99 ± 0.01k,l	1.98 ± 0.03k,l
TOTAL SERUM CREATININE (mg/dl)	M	0.70 ± 0.01m,n	0.71 ± 0.01m,n	0.70 ± 0.01m,n	0.81 ± 0.01q	0.81 ± 0.01q	0.83 ± 0.01q
	F	0.68 ± 0.01m	0.64 ± 0.01m	0.73 ± 0.01n	0.71 ± 0.03m,n	0.82 ± 0.01p	0.77 ± 0.02p
TOTAL AVAILABLE SERUM SUGARS (mg/dl)	M	268.60 ± 0.01s	288.10 ± 0.10v	284.53 ± 0.03u	295.70 ± 0.30x	348.80 ± 0.02a′	349.50 ± 0.50a′
	F	260.57 ± 0.01r	292.20 ± 0.20w	278.82 ± 0.02t	300.25 ± 0.10y,x	345.53 ± 0.01z	350.10 ± 0.50b′

Sample number is fifty (50) broilers per sex.
CTRL = Control (i.e. normal light exposure of 100W incandescent pin bulb / 3.4m^2 floor space).
LIGHT = High light exposure of 400W incandescent pin bulb / 3.4m^2 floor space.
a-b′ Values with different letters from corresponding controls differ significantly by ANOVA ($P < 0.01$).

(v) **Total available serum sugars:** High light exposure had no significant effect ($P > 0.01$) on total available serum sugar levels in male broilers of 8 weeks of age only (348.80 ± 0.02 to 349.50 ± 0.50 mg/dl).

B) Significant effect:

(i) **Total serum cholesterol:** High light exposure had a significant effect on total serum cholesterol levels in male and female broilers at 3 and 5 weeks of age (170.47 ± 0.27 to 184.27 ± 0.51 mg/dl; 167.68 ± 1.10 to 174.99 ± 0.12 mg/dl) and in male broilers at 8 weeks of age only (185.66 ± 0.01 to 189.62 ± 0.03mg/dl). The levels increased above corresponding control levels (170.42 ± 0.27 mg/dl; 167.68 ± 1.10 mg/dl) by about 4% in both male and female broilers at 3 weeks of age (177.12 ± 0.54 mg/dl; 174.92 ± 0.43 mg/dl; 180.41 ± 0.37 mg/dl; 170.42 ± 0.02 mg/dl and 185.66 ± 0.01 mg/dl), by about 2% in male broilers (184.27 ± 0.51 mg/dl) and 3% in female ones (174.99 ± 0.12 mg/dl) and by about 2% in male broilers at 8 weeks of age (189.62 ± 0.03 mg/dl), respectively.

(ii) **Total serum protein:** High light exposure had a significant effect ($P > 0.01$) on total serum protein levels in male broilers (1.60 ± 0.09 to 1.93 ± 0.09 g/dl) and in female ones (1.70 ± 0.05 to 1.84 ± 0.02 g/dl) at 3 weeks of age and in male broilers (1.95 ± 0.01 to 2.05 ± 0.06 g/dl) at 8 weeks of age only. The levels increased above corresponding control levels (1.60 ± 0.09 g/dl; 1.70 ± 0.05 g/dl and 1.95 ± 0.01 g/dl) by about 21% in male broilers (1.93 ± 0.09 g/dl) and 8% in female broilers (1.84 ± 0.02 g/dl) at 3 weeks of age and by about 5% in male broilers (2.05 ± 0.06 g/dl) at 8 weeks of age.

(iii) **Total serum creatinine:** High light exposure had a significant effect ($P < 0.01$) on total serum creatinine level in male broilers (0.70 ± 0.01 to 0.81 ± 0.01 mg/dl) at 5 weeks of age only. The level increased above corresponding control level (0.70 ± 0.01 mg/dl) by about 16% in male broilers (0.81 ± 0.01 mg/dl) at 5 weeks of age.

(iv) **Total available serum sugars:** High light exposure had a significant effect ($P < 0.01$) on total available serum sugar levels in male and female broilers of 3 and 5 weeks of age (268.60 ± 0.01 to 288.10 ± 0.10 mg/dl; 284.53 ± 0.03 to 295.70 ± 0.30 mg/dl; 260.57 ± 0.01 to 292.20 ± 0.20 mg/dl; 278.82 ± 0.02 to 300.25 ± 0.01 mg/dl) and in female broilers at 8 weeks of age only (345.53 ± 0.01 to 350.10 ± 0.05 mg/dl). The levels increased above corresponding control levels (268.60 ± 0.01 mg/dl; 260.57 ± 0.01 mg/dl; 284.53 ± 0.03 mg/dl; 278.82 ± 0.02 mg/dl and 345.53 ± 0.01 mg/dl) by about 7% in male broilers (288.10 ± 0.10 mg/dl) and 12% in female ones (292.20 ± 0.20 mg/dl) at 3 weeks of age, by about 4% in male broilers (295.70 ± 0.03 mg/dl) and 8% in female ones (300.25 ± 0.10 mg/dl) at 5 weeks of age and by about 1% in female broilers (350.10 ± 0.50 mg/dl) at 8 weeks of age.

3.1.4 Effect(s) of high light exposure with ascorbic acid supplementation:

The effect(s) of high light exposure on some biochemical parameters of ascorbic acid-supplemented broilers of different ages (3, 5 and 8 weeks) are as shown (Table 3). Using high light exposure of 400W incandescent pin bulb / 3.4m^2 of floor space / day but without ascorbic acid supplementation as control, high light exposure with ascorbic acid supplementation (300mg / kg diet) had either no significant effect ($P > 0.01$) or a significant effect ($P < 0.01$) on the levels of the biochemical parameters as follows:-

A) No significant effect:

(i) **Haematocrit (PCV):** High light exposure with ascorbic acid supplementation had no significant effect ($P > 0.01$) on haematocrit (PCV) levels in female broilers at 3 weeks of age (30.25 ± 2.62 to 30.50 ± 3.00 %) and in male and female broilers at 5 and 8 weeks of age (27.88 ± 2.18 to 31.25 ± 0.96 %; 29.50 ± 1.58 to 30.13 ± 1.26 %; 28.37 ± 1.88 to 33.35 ± 2.10 % and 29.62 ± 1.50 to 32.63 ± 2.78 %).

(ii) **Total serum cholesterol:** High light exposure with ascorbic acid supplementation had no significant effect ($P > 0.01$) on total serum cholesterol levels in male broilers at 5 and 8 weeks of age only (184.08 ± 0.45 to 184.27 ± 0.51 mg/dl; 187.75 ± 0.78 to 189.62 ± 0.03 mg/dl).

(iii) **Total serum protein:** High light exposure with ascorbic acid supplementation had no significant effect ($P > 0.01$) on total serum protein levels in male and female broilers at 3, 5 and 8 weeks of age (1.84 ± 0.02 to 2.05 ± 0.06 g/dl).

(iv) **Total serum creatinine:** High light exposure with ascorbic acid supplementation had no significant effect ($P > 0.01$) on total serum creatinine levels in male and female broilers at 3 weeks of age (0.65 ± 0.04 to 0.71 ± 0.01 mg/dl; 0.62 ± 0.02 to 0.64 ± 0.01 mg/dl) and in female broilers at 5 and 8 weeks of age only (0.64 ± 0.02 to 0.71 ± 0.03 mg/dl; 0.71 ± 0.02 to 0.77 ± 0.02 mg/dl).

(v) **Total available serum sugars:** High light exposure with ascorbic acid supplementation had no significant effect ($P > 0.01$) on total available serum sugar levels in female broilers of 5 weeks of age only (300.25 ± 0.10 to 305.63 ± 0.03 mg/dl).

B) Significant effect:

(i) **Haematocrit (PCV):** High light exposure with ascorbic acid supplementation had a significant effect ($P , 0.01$) on haematocrit level in male broilers at 3 weeks of age only (28.63 ± 3.06 to 34.00 ± 3.62 %). The level (28.63 ± 3.06 %) decreased below corresponding control level (34.00 ± 3.62 %) by about 16% in male broilers at 3 weeks of age.

(ii) **Total serum cholesterol:** High light exposure with ascorbic acid supplementation had a significant effect ($P < 0.01$) on total serum cholesterol levels in male and female broilers at 3 weeks of age (177.12 ± 0.54 to 184. 77 ± 0.43 mg/dl; 174.92 ± 0.43 to 182.76 ± 0.13 mg/dl) and in female broilers of 5 and 8 weeks of age only (174.99 ± 0.12 to 178.33 ± 0.65 mg/dl; 179.74 ± 0.33 to 188.30 ± 0.35 mg/dl).

TABLE 3: EFFECT OF HIGH LIGHT EXPOSURE ON SOME BIOCHEMICAL PARAMETERS (Mean ± SEM) OF ASCORBIC ACID-SUPPLEMENTED BROILERS OF DIFFERENT AGES

BIOCHEMICAL PARAMETERS	SEX (M/F)	AGE (WEEKS) 3		5		8	
		H/LIGHT	+AA	H/LIGHT	+AA	H/LIGHT	+AA
HAEMATOCRIT (PCV) %	M	34.00 ± 3.62b	28.63 ± 3.06a	31.25 ± 0.96a,b	27.88 ± 2.18a	33.35 ± 2.10a,b	28.37 ± 1.88a
	F	30.25 ± 2.62a,b	30.50 ± 3.00a,b	29.50 ± 1.58a	30.13 ± 1.26a,b	29.62 ± 1.50a	32.63 ± 2.78a,b
TOTAL SERUM CHOLESTEROL (mg/dl)	M	177.12 ± 0.54d	184.77 ± 0.43e	184.27 ± 0.51e	184.08 ± 0.45e	189.62 ± 0.03f	187.75 ± 0.78f
	F	174.92 ± 0.43c	182.76 ± 0.13e	174.99 ± 0.12c	178.33 ± 0.65d	179.74 ± 0.33d	188.30 ± 0.35f
TOTAL SERUM PROTEIN (g/dl)	M	1.93 ± 0.09g	1.97 ± 0.01g	1.97 ± 0.10g	1.93 ± 0.01g	2.05 ± 0.06g	2.01 ± 0.01g
	F	1.84 ± 0.02g	1.96 ± 0.01g	1.88 ± 0.01g	1.97 ± 0.01g	1.98 ± 0.03g	1.96 ± 0.05g
TOTAL SERUM CREATININE (mg/dl)	M	0.71 ± 0.01h,j	0.65 ± 0.04h	0.81 ± 0.01i,j	0.60 ± 0.04h	0.83 ± 0.01i,j	0.63 ± 0.01h
	F	0.64 ± 0.01h	0.62 ± 0.02h	0.71 ± 0.03h,j	0.64 ± 0.02h	0.77 ± 0.02i,j	0.71 ± 0.02h,j
TOTAL AVAILABLE SERUM SUGARS (mg/dl)	M	288.10 ± 0.10l	271.20 ± 0.03k	295.70 ± 0.30m	301.25 ± 0.87n	349.50 ± 0.50p	352.15 ± 0.40q
	F	292.20 ± 0.20m	278.54 ± 0.05l	300.25 ± 0.10n	305.63 ± 0.03n	350.10 ± 0.50q	354.85 ± 0.20r

Sample number is fifty (50) broilers per sex.
H/LIGHT = Control (i.e. high light exposure of 400W incandescent pin bulb/3.4m^2 floor space but without ascorbic acid supplementation).
+AA = High light exposure of 400W incandescent pin bulb/3.4m^2 floor space with ascorbic acid supplementation (300mg / kg diet).
a - r Values with different letters from corresponding controls differ significantly by ANOVA ($P < 0.01$).

The levels increased ($P < 0.01$) above corresponding control levels (177.12 ± 0.54 mg/dl; 174.92 ± 0.43 mg/dl) by about 4% in male broilers (184.77 ± 0.43 mg/dl) and by 5% in female ones (182.76 ± 0.13 mg/dl) at 3 weeks of age. The levels, also, increased ($P < 0.01$) above corresponding control levels (174.99 ± 0.12 mg/dl; 179.74 ± 0.33 mg/dl) by about 2% in female broilers at 5 weeks of age (178.33 ± 0.65 mg/dl) and by 5% in 8 weeks old broilers (188.30 ± 0.35 mg/dl).

(iii) Total serum creatinine: High light exposure with ascorbic acid supplementation had a significant effect ($P < 0.01$) on total serum creatinine levels in male broilers at 5 and 8 weeks of age only (0.60 ± 0.04 to 0.81 ± 0.01 mg/dl; 0.63 ± 0.01 to 0.83 ± 0.01 mg/dl). The levels decreased ($P < 0.01$) below corresponding control levels (0.81 ± 0.01 mg/dl; 0.83 ± 0.01 mg/dl) by about 26% in male broilers at 5 weeks of age (0.60 ± 0.04 mg/dl) and by 24% in male broilers at 8 weeks of age (0.63 ± 0.01 mg/dl).

(iv) Total available serum sugars: High light exposure with ascorbic acid supplementation had a significant effect ($P < 0.01$) on total available serum sugar levels in male broilers (271.20 ± 0.03 to 288.10 ± 0.10 mg/dl; 349.50 ± 0.50 to 352.15 ± 0.40 mg/dl) and in female ones (278.54 ± 0.20 to 292.20 ± 0.20 mg/dl; 350.10 ± 0.50 to 354.85 ± 0.20 mg/dl) at 3 and 8 weeks of age. There was, also, a significant effect ($P < 0.01$) on the levels in male broilers at 5 and 8 weeks of age (295.70 ± 0.30 to 301.25 ± 0.87 mg/dl; 349.50 ± 0.50 to 352.15 ± 0.40 mg/dl). The levels decreased ($P < 0.01$) below corresponding control levels (288.10 ± 0.10 mg/dl; 292.20 ± 0.20 mg/dl) by about 6% in male broilers (271.20 ± 0.03 mg/dl) and 5% in female ones (278.54 ± 0.05 mg/dl) at 3 weeks of age. The level also increased ($P < 0.01$) above corresponding control levels (295.70 ± 0.30 mg/dl; 349.50 ± 0.50 mg/dl; 350.10 ± 0.50 mg/dl) by about 2% in male broilers (301.25 ± 0.87 mg/dl) at 5 weeks of age only and by about 1% each in male broilers (352.15 ± 0.40 mg/dl) and in female ones (354.85 ± 0.20 mg/dl) at 8 weeks of age.

3.1.5 Effect(s) of low light exposure:

The effect(s) of low light exposure on some biochemical parameters in broilers of different ages (3, 5 and 8 weeks) are as shown (Table 4). Using a normal light exposure of 100W incandescent pin bulb / 3.4m^2 of floor space as control, a low light exposure of 60W incandescent pin bulb / 3.4m^2 of floor space had no significant effect ($P > 0.01$) on the levels of the biochemical parameters as follows:-

(i) Haematocrit (PCV): Low light exposure had no significant effect ($P > 0.01$) on haematocrit levels in male and female broilers at 3, 5 and 8 weeks of age (29.88 ± 1.10 to 34.00 ± 3.14 %). The trend showed an insignificant decrease ($P > 0.01$) of 3 weeks old male broiler base line haematocrit level (31.38 ± 1.38 %) by about 5% at 5 weeks of age (29.88 ± 1.10 %) and by 2% at 8 weeks of age (30.88 ± 2.28 %). Similarly, 3 weeks old female broiler base line haematocrit level (34.00 ± 3.14 %) decreased ($P > 0.01$) by about 9% at 5 weeks of age (31.00 ± 0.93 %) and by 4% at 8 weeks of age (32.63 ± 1.04 %).

(ii) Total serum cholesterol: Low light exposure had no significant effect ($P > 0.01$) on total cholesterol levels in male and female broilers at 3, 5 and 8 weeks of age (167.68 ± 1.10 to 185.66 ± 0.01 mg/dl). But, the trend showed a significant increase ($P < 0.01$) of 3 weeks old male broiler base line total cholesterol level (170.47 ± 0.27 mg/dl) by about 6% at 5 weeks (180.41 ± 0.37 mg/dl) and by 9% at 8 weeks of age (185.66 ± 0.01 mg/dl). Similarly, 3 weeks old female broiler base line total cholesterol level (167.68 ± 1.10 mg/dl) increased ($P < 0.01$) by about 2% at 5 weeks (170.42 ± 0.02 mg/dl) and by 8% at 8 weeks of age (180.59 ± 0.01 mg/dl).

(iii) Total serum protein: Low light exposure had no significant effect ($P > 0.01$) on total protein levels in male and female broilers of 3, 5 and 8 weeks of age (1.60 ± 0.09 to 1.99 ± 0.01 g/dl). But, the trend showed a significant increase ($P < 0.01$) of 3 weeks old male broiler base line total protein level (1.60 ± 0.09 g/dl) by about 15% at 5 weeks (1.84 ± 0.08 g/dl) and by 22% at 8 weeks of age (1.95 ± 0.01 g/dl). Similarly, 3 weeks old female broiler base line total protein

TABLE 4: EFFECT OF LOW LIGHT EXPOSURE ON SOME BIOCHEMICAL PARAMETERS (Mean ± SEM) IN BROILERS OF DIFFERENT AGES

BIOCHEMICAL PARAMETERS	SEX (M/F)	AGE (WEEKS) 3 CTRL	3 L/LIGHT	5 CTRL	5 L/LIGHT	8 CTRL	8 L/LIGHT
HAEMATOCRIT (PCV) %	M	31.38 ± 1.38a	31.38 ± 1.38a	29.88 ± 1.10a	29.88 ± 1.10a	30.88 ± 2.28a	30.88 ± 2.28a
	F	34.00 ± 3.14a,b	34.00 ± 3.14a,b	31.00 ± 0.92a	31.00 ± 0.92a	29.25 ± 2.10a	29.25 ± 2.10a
TOTAL SERUM CHOLESTEROL (mg/dl)	M	170.47 ± 0.27d	170.47 ± 0.27d	180.41 ± 0.37e	180.41 ± 0.37e	185.66 ± 0.01g	185.66 ± 0.01g
	F	167.68 ± 1.10c	167.68 ± 1.10c	170.42 ± 0.02d	170.42 ± 0.02d	180.59 ± 0.10f	180.59 ± 0.10f
TOTAL SERUM PROTEIN (g/dl)	M	1.60 ± 0.09g	1.60 ± 0.09h	1.84 ± 0.08j	1.84 ± 0.08j	1.95 ± 0.01k	1.95 ± 0.01k
	F	1.70 ± 0.05h,i	1.70 ± 0.05h,i	1.82 ± 0.02j	1.82 ± 0.02j	1.99 ± 0.01k	1.99 ± 0.01k
TOTAL SERUM CREATININE (mg/dl)	M	0.70 ± 0.01l	0.70 ± 0.01l	0.70 ± 0.01l	0.70 ± 0.01l	0.81 ± 0.01m	0.81 ± 0.01m
	F	0.68 ± 0.01l	0.68 ± 0.01l	0.73 ± 0.01l	0.73 ± 0.01l	0.82 ± 0.01m	0.82 ± 0.01m
TOTAL AVAILABLE SERUM SUGARS (mg/dl)	M	268.60 ± 0.10p	268.60 ± 0.10p	284.53 ± 0.30r	284.53 ± 0.30r	348.80 ± 0.02t	348.80 ± 0.02t
	F	260.57 ± 0.01n	260.57 ± 0.10n	278.82 ± 0.02q	278.82 ± 0.02q	345.53 ± 0.01s	345.53 ± 0.01s

Sample number is fifty (50) broilers per sex.
CTRL = Control (i.e. normal light exposure of 100W incandescent pin bulb/3.4m^2 floor space).
L/LIGHT = Low light exposure of 60W incandescent pin bulb/3.4m^2 floor space).
a-t Values with the same letters as corresponding controls did not differ significantly by ANOVA ($P < 0.01$).

level (1.70 ± 0.05 g/dl) increased ($P < 0.01$) by about 10% at 5 weeks (1.87 ± 0.02 g/dl) and by 17% at 8 weeks of age (1.99 ± 0.01g/dl).

(iv) **Total serum creatinine:** Low light exposure had no significant effect ($P > 0.01$) on total creatinine levels in male and female broilers of 3, 5 and 8 weeks of age (0.68 ± 0.01 to 0.82 ± 0.01 mg/dl). But, the trend showed a significant increase ($P < 0.01$) of 3 weeks old male broiler base line total creatinine level (0.70 ± 0.01 mg/dl) by about 16% only at 8 weeks of age (0.81 ± 0.01 mg/dl). In female broilers, base line total creatinine level at 3 weeks of age (0.68 ± 0.01) increased though insignificantly ($P > 0.01$) by about 7% at 5 weeks (0.73 ± 0.01 mg/dl) and significantly ($P < 0.01$) by 17% at 8 weeks of age (0.82 ± 0.01 mg/dl).

(v) **Total available serum sugars:** Low light exposure had no significant effect ($P > 0.01$) on total sugar levels in male and female broilers at 3, 5 and 8 weeks of age (260.57 ± 0.01 to 348.80 ± 0.02 mg/dl). But, the trend showed a significant increase ($P < 0.01$) of 3 weeks old male broiler base line total sugar level (268.60 ± 0.01 mg/dl) by 6% at 5 weeks (284.53 ± 0.03 mg/dl) and by 30% at 8 weeks (348.80 ± 0.02 mg/dl). For female broilers, base line total sugar level at 3 weeks of age (260.57 ± 0.01 mg/dl) increased ($P < 0.01$) by about 7% at 5 weeks (278.82 ± 0.02 mg/dl) and by 33% at 8 weeks of age (345.53 ± 0.01 mg/dl).

3.1.6 Effect(s) of low light exposure with ascorbic acid supplementation:

The effect(s) of low light exposure on some biochemical parameters of ascorbic acid-supplemented broilers of different ages (3, 5 and 8 weeks) are as shown (Table 5). Using low light exposure of 60W incandescent pin bulb / 3.4m^2 of floor space but without ascorbic acid supplementation as control, low light exposure with ascorbic acid supplementation (300mg / kg diet) had either no significant effect ($P > 0.01$) or a significant effect ($P < 0.01$) on the levels of the biochemical parameters as follows:-

A) No significant effect:

(i) Haematocrit (PCV): Low light exposure with ascorbic acid supplementation had no significant effect ($P > 0.01$) on haematocrit (PCV) levels in male and female broilers of 3, 5 and 8 weeks of age (28.00 ± 3.18 to 34.00 ± 3.14 %).

(ii) Total serum cholesterol: Low light exposure with ascorbic acid supplementation had no significant effect ($P > 0.01$) on total serum cholesterol levels in male broilers (180.41 ± 0.37 to 180.60 ± 0.39 mg/dl) and in female ones (170.42 ± 0.02 to 171.72 ± 1.02 mg/dl) at 5 weeks of age only.

(iii) Total serum protein: Low light exposure with ascorbic acid supplementation had no significant effect ($P > 0.01$) on total serum protein levels in male broilers at 3 weeks of age (1.56 ± 0.01 to 1.60 ± 0.09 g/dl) and in female ones (0.66 ± 0.03 to 0.73 ± 0.01 g/dl; 0.67 ± 0.01 to 0.82 ± 0.01 g/dl) at 5 and 8 weeks of age only.

(iv) Total serum creatinine: Low light exposure with ascorbic acid supplementation had no significant effect ($P > 0.01$) on total serum creatinine levels in male broilers (0.69 ± 0.01 to 0.70 ± 0.01 mg/dl) and in female ones (0.66 ± 0.01 to 0.68 ± 0.01 mg/dl) at 3 weeks of age and in male broilers (0.65 ± 0.01 to 0.70 ± 0.01 mg/dl) at 5 weeks of age only.

B) Significant effect:

(i) Total serum cholesterol: Low light exposure with ascorbic acid supplementation had a significant effect ($P < 0.01$) on total serum cholesterol levels in male broilers (170.47 ± 0.27 to 185.44 ± 0.16 mg/dl; 167.09 ± 0.83 to 185.66 ± 0.01 mg/dl) and in female ones (167.68 ± 1.10 to 184.50 ± 0.18 mg/dl; 180.59 ± 0.10 to 186.42 ± 0.42 mg/dl) at 3 and 8 weeks of age. The levels (170.47 ± 0.27 mg/dl; 167.68 ± 1.10 mg/dl; 180.59 ± 0.10 mg/dl) increased ($P < 0.01$) by about 9% in male broilers (185.44 ± 0.16 mg/dl) and by 10% in female broilers (184.50 ± 0.18 mg/dl) at 3 weeks of age, also by about 3% in female broilers (186.42 ± 0.42 mg/dl) at 8 weeks of age.

TABLE 5: EFFECT OF LOW LIGHT EXPOSURE ON SOME BIOCHEMICAL PARAMETERS (Mean ± SEM) OF ASCORBIC ACID -SUPPLEMENTED BROILERS OF DIFFERENT AGES

BIOCHEMICAL PARAMETERS	SEX (M/F)	AGE (WEEKS) 3		5		8	
		LOW LIGHT	+AA	LOW LIGHT	+AA	LOW LIGHT	+AA
HAEMATOCRIT (PCV) %	M	31.38 ± 1.38a	33.50 ± 1.48a	29.88 ± 1.10a	31.74 ± 0.68a	30.88 ± 2.28a	31.00 ± 2.70a
	F	34.00 ± 3.14a,b	28.00 ± 3.18a	31.00 ± 0.92a	29.25 ± 2.10a	29.25 ± 2.10a	30.00 ± 2.88a
TOTAL SERUM CHOLESTEROL (mg/dl)	M	170.47 ± 0.27d	185.44 ± 0.16f	180.41 ± 0.37e	180.60 ± 0.39e	185.66 ± 0.01f	167.09 ± 0.83c
	F	167.68 ± 1.10c	184.50 ± 0.18f	170.42 ± 0.02d	171.72 ± 1.02d	180.59 ± 0.10e	186.42 ± 0.42f
TOTAL SERUM PROTEIN (g/dl)	M	1.60 ± 0.09g	1.56 ± 0.01g	1.84 ± 0.08h,i	1.60 ± 0.01g	1.95 ± 0.01i	1.76 ± 0.10g,h
	F	1.70 ± 0.05g	1.92 ± 0.01i	1.82 ± 0.02h,i	1.95 ± 0.01i	1.99 ± 0.01i	2.09 ± 0.01i
TOTAL SERUM CREATININE (mg/dl)	M	0.70 ± 0.01j,k	0.69 ± 0.01j	0.70 ± 0.01j,k	0.65 ± 0.01j	0.81 ± 0.01l	0.61 ± 0.04j
	F	0.68 ± 0.01j	0.66 ± 0.01j	0.73 ± 0.01k	0.66 ± 0.03j	0.82 ± 0.01l	0.67 ± 0.01j
TOTAL AVAILABLE SERUM SUGARS (mg/dl)	M	268.60 ± 0.10q	258.51 ± 0.01n	284.53 ± 0.30s	287.10 ± 0.35t	348.80 ± 0.02w	345.20 ± 0.50v
	F	260.57 ± 0.01p	256.65 ± 0.10m	278.82 ± 0.02r	291.40 ± 0.20u	345.53 ± 0.01v	348.25 ± 0.10w

Sample number is fifty (50) broilers per sex.
LOW LIGHT = Control (i.e. low light exposure of 60W incandescent pin bulb/3.4m^2 floor space but without ascorbic acid supplementation).
+AA = Low light exposure of 60W incandescent pin bulb/3.4m^2 floor space with ascorbic acid supplementation (300mg / kg diet).
a-w Values with different letters from corresponding controls differ significantly by ANOVA ($P < 0.01$).

But the levels decreased ($P < 0.01$) below corresponding control level (185.66 ± 0.01 mg/dl) by about 10% in male broilers (167.09 ± 0.83 mg/dl) at 8 weeks of age.

(ii) Total serum protein: Low light exposure with ascorbic acid supplementation had a significant effect ($P < 0.01$) on total serum protein levels in female broilers (1.70 ± 0.05 to 1.92 ± 0.01 g/dl) at 3 weeks of age and in male broilers at 5 and 8 weeks of age (1.60 ± 0.01 to 1.84 ± 0.08 g/dl; 1.76 ± 0.10 to 1.95 ± 0.01 g/dl). The levels increased ($P < 0.01$) above corresponding control level (1.70 ± 0.05 g/dl) by about 13% in female broilers (1.92 ± 0.01 g/dl) at 3 weeks of age, but decreased ($P < 0.01$) below corresponding control levels (1.84 ± 0.08 g/dl; 1.95 ± 0.01 g/dl) by about 13% in male broilers (1.60 ± 0.01 g/dl) at 5 weeks of age and by 10% in male broilers (1.76 ± 0.10 g/dl) at 8 weeks of age.

(iii) Total serum creatinine: Low light exposure with ascorbic acid supplementation had a significant effect ($P < 0.01$) on total serum creatinine levels in female broilers (0.66 ± 0.03 to 0.73 ± 0.01 mg/dl) at 5 weeks of age and in male broilers (0.61 ± 0.04 to 0.81 ± 0.01 mg/dl) and in female ones (0.67 ± 0.01 to 0.82 ± 0.01 mg/dl) at 8 weeks of age . The levels decreased ($P < 0.01$) below corresponding control levels (0.73 ± 0.01 mg/dl; 0.81 ± 0.01 mg/dl; 0.82 ± 0.01 mg/dl) by about 10% in female broilers (0.66 ± 0.03 mg/dl) at 5 weeks of age, by 25% in male broilers (0.61 ± 0.04 mg/dl) and 18% in female broilers (0.67 ± 0.01 mg/dl) at 8 weeks of age.

(iv) Total available serum sugars: Low light exposure with ascorbic acid supplementation had a significant effect ($P < 0.01$) on total available serum sugar levels in male and female broilers at 3, 5 and 8 weeks of age (256.65 ± 0.10 to 348.80 ± 0.02 mg/dl). The levels decreased ($P < 0.01$) below corresponding control levels (268.60 ± 0.10 mg/dl; 260.57 ± 0.01 mg/dl; 348.80 ± 0.02 mg/dl) by about 4% in male broilers (258.51 ± 0.01 mg/dl) and by 2% in female broilers (256.65 ± 0.10 mg/dl) at 3 weeks of age and by about 1% in male ones (345.20 ± 0.50 mg/dl) at 8 weeks of age. But, the levels also increased ($P < 0.01$) above corresponding control levels (284.53 ± 0.30 mg/dl; 278.82 ± 0.02 mg/dl; 345.53 ± 0.01 mg/dl) by about 1% in male broilers

(287.10 ± 0.35 mg/dl) and by 5% in female ones (291.40 ± 0.20 mg/dl) at 5 weeks of age and by about 1% in female broilers (348.25 ± 0.10 mg/dl) at 8 weeks of age.

3.1.7 Effect(s) of number of entries into the pens:

The effect(s) of number of entries into the pens on some biochemical parameters in broilers of different ages (3, 5 and 8 weeks) are as shown (Table 6). Using a normal entry into the pens (2 per day) as control, a high number of entries into the pens (4 per day) had either no significant effect ($P > 0.01$) or a significant effect ($P < 0.01$) on the levels of the biochemical parameters as follows:-

A) **No significant effect:**

(i) **Haematocrit (PCV):** High number of entries into the pens (4 per day) had no significant effect ($P > 0.01$) on haematocrit (PCV) levels in broilers at 3, 5 and 8 weeks of age (26.50 ± 0.78 to 34.00 ± 3.14 %).

(ii) **Total serum cholesterol:** High number of entries into the pens (4 per day) had no significant effect ($P > 0.01$) on total serum cholesterol level in male broilers at 5 weeks of age only (180.41 ± 0.37 to 181.27 ± 3.92 mg/dl).

(iii) **Total serum creatinine:** High number of entries into the pens (4 per day) had no significant effect ($P > 0.01$) on total serum creatinine levels in male broilers (0.64 ± 0.01 to 0.70 ± 0.01 mg/dl) and in female ones (0.67 ± 0.03 to 0.73 ± 0.01 mg/dl) at 5 weeks of age.

B) **Significant effect:**

(i) **Total serum cholesterol:** High number of entries into the pens (4 per day) had a significant effect ($P < 0.01$) on total serum cholesterol levels in male broilers (170.47 ± 0.27 to 197.89 ± 1.86 mg/dl; 181.93 ± 0.65 to 192.64 ± 0.03 mg/dl) and in female ones (167.68 ± 1.10 to 197.30 ± 0.90 mg/dl; 183.49 ± 0.08 to 188.57 ± 1.47 mg/dl) at 3 and 8 weeks of age and in female broilers (170.42 ± 0.02 to 174.80 ± 1.58 mg/dl) at 5 weeks of age only. The levels

TABLE 6: EFFECT OF NUMBER OF ENTRIES INTO THE PENS ON SOME BIOCHEMICAL PARAMETERS (Mean ± SEM) IN BROILERS OF DIFFERENT AGES

BIOCHEMICAL PARAMETERS	SEX (M/F)	AGE (WEEKS) 3 CTRL	3 ENTRY	5 CTRL	5 ENTRY	8 CTRL	8 ENTRY
HAEMATOCRIT (PCV) %	M	31.38 ± 1.38a	31.24 ± 2.14a	29.88 ± 1.10a	26.50 ± 0.78a	29.63 ± 2.50a	29.00 ± 1.96a
	F	34.00 ± 3.14a,b	32.94 ± 1.08a	31.00 ± 0.92a	28.00 ± 0.38a	32.75 ± 4.84a	27.00 ± 3.36a
TOTAL SERUM CHOLESTEROL (mg/dl)	M	170.47 ± 0.27d	197.89 ± 1.86j	180.41 ± 0.37f	181.27 ± 3.92f	192.64 ± 0.03i	181.93 ± 0.65f
	F	167.68 ± 1.10c	197.30 ± 0.90j	170.42 ± 0.02d	174.80 ± 1.58e	183.49 ± 0.08g	188.57 ± 1.47h
TOTAL SERUM PROTEIN (g/dl)	M	1.60 ± 0.09k	1.64 ± 0.25k	1.84 ± 0.08l,m	1.86 ± 0.34l,m	2.00 ± 0.05m	1.83 ± 0.06l
	F	1.70 ± 0.05k,l	1.88 ± 0.02l,m	1.87 ± 0.02l,m	1.75 ± 0.09k,l	2.03 ± 0.02m	1.82 ± 0.07l
TOTAL SERUM CREATININE (mg/dl)	M	0.70 ± 0.01n,p	0.85 ± 0.02q	0.70 ± 0.01n,p	0.64 ± 0.01n	0.81 ± 0.01q	0.65 ± 0.01n
	F	0.68 ± 0.01n	0.86 ± 0.03q	0.73 ± 0.01p	0.67 ± 0.03n,p	0.82 ± 0.01q	0.63 ± 0.02n
TOTAL AVAILABLE SERUM SUGARS (mg/dl)	M	268.60 ± 0.10v	248.49 ± 0.01s	284.53 ± 0.30x	268.52 ± 0.02v	348.80 ± 0.02z	362.53 ± 0.02a′
	F	260.57 ± 0.01t	241.00 ± 0.10r	278.82 ± 0.02w	258.53 ± 0.01u	345.53 ± 0.01y	356.42 ± 0.10b′

Sample number is fifty (50) broilers per sex.
CTRL = Control (i.e. normal entry into the pens = 4 per day).
ENTRY = High number of entries into the pens (4 per day).
a-b′ Values with different letters from corresponding controls differ significantly by ANOVA ($P < 0.01$).

increased (< 0.01) above corresponding control levels (170.47 ± 0.27 mg/dl; 167.68 ± 1.10 mg/dl; 170.42 ± 0.02 mg/dl; 183.49 ± 0.08 mg/dl) by about 16% in male broilers (197.89 ± 1.86 mg/dl) and 18% in female ones (197.30 ± 0.09 mg/dl) at 3 weeks of age and by about 3% each in female broilers (174.80 ± 1.58 mg/dl; 188.57 ± 1.47 mg/dl) at 5 and 8 weeks of age. But, the level decreased ($P < 0.01$) below corresponding control level (192.64 ± 0.03 mg/dl) by about 3% in male broilers (181.93 ± 0.65 mg/dl) at 8 weeks of age.

(ii) **Total serum protein:** High number of entries into the pens (4 per day) had a significant effect ($P < 0.01$) on total serum protein levels in male broilers (1.83 ± 0.06 to 2.00 ± 0.05 g/dl) and in female ones (1.82 ± 0.07 to 2.03 ± 0.02 g/dl) at 8 weeks of age only. The levels decreased ($P < 0.01$) below corresponding control levels (2.00 ± 0.05 g/dl; 2.03 ± 0.02 g/dl) by about 9% in male broilers (1.83 ± 0.06 g/dl) and by 10% in female ones (1.82 ± 0.07 g/dl) at 8 weeks of age.

(iii) **Total serum creatinine:** High number of entries into the pens (4 per day) had a significant effect ($P < 0.01$) on total serum creatinine levels in male broilers (0.70 ± 0.01 to 0.85 ± 0.02 mg/dl; 0.65 ± 0.01 to 0.81 ± 0.01 mg/dl) and in female ones (0.68 ± 0.01 to 0.86 ± 0.03 mg/dl; 0.63 ± 0.02 to 0.82 ± 0.01 mg/dl) at 3 and 8 weeks of age only. The levels increased ($P < 0.01$) above corresponding control levels (0.70 ± 0.01 mg/dl; 0.68 ± 0.01 mg/dl) by about 21% in male broilers (0.85 ± 0.02 mg/dl) and by 26% in female ones (0.86 ± 0.03 mg/dl) at 3 weeks of age. But, the levels decreased ($P < 0.01$) below corresponding control levels (0.81 ± 0.01 mg/dl; 0.82 ± 0.01 mg/dl) by about 20% in male broilers (0.65 ± 0.01 mg/dl) and by 23% in female ones (0.63 ± 0.02 mg/dl) at 8 weeks of age.

(iv) **Total available serum sugars:** High number of entries into the pens (4 per day) had a significant effect ($P < 0.01$) on total available serum sugar levels in male and female broilers of 3, 5 and 8 weeks of age (241.00 ± 0.10 to 362.53 ± 0.02 mg/dl). The levels decreased ($P < 0.01$) below corresponding control levels (268.60 ± 0.10 mg/dl; 260.57 ± 0.01 mg/dl; 284.53 ± 0.30

mg/dl; 278.82 ± 0.02 mg/dl) by about 7% in male broilers (248.49 ± 0.01 mg/dl) and by 8% in female ones (241.00 ± 0.10 mg/dl) at 3 weeks of age and by about 6% in male broilers (268.52 ± 0.02 mg/dl) and 7% in female ones (258.53 ± 0.01 mg/dl) at 5 weeks of age. But, the levels increased ($P < 0.01$) above corresponding control levels (348.80 ± 0.02 mg/dl; 345.53 ± 0.01 mg/dl) by about 4% in male broilers (362.53 ± 0.02 mg/dl) and by 3% in female ones (356.42 ± 0.10 mg/dl) at 8 weeks of age.

3.1.8 Effect(s) of high number of entries into the pens with ascorbic acid supplementation:

The effect(s) of high number of entries into the pens on selected biochemical parameters of ascorbic acid-supplemented broilers of different ages (3, 5 and 8 weeks) are as shown (Table 7). Using high number of entries into the pens (4 per day) but without ascorbic acid supplementation as control, high number of entries into the pens (4 per day) with ascorbic acid supplementation (300mg / kg diet) had either no significant effect ($P > 0.01$) or a significant effect ($P < 0.01$) on the levels of the biochemical parameters as follows:-

A) No significant effect:

(I) Haematocrit (PCV): High number of entries into the pens (4 per day) with ascorbic acid supplementation (300mg / kg diet) had no significant effect ($P > 0.01$) on haematocrit (PCV) levels in male and female broilers at 3, 5 and 8 weeks of age (24.75 ± 1.90 to 32.94 ± 1.08 %).

(II) Total serum cholesterol: High number of entries into the pens (4 per day) with ascorbic acid supplementation (300mg / kg diet) had no significant effect ($P > 0.01$) on total serum cholesterol levels in female broilers (197.30 ± 0.90 to 197.80 ± 1.16 mg/dl) at 3 weeks of age only.

TABLE 7: EFFECT OF HIGH NUMBER OF ENTRIES INTO THE PENS ON SOME BIOCHEMICAL PARAMETERS (Mean ± SEM) OF ASCORBIC ACID-SUPPLEMENTED BROILERS OF DIFFERENT AGES

BIOCHEMICAL PARAMETERS	SEX (M/F)	AGE (WEEKS) 3 ENTRY	3 +AA	5 ENTRY	5 +AA	8 ENTRY	8 +AA
HAEMATOCRIT (PCV) %	M	31.24 ± 2.14b,c	31.28 ± 0.44b,c	26.50 ± 0.78a,b	28.63 ± 1.66ab	29.00 ± 1.96a,b	28.75 ± 3.88a,b
	F	32.94 ± 1.08b,c	31.12 ± 2.16b,c	28.00 ± 0.38a,b	29.00 ± 2.54ab	27.00 ± 3.36a,b	24.75 ± 1.90a
TOTAL SERUM CHOLESTEROL (mg/dl)	M	197.89 ± 1.86h	182.74 ± 0.28e	181.27 ± 3.92e	192.58 ± 3.66g	181.93 ± 0.65e	192.18 ± 1.25g
	F	197.30 ± 0.90h	197.80 ± 1.16h	174.80 ± 1.58d	197.10 ± 0.58h	188.57 ± 1.47f	183.76 ± 1.30e
TOTAL SERUM PROTEIN (g/dl)	M	1.64 ± 0.25i	1.73 ± 0.01i	1.86 ± 0.34i,j	1.77 ± 0.01i	1.83 ± 0.06i,j	1.99 ± 0.04j
	F	1.88 ± 0.02i,j	1.71 ± 0.05i	1.75 ± 0.09i	1.84 ± 0.05ij	1.82 ± 0.07i,j	1.80 ± 0.05i,j
TOTAL SERUM CREATININE (mg/dl)	M	0.85 ± 0.02l	0.66 ± 0.04k	0.64 ± 0.01k	0.67 ± 0.05k	0.65 ± 0.01k	0.67 ± 0.02k
	F	0.86 ± 0.03l	0.68 ± 0.02k	0.67 ± 0.03k	0.61 ± 0.04k	0.63 ± 0.02k	0.67 ± 0.04k
TOTAL AVAILABLE SERUM SUGARS (mg/dl)	M	248.49 ± 0.01s	244.50 ± 0.58r	268.52 ± 0.02u	238.26 ± 0.02p	362.53 ± 0.02y	354.20 ± 0.10w
	F	241.00 ± 0.10q	228.47 ± 0.01n	258.53 ± 0.01t	220.39 ± 0.01m	356.42 ± 0.10x	350.34 ± 0.05v

Sample number is fifty (50) broilers per sex.
ENTRY = Control (i.e. high number of entries into the pens (4 per day) but without ascorbic acid supplementation).
+AA = High number of entries into the pens (4 per day) with ascorbic acid supplementation (300mg / kg diet).
a-y Values with different letters from corresponding controls differ significantly by ANOVA ($P < 0.01$).

(iii) **Total serum protein:** High number of entries into the pens (4 per day) with ascorbic acid supplementation (300mg / kg diet) had no significant effect ($P > 0.01$) on serum protein levels in male and female broilers at 3, 5 and 8 weeks of age (1.64 ± 0.25 to 1.99 ± 0.04 g/dl).

(iv) **Total serum creatinine:** High number of entries into the pens (4 per day) with ascorbic acid supplementation (300mg / kg diet) had no significant effect ($P > 0.01$) on total serum creatinine levels in male broilers (0.64 ± 0.01 to 0.67 ± 0.05 mg/dl; 0.65 ± 0.01 to 0.67 ± 0.02 mg/dl) and female ones (0.61 ±0.04 to 0.67 ± 0.03 mg/dl; 0.63 ± 0.02 to 0.67 ± 0.04 mg/dl) at 5 and 8 weeks of age.

B) Significant effect:

(i) **Total serum cholesterol:** High number of entries into the pens (4 per day) with ascorbic acid supplementation (300mg / kg diet) had a significant effect ($P < 0.01$) on total serum cholesterol levels in male broilers (182.74 ± 0.28 to 197.89 ±1.86 mg/dl) at 3 weeks of age only and in both male broilers (181.27 ±3.92 to 192.58 ± 3.66 mg/dl; 181.93 ± 0.65 to 192.18 ± 1.25 mg/dl) and female ones (174.80 ± 1.58 to 197.10 ± 0.58 mg/dl; 183.76 ± 1.30 to 188.57 ± 1.47 mg/dl) at 5 and 8 weeks of age. The levels decreased ($P < 0.01$) below corresponding control levels (197.89 ± 1.86 mg/dl; 188.57 ± 1.47 mg/dl) by about 8% in male broilers (182.74 ± 0.28 mg/dl) at 3 weeks of age and by 3% in female ones (183.76 ± 1.30 mg/dl) at 8 weeks of age only. But, the levels increased ($P < 0.01$) above corresponding control levels (181.27 ± 3.92 mg/dl; 174.80 ± 1.58 mg/dl; 181.93 ± 0.65 mg/dl) by about 6% in male broilers (192.58 ± 3.66 mg/dl) and 13% in female ones (197.10 ± 0.58 mg/dl) at 5 weeks of age, and by 6% in male broilers (192.18 ± 1.25 mg/dl) at 8 weeks of age.

(ii) **Total serum creatinine:** High number of entries into the pens (4 per day) with ascorbic acid supplementation (300mg / kg diet) had a significant effect ($P < 0.01$) on total serum creatinine levels in male broilers (0.66 ± 0.04 to 0.85 ± 0.02 mg/dl) and in female ones (0.68 ± 0.02 to 0.86 ± 0.03 mg/dl) at 3 weeks of age only. The levels decreased ($P < 0.01$) below

corresponding control levels (0.85 ± 0.02 mg/dl; 0.86 ± 0.03 mg/dl) by about 22% in male broilers (0.66 ± 0.04 mg/dl) and 21% in female ones (0.68 ± 0.02 mg/dl) at 3 weeks of age.

(iii) **Total available serum sugars:** High number of entries into the pens (4 per day) with ascorbic acid supplementation (300mg / kg diet) had a significant effect ($P < 0.01$) on total available serum sugar levels in male and female broilers at 3, 5 and 8 weeks of age (220.39 ± 0.01 to 362.53 ± 0.02 mg/dl). The levels decreased ($P < 0.01$) below corresponding control levels (248.49 ± 0.01 mg/dl; 241.00 ± 0.01 mg/dl; 268.52 ± 0.02 mg/dl; 258.53 ± 0.01 mg/dl; 362.53 ± 0.02 mg/dl; 356.42 ± 0.10 mg/dl) by about 2% in male broilers (244.50 ± 0.58 mg/dl) and 5% in female ones (228.47 ± 0.01 mg/dl) at 3 weeks of age, by about 11% in male broilers (238.26 ± 0.02 mg/dl) and 15% in female ones (220.39 ± 0.01 mg/dl) at 5 weeks of age and by about 2% each in male and female broilers at 8 weeks of age (354.20 ± 0.10 mg/dl; 350.34 ± 0.05 mg/dl).

3.1.9 Effect(s) of low number of entry into the pens:

The effect(s) of low number of entry into the pens on some biochemical parameters in broilers of different ages (3, 5 and 8 weeks) are as shown (Table 8). Using a normal entry into the pens (2 per day) as control, a low number of entry into the pens (1 per day) had no significant effect ($P > 0.01$) on the levels of the biochemical parameters as follows:-

(i) **Haematocrit (PCV):** Low number of entry into the pens (1 per day) had no significant effect ($P > 0.01$) on haematocrit levels in male and female broilers at 3, 5 and 8 weeks of age (29.88 ± 1.10 to 34.00 ± 3.14 %). The trend showed an insignificant decrease ($P > 0.01$) of 3 weeks old male broiler base line haematocrit level (31.38 ± 1.38 %) by about 5% at 5 weeks of age (29.88 ± 1.10 %) and by 2% at 8 weeks of age (30.88 ± 2.28 %). Similarly, 3 weeks old female broiler base line haematocrit level (34.00 ± 3.14 %) decreased ($P > 0.01$) by about 9% at 5 weeks of age (31.00 ± 0.93 %) and by 4% at 8 weeks of age (32.63 ± 1.04 %).

TABLE 8: EFFECT OF LOW NUMBER OF ENTRY INTO THE PENS ON SOME BIOCHEMICAL PARAMETERS (Mean ± SEM) IN BROILERS OF DIFFERENT AGES

BIOCHEMICAL PARAMETERS	SEX (M/F)	AGE (WEEKS) 3		5		8	
		CTRL	LOW ENTRY	CTRL	LOW ENTRY	CTRL	LOW ENTRY
HAEMATOCRIT (PCV) %	M	31.38 ± 1.38a	31.38 ± 1.38a	29.88 ± 1.10a	29.88 ± 1.10a	30.88 ± 2.28a	30.88 ± 2.28a
	F	34.00 ± 3.14a,b	34.00 ± 3.14a,b	31.00 ± 0.92a	31.00 ± 0.92a	29.25 ± 2.10a	29.25 ± 2.10a
TOTAL SERUM CHOLESTEROL (mg/dl)	M	170.47 ± 0.27d	170.47 ± 0.27d	180.41 ± 0.37e	180.41 ± 0.37e	185.66 ± 0.01g	185.66 ± 0.01g
	F	167.68 ± 1.10c	167.68 ± 1.10c	170.42 ± 0.02d	170.42 ± 0.02d	180.59 ± 0.10f	180.59 ± 0.10f
TOTAL SERUM PROTEIN (g/dl)	M	1.60 ± 0.09g	1.60 ± 0.09h	1.84 ± 0.08j	1.84 ± 0.08j	1.95 ± 0.01k	1.95 ± 0.01k
	F	1.70 ± 0.05h,i	1.70 ± 0.05h,i	1.82 ± 0.02j	1.82 ± 0.02j	1.99 ± 0.01k	1.99 ± 0.01k
TOTAL SERUM CREATININE (mg/dl)	M	0.70 ± 0.01l	0.70 ± 0.01l	0.70 ± 0.01l	0.70 ± 0.01l	0.81 ± 0.01m	0.81 ± 0.01m
	F	0.68 ± 0.01l	0.68 ± 0.01l	0.73 ± 0.01l	0.73 ± 0.01l	0.82 ± 0.01m	0.82 ± 0.01m
TOTAL AVAILABLE SERUM SUGARS (mg/dl)	M	268.60 ± 0.10p	268.60 ± 0.10p	284.53 ± 0.30r	284.53 ± 0.30r	348.80 ± 0.02t	348.80 ± 0.02t
	F	260.57 ± 0.01n	260.57 ± 0.10n	278.82 ± 0.02q	278.82 ± 0.02q	345.53 ± 0.01s	345.53 ± 0.01s

Sample number is fifty (50) broilers per sex.
CTRL = Control (i.e. normal entry into the pens = 2 per day).
LOW ENTRY = Low entry into the pens (1 per day).
a-t Values with the same letters as corresponding controls did not differ significantly by ANOVA ($P < 0.01$).

(ii) **Total serum cholesterol:** Low number of entry into the pens (1 per day) had no significant effect ($P > 0.01$) on total cholesterol levels in male and female broilers at 3, 5 and 8 weeks of age (167.68 ± 1.10 to 185.66 ± 0.01 mg/dl). But, the trend showed a significant increase ($P < 0.01$) of 3 weeks old male broiler base line total cholesterol level (170.47 ± 0.27 mg/dl) by about 6% at 5 weeks (180.41 ± 0.37 mg/dl) and by 9% at 8 weeks of age (185.66 ± 0.01 mg/dl). Similarly, 3 weeks old female broiler base line total cholesterol level (167.68 ± 1.10 mg/dl) increased ($P < 0.01$) by about 2% at 5 weeks (170.42 ± 0.02 mg/dl) and by 8% at 8 weeks of age (180.59 ± 0.01 mg/dl).

(iii) **Total serum protein:** Low number of entry into the pens (1 per day) had no significant effect ($P > 0.01$) on total protein levels in male and female broilers of 3, 5 and 8 weeks of age (1.60 ± 0.09 to 1.99 ± 0.01 g/dl). But, the trend showed a significant increase ($P < 0.01$) of 3 weeks old male broiler base line total protein level (1.60 ± 0.09 g/dl) by about 15% at 5 weeks (1.84 ± 0.08 g/dl) and by 22% at 8 weeks of age (1.95 ± 0.01 g/dl). Similarly, 3 weeks old female broiler base line total protein level (1.70 ± 0.05 g/dl) increased ($P < 0.01$) by about 10% at 5 weeks (1.87 ± 0.02 g/dl) and by 17% at 8 weeks of age (1.99 ± 0.01g/dl).

(iv) **Total serum creatinine:** Low number of entry into the pens (1 per day) had no significant effect ($P > 0.01$) on total creatinine levels in male and female broilers of 3, 5 and 8 weeks of age (0.68 ± 0.01 to 0.82 ± 0.01 mg/dl). But, the trend showed a significant increase ($P < 0.01$) of 3 weeks old male broiler base line total creatinine level (0.70 ± 0.01 mg/dl) by about 16% only at 8 weeks of age (0.81 ± 0.01 mg/dl). In female broilers, base line total creatinine level at 3 weeks of age (0.68 ± 0.01) increased though insignificantly ($P > 0.01$) by about 7% at 5 weeks (0.73 ± 0.01 mg/dl) and significantly ($P < 0.01$) by 17% at 8 weeks of age (0.82 ± 0.01 mg/dl).

(v) **Total available serum sugars:** Low number of entry into the pens (1 per day) had no significant effect ($P > 0.01$) on total sugar levels in male and female broilers at 3, 5 and 8 weeks

significant effect ($P > 0.01$) on total sugar levels in male and female broilers at 3, 5 and 8 weeks of age (260.57 ± 0.01 to 348.80 ± 0.02 mg/dl). But, the trend showed a significant increase ($P < 0.01$) of 3 weeks old male broiler base line total sugar level (268.60 ± 0.01 mg/dl) by 6% at 5 weeks (284.53 ± 0.03 mg/dl) and by 30% at 8 weeks (348.80 ± 0.02 mg/dl). For female broilers, base line total sugar level at 3 weeks of age (260.57 ± o.o1 mg/dl) increased ($P < 0.01$) by about 7% at 5 weeks (278.82 ± 0.02 mg/dl) and by 33% at 8 weeks of age (345.53 ± 0.01 mg/dl).

3.1.10 Effect(s) of low number of entry into the pens with ascorbic acid supplementation:

The effect(s) of low number of entry into the pens on some biochemical parameters of ascorbic acid-supplemented broilers of different ages (3, 5 and 8 weeks) are as shown (Table 9). Using a low number of entry into the pens (1 per day) but without ascorbic acid supplementation as control, a low number of entry into the pens (1 per day) with ascorbic acid supplementation (300mg / kg diet) had either no significant effect ($P > 0.01$) or a significant effect ($P < 0.01$) on the levels of the biochemical parameters as follows:-

A) **No significant effect:**

(i) **Haematocrit (PCV):** Low number of entry into the pens (1 per day) with ascorbic acid supplementation (300mg / kg diet) had no significant effect ($P > 0.01$) on haematocrit (PCV) levels in male and female broilers of 3, 5 and 8 weeks of age (28.63 ± 3.14 to 34.00 ± 2.14 %).

(ii) **Total serum cholesterol:** Low number of entry into the pens (1 per day) with ascorbic acid supplementation (300mg / kg diet) had no significant effect ($P > 0.01$) on total serum cholesterol levels in male broilers (180.41 ± 0.37 to 184.63 ± 3.76 mg/dl) at 5 weeks of age and in female ones (183.49 ± 0.08 to 185.00 ± 0.68 mg/dl) at 8 weeks of age only.

(iii) **Total serum protein:** Low number of entry into the pens (1 per day) with ascorbic acid supplementation (300mg / kg diet) had no significant effect ($P > 0.01$) on total serum protein

TABLE 9: EFFECT OF NUMBER OF ENTRY INTO THE PENS ON SOME BIOCHEMICAL PARAMETERS (Mean ± SEM) OF ASCORBIC ACID-SUPPLEMENTED BROILERS OF DIFFERENT AGES

BIOCHEMICAL PARAMETERS	SEX (M/F)	AGE (WEEKS) 3		5		8	
		LOW ENTRY	+AA	LOW ENTRY	+AA	LOW ENTRY	+AA
HAEMATOCRIT (PCV) %	M	31.38 ± 1.38a	32.25 ± 0.90a	29.88 ± 1.10a	28.63 ± 4.74a	29.63 ± 2.50a	28.63 ± 3.14a
	F	34.00 ± 3.14a,b	32.50 ± 1.42a	31.00 ± 0.92a	28.88 ± 1.44a	32.75 ± 4.84a	29.75 ± 0.18a
TOTAL SERUM CHOLESTEROL (mg/dl)	M	170.47 ± 0.27d	190.10 ± 0.98h	180.41 ± 0.37f	184.63 ± 3.76f,g	192.64 ± 0.03i	186.99 ± 0.63g
	F	167.68 ± 1.10c	201.27 ± 0.02i	170.42 ± 0.02d	175.49 ± 1.54e	183.49 ± 0.08g	185.00 ± 0.68g
TOTAL SERUM PROTEIN (g/dl)	M	1.60 ± 0.09j	1.57 ± 0.01j	1.84 ± 0.08l,m	1.79 ± 0.05j,k	2.00 ± 0.05m	1.95 ± 0.06m
	F	1.70 ± 0.05j,k	1.59 ± 0.10j	1.87 ± 0.02l,m	1.73 ± 0.17j,k	2.03 ± 0.02m	1.73 ± 0.03j,k
TOTAL SERUM CREATININE (mg/dl)	M	0.70 ± 0.01p	0.78 ± 0.01p,q	0.70 ± 0.01p	0.70 ± 0.02p	0.81 ± 0.01q	0.72 ± 0.02p
	F	0.68 ± 0.01p	0.84 ± 0.01q	0.73 ± 0.01p	0.66 ± 0.01p	0.82 ± 0.01q	0.66 ± 0.02p
TOTAL AVAILABLE SERUM SUGARS (mg/dl)	M	268.60 ± 0.10u	263.56 ± 0.10t	284.53 ± 0.30w	288.39 ± 0.01x	348.80 ± 0.02a′	342.54 ± 0.10z
	F	260.57 ± 0.01s	258.51 ± 0.01r	278.82 ± 0.02v	278.34 ± 0.02v	345.53 ± 0.01b′	335.00 ± 0.02y

Sample number is fifty (50) broilers per sex.
LOW ENTRY = Control (i.e. low number of entry into the pens (1 per day) but without ascorbic acid supplementation).
+AA = Low number of entry into the pens (1 per day) with ascorbic acid supplementation (300mg / kg diet).
a-b′ Values with different letters from corresponding controls differ significantly by ANOVA ($P < 0.01$).

levels in male broilers (1.57 ± 0.01 to 1.60 ± 0.09 g/dl) and in female ones (1.59 ± 0.10 to 1.70 ± 0.05 g/dl) at 3 weeks of age and in male broilers (1.95 ± 0.06 to 2.00 ± 0.05 g/dl) at 8 weeks of age only.

(iv) **Total serum creatinine:** Low number of entry into the pens (1 per day) with ascorbic acid supplementation (300mg / kg diet) had no significant effect ($P > 0.01$) on total serum creatinine levels in male broilers (0.70 ± 0.01 to 0.78 ± 0.01 mg/dl) at 3 weeks of age and in male broilers (0.70 ± 0.01 to 0.70 ±0.02 mg/dl) and in female ones (0.66 ± 0.01 to 0.73 ± 0.01 mg/dl) at 5 weeks of age only.

(v) **Total available serum sugars:** Low number of entry into the pens (1 per day) with ascorbic acid supplementation (300mg / kg diet) had no significant effect ($P > 0.01$) on total available serum sugar level in female broilers (278.34 ± 0.02 to 278.82 ± 0.02 mg/dl) at 5 weeks of age only.

B) Significant effect:

(i) **Total serum cholesterol:** Low number of entry into the pens (1 per day) with ascorbic acid supplementation (300mg / kg diet) had a significant effect ($P < 0.01$) on total serum cholesterol levels in male broilers (170.47 ± 0.27 to 190.10 ± 0.98 mg/dl) and in female ones (167.68 ± 1.10 to 201.27 ± 0.02 mg/dl) at 3 weeks of age, in female broilers (170.42 ± 0.02 to 175.49 ± 1.54 mg/dl) at 5 weeks of age and in male ones (186.99 ± 0.63 to 192.64 ± 0.03 mg/dl) at 8 weeks of age. The levels increased ($P < 0.01$) above corresponding control levels (170.42 ± 0.02 mg/dl; 167.68 ± 1.10 mg/dl; 170.42 ± 0.02 mg/dl) by about 12% in male broilers (190.10 ± 0.98 mg/dl) and by 20% in female ones (201.27 ± 0.02 mg/dl) at 3 weeks of age and by about 3% in female broilers (175.49 ± 1.54 mg/dl) at 5 weeks of age. But, the level decreased ($P< 0.01$) below corresponding control level (192.64 ± 0.03 mg/dl) by about 3% in male broilers (186.99 ± 0.63 mg/dl) at 8 weeks of age.

(ii) **Total serum protein:** Low number of entry into the pens (1 per day) with ascorbic acid

supplementation (300mg / kg diet) had a significant effect ($P < 0.01$) on total serum protein levels in male broilers (1.79 ± 0.05 to 1.84 ± 0.08 g/dl) and in female ones (1.73 ± 0.17 to 1.87 ± 0.02 g/dl) at 5 weeks of age and in female broilers (1.73 ± 0.03 to 2.03 ± 0.02 g/dl) at 8 weeks of age only. The levels decreased ($P < 0.01$) below corresponding control levels (1.84 ± 0.08 g/dl; 1.87 ± 0.02 g/dl; 2.03 ± 0.02 g/dl) by about 3% in male broilers (1.79 ± 0.05 g/dl) and 7% in female ones (1.73 ± 0.17 g/dl) at 5 weeks of age and by about 15% in female broilers (1.73 ± 0.03 g/dl) at 8 weeks of age.

(iii) **Total serum creatinine:** Low number of entry into the pens (1 per day) with ascorbic acid supplementation (300mg / kg diet) had a significant effect ($P < 0.01$) on total serum creatinine levels in male broilers (0.70 ± 0.01 to 0.78 ± 0.01 mg/dl) at 3 weeks of age and in male broilers (0.72 ± 0.02 to 0.81 ± 0.01 mg/dl) and in female broilers (0.66 ± 0.02 to 0.82 ± 0.01 mg/dl) at 8 weeks of age only. The level increased ($P < 0.01$) above corresponding control level (0.68 ± 0.01 mg/dl) by about 24% in female broilers (0.84 ± 0.01 mg/dl) at 3 weeks of age. But, the level decreased ($P < 0.01$) below corresponding control levels (0.81 ± 0.01 mg/dl; 0.82 ± 0.01 mg/dl) by about 11% in male broilers (0.72 ± 0.02 mg/dl) and by 20% in female ones (0.66 ± 0.02 mg/dl) at 8 weeks of age.

(iv) **Total available serum sugars:** Low number of entry into the pens (1 per day) with ascorbic acid supplementation (300mg / kg diet) had a significant effect ($P < 0.01$) on total available serum sugar levels in male broilers (263.56 ± 0.10 to 268.60 ± 0.10 mg/dl; 284.53 ± 0.30 to 288.39 ± 0.01 mg/dl) and in female ones (258.51 ± 51 0.01 to 260.57 ± 0.01 mg/dl; 278.34 ± 0.02 to 278.82 ± 0.02 mg/dl) at 3 and 5 weeks of age and in male broilers (342.54 ± 0.10 to 348.80 ± 0.02 mg/dl) and in female ones (335.00 ± 0.02 to 345.53 ± 0.01 mg/dl) at 8 weeks of age only. The levels decreased ($P < 0.01$) below corresponding control levels (268.60 ± 0.10 mg/dl; 260.57 ± 0.01 mg/dl; 348.80 ± 0.02 mg/dl; 345.53 ± 0.01 mg/dl) by about 2% in male broilers (263.56 ± 0.10 mg/dl) and 1% in female ones (258.51 ± 0.01 mg/dl) at 3 weeks of

age and by 2% in male broilers (342.54 ± 0.10 mg/dl) and 3% in female ones (335.00 ± 0.02 mg/dl) at 8 weeks of age. But, the level increased ($P < 0.01$) above corresponding control level (284.53 ± 0.30 mg/dl) by about 1% in male broilers (288.39 ± 0.01 mg/dl) at 5 weeks of age.

3.1.11 Effect(s) of transportation:

The effect(s) of transportation (30km / day) on some biochemical parameters in broilers of 8 weeks of age are as shown (Table 10). Using not transported broilers as control, transportation (30km / day) had either no significant effect ($P > 0.01$) or a significant effect ($P < 0.01$) on the levels of the biochemical parameters as follows:-

A) No significant effect:

(i) Haematocrit (PCV): Transportation (30km / day) had no significant effect ($P > 0.01$) on haematocrit (PCV) levels in broilers at 8 weeks of age (27.50 ± 2.42 to 32.75 ± 4.84 %).

(ii) Total serum protein: Transportation (30km / day) had no significant effect ($P > 0.01$) on total serum protein levels in male broilers (1.76 ± 0.01 to 2.00 ± 0.05 g/dl) and in female ones (1.93 ± 0.03 to 2.03 ± 0.02 g/dl) at 8 weeks of age.

(iii) Total serum creatinine: Transportation (30km / day) had no significant effect ($P > 0.01$) on total serum creatinine levels in male broilers (0.81 ± 0.01 to 0.84 ± 0.03 mg/dl) and in female ones (0.82 ± 0.01 to 0.83 ± 0.02 mg/dl) at 8 weeks of age.

B) Significant effect:

(i) Total serum cholesterol: Transportation had a significant effect ($P < 0.01$) on total serum cholesterol levels in male broilers (192.64 ± 0.03 to 210.59 ± 0.56 mg/dl) and in female ones (183.49 ± 0.08 to 188.12 ± 0.36 mg/dl) at 8 weeks of age. The levels increased ($P < 0.01$) above corresponding control levels (192.64 ± 0.03 mg/dl; 183.49 ± 0.08 mg/dl) by about 9% in male broilers (210.59 ± 0.56 mg/dl) and by 3% in female ones (188.12 ± 0.36 mg/dl) at 8 weeks of age.

TABLE 10: EFFECT OF TRANSPORTATION ON SOME BIOCHEMICAL PARAMETERS (Mean ± SEM) IN BROILERS AT 8 WEEKS OF AGE

BIOCHEMICAL PARAMETERS	SEX (M/F)	CONTROL	TRANSPORTATION
HAEMATOCRIT (PCV) %	M	29.63 ± 2.50a	27.50 ± 2.42a
	F	32.75 ± 4.84a,b	29.13 ± 2.84a
TOTAL SERUM CHOLESTEROL (mg/dl)	M	192.64 ± 0.03e	210.59 ± 0.56f
	F	183.49 ± 0.08c	188.12 ± 0.36d
TOTAL SERUM PROTEIN (g/dl)	M	2.00 ± 0.05e,f	1.76 ± 0.01e
	F	2.03 ± 0.02e,f	1.93 ± 0.03e,f
TOTAL SERUM CREATININE (mg/dl)	M	0.81 ± 0.01g	0.84 ± 0.03g
	F	0.82 ± 0.01g	0.83 ± 0.02g
TOTAL AVAILABLE SERUM SUGARS (mg/dl)	M	348.80 ± 0.02i	357.45 ± 0.23j
	F	345.53 ± 0.01h	360.12 ± 0.30k

Sample number is fifty (50) broilers per sex.
CONTROL = Not transported broilers.
TRANSPORTATION = Transported (30km / day) broilers.
a-k Values with different letters from corresponding controls differ significantly by ANOVA ($P < 0.01$).

(ii) **Total available serum sugars:** Transportation (30km / day) had a significant effect ($P < 0.01$) on total available serum sugar levels in male broilers (348.80 ± 0.02 to 357.45 ± 0.23 mg/dl) and in female ones (345.53 ± 0.01 to 360.12 ± 0.30 mg/dl) at 8 weeks of age. The levels increased ($P < 0.01$) above corresponding control levels (348.80 ± 0.02 mg/dl; 345.53 ± 0.01 mg/dl) by about 2% in male broilers (357.45 ± 0.23 mg/dl) and by 4% in female ones (360.12 ± 0.30 mg/dl) at 8 weeks of age.

3.1.12 Effect(s) of transportation with ascorbic acid supplementation:

The effect(s) of transportation (30km / day) on some biochemical parameters in ascorbic acid-supplemented broilers at 8 weeks of age are as shown (Table 11). Using transportation (30km / day) but without ascorbic acid supplementation as control, transportation with ascorbic acid supplementation (300mg / kg diet) had either no significant effect ($P > 0.01$) or a significant effect ($P < 0.01$) on the levels of the biochemical parameters as follows:-

A) No significant effect:

(i) **Haematocrit (PCV):** Transportation (30km / day) with ascorbic acid supplementation (300mg / kg diet) had no significant effect ($P > 0.01$) on haematocrit (PCV) levels in male broilers (25.75 ± 1.94 to 27.50 ± 2.42 %) and in female ones (29.13 ± 2.84 to 29.88 ± 3.04 %) at 8 weeks of age.

(ii) **Total serum protein:** Transportation (30km / day) with ascorbic acid supplementation (300mg / kg diet) had no significant effect ($P > 0.01$) on total serum protein levels in male broilers (1.76 ± 0.01 to 2.04 ± 0.33 g/dl) and in female ones (1.93 ± 0.03 to 2.22 ± 0.05 g/dl) at 8 weeks of age.

(iii) **Total available serum sugars:** Transportation (30km / day) with ascorbic acid supplementation (300mg / kg diet) had no significant effect ($P > 0.01$) on total available serum sugar levels in female broilers (358.65 ± 0.02 to 360.12 ± 0.30 mg/dl) at 8 weeks of age.

TABLE 11: EFFECT OF TRANSPORTATION ON SOME BIOCHEMICAL PARAMETERS (Mean ± SEM) OF ASCORBIC ACID - SUPPLEMENTED BROILERS OF 8 WEEKS OF AGE.

BIOCHEMICAL PARAMETERS	SEX (M/F)	CONTROL	+AA
HAEMATOCRIT (PCV) %	M	27.50 ± 2.42a	25.75 ± 1.94a
	F	29.13 ± 2.84a	29.88 ± 3.04a,b
TOTAL SERUM CHOLESTEROL (mg/dl)	M	210.59 ± 0.56f	189.01 ± 0.61d
	F	188.12 ± 0.36d	176.44 ± 0.04c
TOTAL SERUM PROTEIN (g/dl)	M	1.76 ± 0.01f,g	2.04 ± 0.33g
	F	1.93 ± 0.03g	2.22 ± 0.05g
TOTAL SERUM CREATININE (mg/dl)	M	0.84 ± 0.03i	0.76 ± 0.01h
	F	0.83 ± 0.02i	0.73 ± 0.01h
TOTAL AVAILABLE SERUM SUGARS (mg/dl)	M	357.45 ± 0.23k	354.43 ± 0.03j
	F	360.12 ± 0.30k,l	358.65 ± 0.02k

Sample number is fifty (50) broilers per sex.
CONTROL = Transportation (30km / day) but without ascorbic acid supplementation).
+AA = Transportation (30km / day) with ascorbic acid supplementation (i.e. 300mg / kg diet).
a - l Values with different letters from corresponding controls differ significantly by ANOVA ($P < 0.01$).

But, the trend appeared to favor an insignificant decrease ($P > 0.01$) of level with ascorbic acid supplementation.

B) **Significant effect:**

(I) **Total serum cholesterol:** Transportation (30km / day) with ascorbic acid supplementation (300mg / kg diet) had a significant effect ($P < 0.01$) on total serum cholesterol levels in male broilers (189.01 ± 0.61 to 210.59 ± 0.56 mg/dl) and in female ones (176.44 ± 0.04 to 188.12 ± 0.36 mg/dl) at 8 weeks of age. The levels decreased ($P < 0.01$) below corresponding control levels (210.59 ± 0.56 mg/dl; 188.12 ± 0.36 mg/dl) by about 10% in male broilers (189.01 ± 0.61 mg/dl) and 6% in female ones (176.44 ± 0.04 mg/dl) at 8 weeks of age.

(ii) **Total serum creatinine:** Transportation (30km / day) with ascorbic acid supplementation (300mg vit.C / kg diet / day) had a significant effect on total serum creatinine levels in male broilers (0.76 ± 0.01 to 0.84 ± 0.03 mg/dl) and in female ones (0.73 ± 0.01 to 0.83 ± 0.02 mg/dl) at 8 weeks of age. The levels decreased ($P < 0.01$) below corresponding control levels (0.84 ± 0.03 mg/dl; 0.83 ± 0.02 mg/dl) by about 10% in male broilers (0.76 ± 0.01 mg/dl) and by 12% in female ones (0.73 ± 0.01) at 8 weeks of age.

(iii)**Total available serum sugars:** Transportation (30km / day) with ascorbic acid supplementation (300mg / kg diet) had a significant effect ($P < 0.01$) on total available serum sugar levels in male broilers (354.43 ± 0.03 to 357.45 ± 0.23 mg/dl) at 8 weeks of age. The level decreased ($P < 0.01$) below corresponding control level (357.43 ± 0.23 mg/dl) by only about 1% in male ones (354.43 ± 0.03 mg/dl) at 8 weeks of age.

3.2 DISCUSSION

3.2.1 Effect(s) of age:

The results of this investigation shows that age and sex are two important physiologic variables upon which classification of the levels of biochemical parameters in broilers could be based. Of the two, age was regarded as the independent variable while sex (male or female) was the dependent variable. Of these biochemical parameters, only total serum cholesterol and total available serum sugar levels were significantly responsive ($P < 0.01$) to age effect. The levels were lowest in broilers of 3 weeks of age and highest in broilers of 8 weeks of age (Table 1). These observed levels of the biochemical parameters, being due to age effect, could have been of the magnitude of normal variations only intended to maintain homeostasis in the physiology and biochemistry of the domestic fowl. This position is buttressed by the fact that the broilers were apparently healthy and devoid of any drastic change, outside of normal variations, in the levels of biochemical parameters. Otherwise, adverse effects on homeostasis in broilers as had been reported by some researchers would have been encountered (Thaxton, 1978; Wels, 1966; Brake and Thaxton, 1979).

3.2.2 Effect(s) of sex:

Being a dependent variable, sex effect makes possible classification of the levels of biochemical parameters according to male or female sex in order to indicate any sex differences. In the broilers in which levels were significantly responsive ($P < 0.01$) to sex effect, males appeared to have had higher levels of some of the biochemical parameters such as total serum cholesterol at 5 and 8 weeks of age and total available serum sugars at 3 and 5 weeks of age than their corresponding female counterparts (Table 1). These higher levels that were observed in the male broilers relative to female ones are in agreement with literature documentation of normal values of similar biochemical parameters as being higher in the male specie of many

animals, including man (Goodale and Widmann, 1969). The reason could be due to their genetic predisposition.

3.2.3 Effect(s) of light exposure:

Apart from haematocrit (PCV) levels which were consistently non-responsive ($P > 0.01$) to the effect of high light exposure, the levels of all the other biochemical parameters (total serum cholesterol, total serum protein, total serum creatinine and total available serum sugars) were significantly increased in those instances in which the broilers responded to the effect of high light exposure. These findings appeared to be consistent with earlier report of light that is too bright being a stressor in broilers (Oluyemi and Roberts, 1979). However, high light exposure even as a stressor did not result in equal magnitude of increase in the levels of the biochemical parameters relative to their corresponding control levels (Table 2). Going by the magnitude of these increases, it is possible to suggest that in the broilers some of the biochemical parameters (e.g. total serum protein, total serum creatinine and total available serum sugars) are better indices of stress due to high light exposure than others (e.g. total serum cholesterol and the non-responsive haematocrit (PCV)). In this regard, broilers at 3 and 5 weeks of age (and male broilers in particular) had higher levels of the biochemical parameters which had responded to high light exposure than their relatively more matured counterpart broilers at 8 weeks of age. The reason for this might be that at 8 weeks of age, broilers are relatively better adapted to the stress factor than at 3 and 5 weeks of age. Therefore, the younger broilers may have exhibited a condition similar to the type described in the literature as a failure of adaptation to stress (Selye, 1976).

3.2.4 Effect(s) of highlight exposure with ascorbic acid supplementation:

The results shows that the non-responsiveness of haematocrit (PCV) levels to high light exposure was, to a large extent, not altered ($P > 0.01$) with ascorbic acid supplementation of the broilers (Table 3). However, with ascorbic acid supplementation of the broilers, the levels of many of the other biochemical parameters which had hitherto significantly increased ($P < 0.01$) due to high light exposure only were observed to either not differ ($P > 0.01$) or to have differed ($P < 0.01$) from their corresponding control levels. For example, ascorbic acid supplementation could have been ameliorative of the adverse effects of high light exposure in those instances in which levels did not significantly differ ($P > 0.01$) from corresponding control levels. The mechanism of this effect may have involved maintenance of homeostatic levels of the biochemical parameters. This was observed of total serum cholesterol in male broilers at 5 and 8 weeks of age, total serum protein in male and female broilers at 3, 5 and 8 weeks of age, total serum creatinine in male and female broilers at 3 weeks of age and in female broilers at 5 and 8 weeks of age and total available serum sugars in female broilers at 5 weeks of age, respectively. It could also involve a significant decrease ($P < 0.01$) of the levels in those other instances in which levels had significantly differed ($P < 0.01$) from corresponding control levels. This was observed of haematocrit (PCV) in male broilers at 3 weeks of age, total serum creatinine in male broilers at 5 and 8 weeks of age and total available serum sugars in male and female broilers at 3 weeks of age, respectively. But, the foregoing were not the only effects of significance. It is possible that a third mechanism of action existed which involved a significant increase ($P < 0.01$), by high light exposure with ascorbic acid supplementation, of the levels of some of the biochemical parameters. This was observed in the significant increase ($P < 0.01$) of levels above corresponding control levels of total serum cholesterol in male and female broilers at 3 weeks of age and in female broilers at 5 and 8 weeks of age, and total available serum sugars in male and female broilers at 3 weeks of age and in male broilers at 5 and 8 weeks

of age, respectively (Table 3). The benefit of such a significant increase ($P < 0.01$) in the levels of some of the biochemical parameters may be limited when viewed against the back-drop of literature evidence that persistently high plasma cholesterol and / or plasma glucose concentrations could lead to high cardiovascular (CVD) mortality and other metabolic disorders, particularly in old-age (Gurr et. al., 1989, Green, 1982; Goodale and Widmann, 1969). This observation might be an important pointer to the need for caution against indiscriminate use of 'mega-doses' of ascorbic acid for the purpose of supplementation of broilers. It has been known for some time that in the laboratory vitamin C can not only act as an antioxidant but under some conditions it can have the opposite effect. It is this type of knowledge that has prompted on-going studies in order to find out how to maximize the potentially beneficial effects of vitamin C while minimising possible harmful ones (see Anonymous, 1998).

3.2.5 Effect(s) of low light exposure:

The results show that low light exposure did not significantly change ($P > 0.01$) the levels of the biochemical parameters from corresponding control levels (Table 4). However, some significant changes ($P < 0.01$) occurred in the trend between base line levels of the biochemical parameters in young broilers at 3 weeks of age and in the more matured ones at 5 and 8 weeks of age. These observed homeostatic levels ($P > 0.01$) could suggest that the broilers generally coped with the effects of low light exposure. Such a capacity to cope with stress might have been relatively enhanced in broilers as shown by some of the significant changes ($P < 0.01$) in the trend of levels (Table 4). These effects of low light exposure in the broilers seemed to be very similar to those of low number of entry into the pens (Table 8).

3.2.6 Effect(s) of low light exposure with ascorbic acid supplementation:

The results, also, shows that the haematocrit (PCV) levels in the broilers were not significantly ($P > 0.01$) altered due to low light exposure with ascorbic acid supplementation (Table 5). In other words, homeostatic levels of haematocrit (PCV) were maintained with ascorbic acid supplementation, despite the stress of low light exposure. Homeostatic levels were, also, maintained of total serum cholesterol in male and female broilers of 5 weeks of age, total serum protein in male broilers of 3 weeks of age and in female broilers of 5 and 8 weeks of age, and of total creatinine in male and female broilers of 3 weeks of age and in male broilers of 5 weeks of age, respectively. These levels show that ascorbic acid has been ameliorative of the adverse effects of stress due to low light exposure in the broilers. That, to some extent, ascorbic acid supplementation is ameliorative of the adverse effects of stress due to low light exposure is further buttressed by the significant decrease ($P < 0.01$), below corresponding control levels, of the levels of some biochemical parameters in broilers. Such decrease ($P < 0.01$) of levels has been to varying degrees in some cases (e.g. total serum cholesterol in male broilers at 8 weeks of age, total serum protein in male broilers at 5 and 8 weeks of age, total serum creatinine in female broilers at 5 weeks of age and in male and female broilers at 8 weeks of age, and total available serum sugars in male and female broilers at 3 weeks of age and in male broilers at 8 weeks of age, respectively). The significant increase ($P < 0.01$) in the levels of total serum cholesterol in male and female broilers at 3 weeks of age and in female broilers at 8 weeks of age, total serum protein in female broilers at 3 weeks of age and total available serum sugars in male and female broilers at 5 weeks of age and in female broilers at weeks of age; could also suggest a limitation to the extent to which ascorbic acid supplementation (300mg / kg diet) is beneficial with low light exposure of broilers.

3.2.7 Effect(s) of number of entries into the pens:

The results show that haematocrit (PCV) levels in the broilers were not significantly altered ($P > 0.01$) by high number of entries into the pens (Table 6). Similarly, the levels of total serum cholesterol in male broilers at 5 weeks of age, total serum protein in male and female broilers up to 5 weeks of age and total serum creatinine in male and female broilers at 5 weeks of age were not significantly affected ($P > 0.01$). These levels which were non-responsive to the effect of high number of entries into the pens (4 per day) might have been essential to the maintenance of homeostasis required for normal biochemical function (Isaacs, 1971). This situation may be limited to those broilers in which the levels had remained non-responsive. Besides this maintenance of level constancy (Table 6), the effect of high number of entries into the pens is also shown in two other ways. There could be a significant increase ($P < 0.01$) of levels of the biochemical parameters in the broilers (e.g. total serum cholesterol in male and female broilers at 3 weeks of age and in female broilers at 5 and 8 weeks of age, total serum creatinine in male and female broilers at 3 weeks of age and of total available serum sugars in male and female broilers at 8 weeks of age, respectively). There could, also, be a significant decrease ($P < 0.01$) of levels of the biochemical parameters in the broilers (e.g. total serum cholesterol in male broilers at 8 weeks of age, total serum protein in male and female broilers at 8 weeks of age, total serum creatinine in male and female broilers at 8 weeks of age and total available serum sugars in male and female broilers at 3 and 5 weeks of age, respectively). These significant change ($P < 0.01$) of levels and maintenance of homeostatic levels ($P > 0.01$) of the biochemical parameters in the broilers may serve as indices of the effects due to high number of entries into the pens.

3.2.8 Effect(s) of high number of entries into the pens with ascorbic acid supplementation:

The results show that haematocrit (PCV) and total serum protein levels in the broilers of different ages (3, 5 and 8 weeks) remained significantly unchanged ($P > 0.01$) by high number of entries into the pens with ascorbic acid supplementation (Table 7). The levels of total serum cholesterol in female broilers at 3 weeks of age and of total serum creatinine in male and female broilers at 5 and 8 weeks of age were, also, not significantly changed ($P > 0.01$). These unchanged ($P > 0.01$) levels of some of the biochemical parameters might be attributed to the protective roles of ascorbic acid supplementation; since high number of entries into the pens elicited some changes in these parameters (Table 6). However, there was lack of consistency in the levels of some of the biochemical parameters (e.g. total serum cholesterol and total available serum sugars). This observation could be said to agree with literature report of the behavior of some biological materials which might not always be consistent (Cafantaris and Seed, 1992). Such behavior of some biological materials may be due to biochemical individuality, genetic and environmental factors.

3.2.9 Effect(s) of low number of entry into the pens:

The results show that low number of entry into the pens did not significantly change ($P > 0.01$) the levels of the biochemical parameters from corresponding control levels (Table 8). However, some significant changes ($P < 0.01$) occurred in the trend between base line levels of the biochemical parameters in young broilers at 3 weeks of age and in the more matured ones at 5 and 8 weeks of age. These observed homeostatic levels ($P > 0.01$) could suggest that the broilers generally coped with the effects of low number of entry into the pens. Such a capacity to cope with stress might have been relatively enhanced in broilers as shown by some of the significant changes ($P < 0.01$) in the trend of levels (Table 8). These effects of low number of entry into the pens in the broilers seemed to be very similar to those of low light exposure (Table 4).

3.2.10 Effect(s) of low number of entries into the pens with ascorbic acid supplementation:

The results show that only haematocrit (PCV) levels in the broilers of different ages (3, 5 and 8 weeks) were significantly non-responsive ($P > 0.01$) to low number of entries into the pens with ascorbic acid supplementation (Table 9). The levels of the other biochemical parameters show varying degrees of response. The maintenance of homeostatic levels ($P > 0.01$) which might be a coping strategy against this production stress occurred in some cases. These included total cholesterol levels in male broilers at 5 weeks of age and in female broilers at 8 weeks of age, total serum protein levels in male and female broilers at 3 weeks of age and in male broilers at 8 weeks of age, total serum creatinine levels in male broilers at 3 weeks of age and in male and female broilers at 5 weeks of age and total available serum sugar level in female broilers at 5 weeks of age, respectively. Ascorbic acid supplementation (300mg / kg diet) might have had protective roles of the levels. By possibly drawing on other mechanisms of action, ascorbic acid supplementation of broilers exposed to low number of entry into the pens could trigger significant increase or decrease ($P < 0.01$) in the levels of some of the biochemical parameters. The levels of total cholesterol in male and female broilers at 3 weeks of age, in female broilers at 5 weeks of age and in male broilers at 8 weeks of age are good examples of such a significant increase ($P < 0.01$). The levels of total serum protein in male and female broilers at 5 weeks of age and in female broilers at 8 weeks of age are, also, good examples of such a significant decrease ($P < 0.01$). While, these change ($P < 0.01$) of levels of the biochemical parameters may be indices of the adverse effects of low number of entry into the pens with ascorbic acid supplementation; again, not all change of levels of the biochemical parameters as a result of ascorbic acid supplementation (300mg / kg diet) might have been beneficial in the broilers.

3.2.11 Effect(s) of transportation:

The results show that the levels of haematocrit (PCV), total serum protein and total serum creatinine were not significantly changed ($P > 0.01$) from their corresponding control levels by transportation through a distance of 30km / day (Table 10). Total cholesterol and sugar levels are the only sensitive indices of stress due to transportation of the broilers. These adverse effects occurred as a significant increase ($P < 0.01$) of the levels above their corresponding control levels in male and female broilers at 8 weeks of age.

Consideration of the overall effects of transportation (30km / day) on the levels of the biochemical parameters show varying effects, some of which seemed to agree with a literature report of lack of consistent stress response in transported birds (Halliday et. al., 1977); particularly where distances of less than 224km / day were involved (Freeman, 1984).

3.2.12 Effect(s) of transportation with ascorbic acid supplementation:

The results show that the levels of haematocrit (PCV) and total protein in male and female broilers at 8 weeks of age and sugars in female broilers at 8 weeks of age were not significantly changed ($P > 0.01$) from their corresponding control levels by transportation with ascorbic acid supplementation (Table 11). These maintenance of homeostatic levels could have been due to the protective roles of ascorbic acid supplementation (300mg / kg diet) in ameliorating the adverse effects of transportation stress on the biochemical parameters.

These seemingly protective roles might have occurred further as a significant decrease ($P < 0.01$) of the levels of total cholesterol and creatinine in both male broilers and female ones at 8 weeks of age and of total sugars in male broilers at 8 weeks of age. For example, the decrease ($P < 0.01$) of levels observed of total creatinine might have implied that, with ascorbic acid supplementation, transportation stress could not cause much break-down of skeletal muscle mass in the broilers (see ARC/MRC Committee, 1974). Such break-down of total serum

creatinine might have occurred as an index of transportation stress sufficient even to account for the homeostatic levels that had been observed earlier (Table 10), when the broilers were transported without ascorbic acid supplementation.

CHAPTER FOUR

4.0 CONCLUSION AND SUGGESTIONS

4.1 CONCLUSION

The effects of age, sex, production stresses and ascorbic acid supplementation on some biochemical parameters in broilers of different ages have been evaluated. It is observed that age as an independent variable did not have any significant effect ($P > 0.01$) on haematocrit (PCV) levels. But levels of total cholesterol, protein, creatinine and sugars showed some significant changes ($P < 0.01$) in the broilers. In some cases, the changes are sex dependent. Male and female broilers at 3, 5 and 8 weeks of age are relatively prone to the effects of the production stresses with or without ascorbic acid supplementation. It is concluded that, to some extent, ascorbic acid supplementation (300mg / kg diet) is ameliorative of the adverse effects of the production stresses in broiler.

4.2 SUGGESTIONS

As the levels of the some biochemical parameters in male and female broilers of different ages (3, 5 and 8 weeks) could be relatively susceptible as indices of production stresses (high / low light exposure, high / low number of entries into the pens and transportation), it is suggested that improved poultry feed regimes with different levels of ascorbic acid supplementation (e.g. 100, 200, 250, 300, 350, 400, 450 and 500mg / Kg diet, etc) should be investigated further in order to ascertain the level(s) which best ameliorate(s) the adverse effects of production stresses in the broilers.

As exposure of broilers to light that is very much outside the range of normal light exposure (i.e. 100W incandescent pin bulb per $3.4m^2$ of floor space) could be a production stress factor, lighting should be sufficient only for the broilers to be able to see their feeds clearly, eat and keep warm without stress. In the alternative, intermittent lighting should be considered as

a standard programme as had been reported elsewhere (World Poultry, 1998).

As entries into the pens that is very much outside the range of normal entry (2 per day) could be another production stress factor, only an acceptable number of entries into the pens for essential purposes of either cleaning, giving of water, vaccines or feeds should be permitted. This might be achieved through improved and modern poultry husbandry techniques.

Transportation over long distance (≥ 30km) could, also, be a stressor in broilers. This should be discouraged by allowing for a shorter distance to be covered at a time. Unfriendly carriage of broilers should be avoided.

This research has brought into sharp focus the need "to promote, among others, the advancement of knowledge of all aspects of poultry science and the poultry industry" (W.P.S.A.C., 1984). It is, hereby, suggested that further studies on these and other biochemical parameters (such as lipoprotein, hormones, catecholamine, enzymes and other micro- nutrient elements) should be undertaken in broilers exposed to production stresses in order to open up new frontiers for updating the present state of knowledge.

Finally, effective public enlightenment is advocated in order to minimize the adverse effects of production stresses in broilers so that their production performance can be enhanced.

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APPENDIX I

THE SPSS/PC+ COMMANDS USED TO PRODUCE THE OUTPUT IN THIS RESEARCH

```
data list matrix free / haemato trt.
var labels haemato "Haematocrit of male & female broilers at 3 wks"
/trt "Treatment & Light exposure".
Value Labels trt 1"ControM" 2"ControF" 3"LightM" 4"LightF".
begin data.
50.     50.     50.     50.
31.38  34.00  34.00  30.25
 1.38   3.14   3.62   2.62
end data.

Oneway haemato by trt (1,4)
      / ranges = Scheffe'(0.01)
 *    / options = 6,7
** / statistics = 1,3.
```

Legend:

* Formatting Options

Option 6: *Display value labels for groups*: Use the first eight characters of the value labels defined for the independent variable as group labels.

Option 7: *Read matrix of counts, means and standard deviations.* ONEWAY expects a vector of counts for each group, followed by a vector of group means and a vector of group standanrd deviations.

** Optional Statistics

Statistic 1: *Group descriptive statistics*: For each group, the count, mean, standard deviation, standard error, minimum, maximum, and 95% confidence interval are displayed for each dependent variable.

Statistic 3: *Homogeneity-of-variance-tests*: Cochran's C, the Bartlett-Box *F*, and Hartley's *F* Max are displayed (Norusis, 1988).

(Similar programme was used to analyze the other biochemical parameters).

APPENDIX II

ANOVA TABLE

Critical values of *F*

The obtained F is significant at a given level if it is equal to or greater than the value shown in the table.
0.05 (light row) and 0.01 (dark row) points for the distribution of F

Degrees of freedom for lesser mean square	Degrees of freedom for greater mean square																							
	1	2	3	4	5	6	7	8	9	10	11	12	14	16	20	24	30	40	50	75	100	200	500	∞
1	161 4052	200 4999	216 5403	225 5625	230 5764	234 5859	237 5928	239 5981	241 6022	242 6056	243 6082	244 6106	245 6142	246 6169	248 6208	249 6234	250 6258	251 6286	252 6302	253 6323	253 6334	254 6352	254 6361	254 6366
2	18.51 98.49	19.00 99.01	19.16 99.17	19.25 99.25	19.30 99.30	19.33 99.33	19.36 99.34	19.37 99.36	19.38 99.38	19.39 99.40	19.40 99.41	19.41 99.42	19.42 99.43	19.43 99.44	19.44 99.45	19.45 99.46	19.46 99.47	19.47 99.48	19.47 99.48	19.48 99.49	19.49 99.49	19.49 99.49	19.50 99.50	19.50 99.50
3	10.13 34.12	9.55 30.81	9.28 29.46	9.12 28.71	9.01 28.24	8.94 27.91	8.88 27.67	8.84 27.49	8.81 27.34	8.78 27.23	8.76 27.13	8.74 27.05	8.71 26.92	8.69 26.83	8.66 26.69	8.64 26.60	8.62 26.50	8.60 26.41	8.58 26.30	8.57 26.27	8.56 26.23	8.54 26.18	8.54 26.14	8.53 26.12
4	7.71 21.20	6.94 18.00	6.59 16.69	6.39 15.98	6.26 15.52	6.16 15.21	6.09 14.98	6.04 14.80	6.00 14.66	5.96 14.54	5.93 14.45	5.91 14.37	5.87 14.24	5.84 14.15	5.80 14.02	5.77 13.93	5.74 13.83	5.71 13.74	5.70 13.69	5.68 13.61	5.66 13.57	5.65 13.52	5.64 13.48	5.63 13.46
5	6.61 16.26	5.79 13.27	5.41 12.06	5.19 11.39	5.05 10.97	4.95 10.67	4.88 10.45	4.82 10.27	4.78 10.15	4.74 10.05	4.70 9.96	4.68 9.89	4.64 9.77	4.60 9.68	4.56 9.55	4.53 9.47	4.50 9.38	4.46 9.29	4.44 9.24	4.42 9.17	4.40 9.13	4.38 9.07	4.37 9.04	4.36 9.02
6	5.99 13.74	5.14 10.92	4.76 9.78	4.53 9.15	4.39 8.75	4.28 8.47	4.21 8.26	4.15 8.10	4.10 7.98	4.06 7.87	4.03 7.79	4.00 7.72	3.96 7.60	3.92 7.52	3.87 7.39	3.84 7.31	3.81 7.23	3.77 7.14	3.75 7.09	3.72 7.02	3.71 6.99	3.69 6.94	3.68 6.90	3.67 6.88
7	5.59 12.25	4.74 9.55	4.35 8.45	4.12 7.85	3.97 7.46	3.87 7.19	3.79 7.00	3.73 6.84	3.68 6.71	3.63 6.62	3.60 6.54	3.57 6.47	3.52 6.35	3.49 6.27	3.44 6.15	3.41 6.07	3.38 5.98	3.34 5.90	3.32 5.85	3.29 5.78	3.28 5.75	3.25 5.70	3.24 5.67	3.23 5.65
8	5.32 11.26	4.46 8.65	4.07 7.59	3.84 7.01	3.69 6.63	3.58 6.37	3.50 6.19	3.44 6.03	3.39 5.91	3.34 5.82	3.31 5.74	3.28 5.67	3.23 5.56	3.20 5.48	3.15 5.36	3.12 5.28	3.08 5.20	3.05 5.11	3.03 5.06	3.00 5.00	2.98 4.96	2.96 4.91	2.94 4.88	2.93 4.86
9	5.12 10.56	4.26 8.02	3.86 6.99	3.63 6.42	3.48 6.06	3.37 5.80	3.29 5.62	3.23 5.47	3.18 5.35	3.13 5.26	3.10 5.18	3.07 5.11	3.02 5.00	2.98 4.92	2.93 4.80	2.90 4.73	2.86 4.64	2.82 4.56	2.80 4.51	2.77 4.45	2.76 4.41	2.73 4.36	2.72 4.33	2.71 4.31
10	4.96 10.04	4.10 7.56	3.71 6.55	3.48 5.99	3.33 5.64	3.22 5.39	3.14 5.21	3.07 5.06	3.02 4.95	2.97 4.85	2.94 4.78	2.91 4.71	2.86 4.60	2.82 4.52	2.77 4.41	2.74 4.33	2.70 4.25	2.67 4.17	2.64 4.12	2.61 4.05	2.59 4.01	2.56 3.96	2.55 3.93	2.54 3.91
11	4.84 9.65	3.98 7.20	3.59 6.22	3.36 5.67	3.20 5.32	3.09 5.07	3.01 4.88	2.95 4.74	2.90 4.63	2.86 4.54	2.82 4.46	2.79 4.40	2.74 4.29	2.70 4.21	2.65 4.10	2.61 4.02	2.57 3.94	2.53 3.86	2.50 3.80	2.47 3.74	2.45 3.70	2.42 3.66	2.41 3.62	2.40 3.60
12	4.75 9.33	3.88 6.93	3.49 5.95	3.26 5.41	3.11 5.06	3.00 4.82	2.92 4.65	2.85 4.50	2.80 4.39	2.76 4.30	2.72 4.22	2.69 4.16	2.64 4.05	2.60 3.98	2.54 3.86	2.50 3.78	2.46 3.70	2.42 3.61	2.40 3.56	2.36 3.49	2.35 3.46	2.32 3.41	2.31 3.38	2.30 3.36
13	4.67 9.07	3.80 6.70	3.41 5.74	3.18 5.20	3.02 4.86	2.92 4.62	2.84 4.44	2.77 4.30	2.72 4.19	2.67 4.10	2.63 4.02	2.60 3.96	2.55 3.85	2.51 3.78	2.46 3.67	2.42 3.59	2.38 3.51	2.34 3.42	2.32 3.37	2.28 3.30	2.26 3.27	2.24 3.21	2.22 3.18	2.21 3.16
14	4.60 8.86	3.74 6.51	3.34 5.56	3.11 5.03	2.96 4.69	2.85 4.46	2.77 4.28	2.70 4.14	2.65 4.03	2.60 3.94	2.56 3.86	2.53 3.80	2.48 3.70	2.44 3.62	2.39 3.51	2.35 3.43	2.31 3.34	2.27 3.26	2.24 3.21	2.21 3.14	2.19 3.11	2.16 3.06	2.14 3.02	2.13 3.00
15	4.54 8.68	3.68 6.36	3.29 5.42	3.06 4.89	2.90 4.56	2.79 4.32	2.70 4.14	2.64 4.00	2.59 3.89	2.55 3.80	2.51 3.73	2.48 3.67	2.43 3.56	2.39 3.48	2.33 3.36	2.29 3.29	2.25 3.20	2.21 3.12	2.18 3.07	2.15 3.00	2.12 2.97	2.10 2.92	2.08 2.89	2.07 2.87

(Continued)

Degrees of freedom for lesser mean square																								
16	4.49	3.63	3.24	3.01	2.85	2.74	2.66	2.59	2.54	2.49	2.45	2.42	2.37	2.33	2.28	2.24	2.20	2.16	2.13	2.09	2.07	2.04	2.02	2.01
	8.53	6.23	5.29	4.77	4.44	4.20	4.03	3.89	3.78	3.69	3.61	3.55	3.45	3.37	3.25	3.18	3.10	3.01	2.96	2.89	2.86	2.80	2.77	2.75
17	4.45	3.59	3.20	2.96	2.81	2.70	2.62	2.55	2.50	2.45	2.41	2.38	2.33	2.29	2.23	2.19	2.15	2.11	2.08	2.04	2.02	1.99	1.97	1.96
	8.40	6.11	5.18	4.67	4.34	4.10	3.93	3.79	3.68	3.59	3.52	3.45	3.35	3.27	3.16	3.08	3.00	2.92	2.86	2.79	2.76	2.70	2.67	2.65
18	4.41	3.55	3.16	2.93	2.77	2.66	2.58	2.51	2.46	2.41	2.37	2.34	2.29	2.25	2.19	2.15	2.11	2.07	2.04	2.00	1.98	1.95	1.93	1.92
	8.28	6.01	5.09	4.58	4.25	4.01	3.85	3.71	3.60	3.51	3.44	3.37	3.27	3.19	3.07	3.00	2.91	2.83	2.78	2.71	2.68	2.62	2.59	2.57
19	4.38	3.52	3.13	2.90	2.74	2.63	2.55	2.48	2.43	2.38	2.34	2.31	2.26	2.21	2.15	2.11	2.07	2.02	2.00	1.96	1.94	1.91	1.90	1.88
	8.18	5.93	5.01	4.50	4.17	3.94	3.77	3.63	3.52	3.43	3.36	3.30	3.19	3.12	3.00	2.92	2.84	2.76	2.70	2.63	2.60	2.54	2.51	2.49
20	4.35	3.49	3.10	2.87	2.71	2.60	2.52	2.45	2.40	2.35	2.31	2.28	2.23	2.18	2.12	2.08	2.04	1.99	1.96	1.92	1.90	1.87	1.85	1.84
	8.10	5.85	4.94	4.43	4.10	3.87	3.71	3.56	3.45	3.37	3.30	3.23	3.13	3.05	2.94	2.86	2.77	2.69	2.63	2.56	2.53	2.47	2.44	2.42
21	4.32	3.47	3.07	2.84	2.68	2.57	2.49	2.42	2.37	2.32	2.28	2.25	2.20	2.15	2.09	2.05	2.00	1.96	1.93	1.80	1.87	1.84	1.82	1.81
	8.02	5.78	4.87	4.37	4.04	3.81	3.65	3.51	3.40	3.31	3.24	3.17	3.07	2.99	2.88	2.80	2.72	2.63	2.58	2.51	2.47	2.42	2.38	2.36
22	4.30	3.44	3.05	2.82	2.66	2.55	2.47	2.40	2.35	2.30	2.26	2.23	2.18	2.13	2.07	2.03	1.98	1.93	1.91	1.87	1.84	1.81	1.80	1.78
	7.94	5.72	4.82	4.31	3.99	3.76	3.59	3.45	3.35	3.26	3.18	3.12	3.02	2.94	2.83	2.75	2.67	2.58	2.53	2.46	2.42	2.37	2.33	2.31
23	4.28	3.42	3.03	2.80	2.64	2.53	2.45	2.38	2.32	2.28	2.24	2.20	2.14	2.10	2.04	2.00	1.96	1.91	1.88	1.84	1.82	1.79	1.77	1.76
	7.88	5.66	4.76	4.26	3.94	3.71	3.54	3.41	3.30	3.21	3.14	3.07	2.97	2.89	2.78	2.70	2.62	2.53	2.48	2.41	2.37	2.32	2.28	2.26
24	4.26	3.40	3.01	2.78	2.62	2.51	2.43	2.36	2.30	2.26	2.22	2.18	2.13	2.09	2.02	1.98	1.94	1.89	1.86	1.82	1.80	1.76	1.74	1.73
	7.82	5.61	4.72	4.22	3.90	3.67	3.50	3.36	3.25	3.17	3.09	3.03	2.93	2.85	2.74	2.66	2.58	2.49	2.44	2.36	2.33	2.27	2.23	2.21
25	4.24	3.38	2.99	2.76	2.60	2.49	2.41	2.34	2.28	2.24	2.20	2.16	2.11	2.06	2.00	1.96	1.92	1.87	1.84	1.80	1.77	1.74	1.72	1.71
	7.77	5.57	4.68	4.18	3.86	3.63	3.46	3.32	3.21	3.13	3.05	2.99	2.89	2.81	2.70	2.62	2.54	2.45	2.40	2.32	2.29	2.23	2.19	2.17
26	4.22	3.37	2.89	2.74	2.59	2.47	2.39	2.32	2.27	2.22	2.18	2.15	2.10	2.05	1.99	1.95	1.90	1.85	1.82	1.78	1.76	1.72	1.70	1.69
	7.72	5.53	4.64	4.14	3.82	3.59	3.42	3.29	3.17	3.09	3.02	2.96	2.86	2.77	2.66	2.58	2.50	2.41	2.36	2.28	2.25	2.19	2.15	2.13
27	4.21	3.35	2.96	2.73	2.57	2.46	2.37	2.30	2.25	2.20	2.16	2.13	2.08	2.03	1.97	1.93	1.88	1.84	1.80	1.76	1.74	1.71	1.68	1.67
	7.68	5.49	4.60	4.11	3.79	3.56	3.39	3.26	3.14	3.06	2.98	2.93	2.83	2.74	2.63	2.55	2.47	2.38	2.33	2.25	2.21	2.16	2.12	2.10
28	4.20	3.34	2.95	2.71	2.56	2.44	2.36	2.29	2.24	2.19	2.15	2.12	2.06	2.02	1.96	1.91	1.87	1.81	1.78	1.75	1.72	1.69	1.67	1.65
	7.64	5.45	4.57	4.07	3.76	3.53	3.36	3.23	3.11	3.03	2.95	2.90	2.80	2.71	2.60	2.52	2.44	2.35	2.30	2.22	2.18	2.13	2.09	2.06
29	4.18	3.33	2.93	2.70	2.54	2.43	2.35	2.28	2.22	2.18	2.14	2.10	2.05	2.00	1.94	1.90	1.85	1.80	1.77	1.73	1.71	1.68	1.65	1.64
	7.60	5.52	4.54	4.04	3.73	3.50	3.32	3.20	3.08	3.00	2.92	2.87	2.77	2.68	2.57	2.49	2.41	2.32	2.27	2.19	2.15	2.10	2.06	2.03
30	4.17	3.32	2.92	2.69	2.53	2.42	2.34	2.27	2.21	2.16	2.12	2.09	2.04	1.99	1.93	1.89	1.84	1.79	1.76	1.72	1.69	1.66	1.64	1.62
	7.56	5.39	4.51	4.02	3.70	3.47	3.30	3.17	3.06	2.98	2.90	2.84	2.74	2.66	2.55	2.47	2.38	2.29	2.24	2.16	2.13	2.07	2.03	2.01

(Continued)

0.05 (light row) and 0.01 (dark row) points for the distribution of F

Degrees of freedom for lesser mean square	Degrees of freedom for greater mean square																							
	1	2	3	4	5	6	7	8	9	10	11	12	14	16	20	24	30	40	50	75	100	200	500	∞
32	4.15	3.30	2.90	2.67	2.51	2.40	2.32	2.25	2.19	2.14	2.10	2.07	2.02	1.97	1.91	1.86	1.82	1.76	1.74	1.69	1.67	1.64	1.61	1.59
	7.50	5.34	4.46	3.97	3.66	3.42	3.25	3.12	3.01	2.94	2.86	2.80	2.70	2.62	2.51	2.42	2.34	2.25	2.20	2.12	2.08	2.02	1.98	1.96
34	4.13	3.28	2.88	2.65	2.49	2.38	2.30	2.23	2.17	2.12	2.08	2.05	2.00	1.95	1.89	1.84	1.80	1.74	1.71	1.67	1.64	1.61	1.59	1.57
	7.44	5.29	4.42	3.93	3.61	3.38	3.21	3.08	2.97	2.89	2.82	2.76	2.66	2.58	2.47	2.38	2.30	2.21	2.15	2.08	2.04	1.98	1.94	1.91
36	4.11	3.26	2.86	2.63	2.48	2.36	2.28	2.21	2.15	2.10	2.06	2.03	1.89	1.93	1.87	1.82	1.78	1.72	1.69	1.65	1.62	1.59	1.56	1.55
	7.39	5.25	4.38	3.89	3.58	3.35	3.18	3.04	2.94	2.86	2.78	2.72	2.62	2.54	2.43	2.35	2.26	2.17	2.12	2.04	2.00	1.94	1.90	1.87
38	4.10	3.25	2.85	2.62	2.46	2.35	2.26	2.19	2.14	2.09	2.05	2.02	1.96	1.92	1.85	1.80	1.76	1.71	1.67	1.63	1.60	1.57	1.54	1.53
	7.35	5.21	4.34	3.86	3.54	3.32	3.15	3.02	2.91	2.82	2.75	2.69	2.59	2.51	2.40	2.32	2.22	2.14	2.08	2.00	1.97	1.90	1.86	1.84
40	4.08	3.23	2.84	2.61	2.45	2.34	2.25	2.18	2.12	2.07	2.04	2.00	1.95	1.90	1.84	1.79	1.74	1.69	1.66	1.61	1.59	1.55	1.53	1.51
	7.31	5.18	4.31	3.83	3.51	3.29	3.12	2.99	2.88	2.80	2.73	2.66	2.56	2.49	2.37	2.29	2.20	2.11	2.05	1.97	1.94	1.88	1.84	1.81
42	4.07	3.22	2.83	2.59	2.44	2.32	2.24	2.17	2.11	2.06	2.02	1.90	1.94	1.89	1.82	1.78	1.73	1.68	1.64	1.60	1.57	1.54	1.51	1.49
	7.27	5.15	4.29	3.80	3.49	3.26	3.10	2.96	2.86	2.77	2.70	2.64	2.54	2.46	2.35	2.26	2.17	2.08	2.02	1.94	1.91	1.85	1.80	1.78
44	4.06	3.21	2.82	2.58	2.43	2.31	2.23	2.16	2.10	2.05	2.01	1.98	1.92	1.88	1.81	1.76	1.72	1.66	1.63	1.58	1.56	1.52	1.50	1.48
	7.24	5.12	4.26	3.78	3.46	3.24	3.07	2.94	2.84	2.75	2.68	2.62	2.52	2.44	2.32	2.24	2.15	2.06	2.09	1.92	1.88	1.82	1.78	1.75
46	4.05	3.20	2.81	2.57	2.42	2.30	2.22	2.14	2.09	2.04	2.00	1.97	1.91	1.87	1.80	1.75	1.71	1.65	1.62	1.57	1.54	1.51	1.48	1.46
	7.21	5.10	4.24	3.76	3.44	3.22	3.05	2.92	2.82	2.73	2.66	2.60	2.50	2.42	2.30	2.22	2.13	2.04	1.98	1.90	1.86	1.80	1.76	1.72
48	4.04	3.19	2.80	2.56	2.41	2.30	2.21	2.14	2.08	2.03	1.99	1.96	1.90	1.86	1.79	1.74	1.70	1.64	1.61	1.56	1.53	1.50	1.47	1.45
	7.19	5.08	4.22	3.74	3.42	3.20	3.04	2.90	2.80	2.71	2.64	2.58	2.48	2.40	2.28	2.20	2.11	2.02	1.96	1.88	1.84	1.78	1.73	1.70
50	4.03	3.18	2.79	2.56	2.40	2.29	2.20	2.13	2.07	2.02	1.98	1.95	1.90	1.85	1.78	1.74	1.69	1.63	1.60	1.55	1.52	1.48	1.46	1.44
	7.17	5.06	4.20	3.72	3.41	3.18	3.02	2.88	2.78	2.70	2.62	2.56	2.46	2.39	2.26	2.18	2.10	2.00	1.94	1.86	1.82	1.76	1.71	1.68
55	4.02	3.17	2.78	2.54	2.38	2.27	2.18	2.11	2.05	2.00	1.97	1.93	1.88	1.83	1.76	1.72	1.67	1.61	1.58	1.52	1.50	1.46	1.43	1.41
	7.12	5.01	4.16	3.68	3.37	3.15	2.98	2.85	2.75	2.66	2.59	2.53	2.43	2.35	2.23	2.15	2.06	1.96	1.90	1.82	1.78	1.71	1.66	1.64
60	4.00	3.15	2.76	2.52	2.37	2.25	2.17	2.10	2.04	1.99	1.95	1.92	1.86	1.81	1.75	1.70	1.65	1.59	1.56	1.50	1.48	1.44	1.41	1.39
	7.08	4.98	4.13	3.65	3.34	3.12	2.95	2.82	2.72	2.63	2.56	2.50	2.40	2.32	2.20	2.12	2.03	1.93	1.87	1.79	1.74	1.68	1.63	1.60
65	3.99	3.14	2.75	2.51	2.36	2.24	2.15	2.08	2.02	1.98	1.94	1.90	1.85	1.80	1.73	1.68	1.63	1.57	1.54	1.49	1.46	1.42	1.39	1.37
	7.04	4.95	4.10	3.62	3.31	3.09	2.93	2.79	2.70	2.61	2.54	2.47	2.37	2.30	2.18	2.09	2.00	1.90	1.84	1.76	1.71	1.64	1.60	1.56
70	3.98	3.13	2.74	2.50	2.35	2.32	2.14	2.07	2.01	1.97	1.93	1.89	1.84	1.79	1.72	1.67	1.62	1.56	1.53	1.47	1.45	1.40	1.37	1.35
	7.01	4.92	4.08	3.60	3.29	3.07	2.91	2.77	2.67	2.59	2.51	2.45	2.35	2.28	2.15	2.07	1.98	1.88	1.82	1.74	1.69	1.62	1.56	1.53
80	3.96	3.11	2.72	2.48	2.33	2.21	2.12	2.05	1.99	1.95	1.91	1.88	1.82	1.77	1.70	1.65	1.60	1.54	1.51	1.45	1.42	1.38	1.35	1.32
	6.96	4.88	4.04	3.56	3.25	3.04	2.87	2.74	2.64	2.55	2.48	2.41	2.32	2.24	2.11	2.03	1.94	1.84	1.78	1.70	1.65	1.57	1.52	1.49

(Continued)

Degrees of freedom for lesser mean square																								
100	3.94 6.90	3.09 4.82	2.70 3.98	2.46 3.51	2.30 3.20	2.19 2.99	2.10 2.82	2.03 2.69	1.97 2.59	1.92 2.51	1.88 2.43	1.85 2.36	1.79 2.26	1.75 2.19	1.68 2.06	1.63 1.98	1.57 1.89	1.51 1.79	1.48 1.73	1.42 1.64	1.39 1.59	1.34 1.51	1.30 1.46	1.28 1.43
125	3.92 6.84	3.07 4.78	2.68 3.94	2.44 3.47	2.29 3.17	2.17 2.95	2.08 2.79	2.01 2.65	1.95 2.56	1.90 2.47	1.84 2.40	1.83 2.33	1.77 2.23	1.72 2.15	1.65 2.03	1.60 1.94	1.55 1.85	1.49 1.75	1.45 1.68	1.39 1.59	1.36 1.54	1.31 1.46	1.27 1.40	1.25 1.37
150	3.91 6.81	3.06 4.75	2.67 3.91	2.43 3.44	2.27 3.13	2.16 2.92	2.07 2.76	2.00 2.62	1.94 2.53	1.89 2.44	1.85 2.37	1.82 2.30	1.76 2.20	1.71 2.12	1.64 2.00	1.59 1.91	1.54 1.83	1.47 1.72	1.44 1.66	1.37 1.56	1.34 1.51	1.29 1.43	1.25 1.37	1.22 1.33
200	3.89 6.76	3.04 4.71	2.65 3.88	2.41 3.41	2.26 3.11	2.14 2.90	2.05 2.73	1.98 2.60	1.92 2.50	1.87 2.41	1.83 2.34	1.80 2.28	1.74 1.17	1.69 2.09	1.62 1.97	1.57 1.88	1.52 1.79	1.45 1.69	1.42 1.62	1.35 1.52	1.32 1.48	1.26 1.39	1.22 1.33	1.19 1.28
400	3.86 6.70	3.02 4.66	2.62 3.83	2.39 3.36	2.22 3.06	2.12 2.85	2.03 2.69	1.96 2.55	1.90 2.46	1.85 2.37	1.81 2.29	1.78 2.23	1.72 2.12	1.67 2.04	1.60 1.92	1.54 1.84	1.49 1.74	1.42 1.64	1.38 1.57	1.32 1.47	1.28 1.42	1.22 1.32	1.16 1.24	1.13 1.19
1000	3.85 6.66	3.00 4.62	2.61 3.80	2.38 3.34	2.22 3.04	2.10 2.82	2.02 2.66	1.95 2.53	1.89 2.43	1.84 2.34	1.80 2.26	1.76 2.20	1.70 2.09	1.65 2.01	1.58 1.89	1.53 1.81	1.47 1.71	1.41 1.61	1.36 1.54	1.30 1.44	1.26 1.38	1.19 1.28	1.13 1.19	1.08 1.11
∞	3.84 6.64	2.99 4.60	2.60 3.78	2.37 3.32	2.21 3.02	2.09 2.80	2.01 2.64	1.94 2.51	1.88 2.41	1.83 2.32	1.79 2.24	1.75 2.18	1.69 2.07	1.64 1.99	1.57 1.87	1.52 1.79	1.46 1.69	1.40 1.59	1.35 1.52	1.28 1.41	1.24 1.36	1.17 1.25	1.11 1.15	1.00 1.00

Source: HABER, A. and RUNYON, R.P. (1980).