EFFECTS OF PROPRIETARY VITAMIN-MINERAL PREMIXES AND HOUSING SYSTEMS ON LAYING CHICKENS EGG PRODUCTION AND QUALITY INDICES

Akinola Yinka Paul OJELADE

Matriculation Number: 42346

`

EFFECTS OF PROPRIETARY VITAMIN-MINERAL PREMIXES AND HOUSING SYSTEMS ON LAYING CHICKENS EGG PRODUCTION AND QUALITY INDICES

By

Akinola Yinka Paul **OJELADE**
B.Sc. (Hons.) Agriculture (Animal Science)
M.Sc. Agricultural Biochemistry and Nutrition (Ibadan)
PGDE Curriculum Studies (Lagos)
Matriculation Number: 42346

Submitted to the Department of Animal Science,
Faculty of Agriculture and Forestry,
University of Ibadan, Ibadan

In partial fulfillment of the award of
Doctor of Philosophy
in
Agricultural Biochemistry and Nutrition

November, 2016

CERTIFICATION

We certify that this project was carried out by **OJELADE** Akinola Yinka Paul with Matriculation Number 42346 in the Department of Animal Science, University of Ibadan, Ibadan, Nigeria, under our supervision

……
Supervisor
Professor A. O. Akinsoyinu
B.Sc. (Hons.), Ph.D. (Ibadan), RAS, FNSAP
Agricultural Biochemistry and Nutrition Unit
Department of Animal Science
University of Ibadan, Ibadan, Nigeria

……
Date

……
Supervisor
Dr. O. A. Ogunwole
B.Sc. **(**Hons.), M. Sc., Ph.D., RAS, FCASN
Senior Lecturer,
Vitamin-Mineral & Amino Acid Metabolism
Agricultural Biochemistry and Nutrition Unit
Department of Animal Science
University of Ibadan, Ibadan, Nigeria

……
Date

DEDICATION

This thesis is dedicated to the Glory of God, The Father, The Son and The Holy Spirit

ACKNOWLEDGEMENT

To God be the glory for great things He has done. All thanks and adoration is to Almighty God for His grace and mercies that saw me through this PhD Programme. I praise God for the wisdom, knowledge and protection in the course of the programme. I am most grateful to A. O. Akinsoyinu; an erudite Professor of Agricultural Biochemistry and Nutrition, former Head of Department of Animal Science and Dean, Faculty of Agriculture and Forestry, University of Ibadan and currently, Dean, School of Agriculture and Industrial Technology, Babcock University, Ilishan-Remo, Ogun State, Nigeria. Also, my sincere appreciation goes to Dr. O. A. Ogunwole for his roles at the onset, execution and eventual completion of this research. I will forever remain appreciative to him for of his enthusiastic support and encouragement. The unalloyed and consistent support of my supervisors in the course of this research impacted greatly on the results obtained. I say thank you for your support and mentorship.

I am grateful to Professor O. J. Babayemi, the Head of Department of Animal Science, Dr. E. O. Ewuola, the Sub-Dean (Postgraduate), Faculty of Agriculture and Forestry and Dr. Olufuunmilayo Adeleye, the Postgraduate Coordinator, Department of Animal Science for their understnding academic contributions to this thesis. I sincerely thank Professors O. O. Tewe, Oyebiodun Longe, A. D. Ologhobo and E. A. Iyayi for the various. I thank Drs. O. A. Abu, Adebisi Agboola, O. Odu and R. Omidiwura for their roles during the different semeniar presentations. My warm appreciation goes to Professor S. S. Abiola of the Federal University of Agriculture, Abeokuta, Ogun State, Nigeria, who was on sabbatical leave in the Department for his encouragement in the course of this research. I thank Professors A. B. Omojola, A. E. Salako, and M. K. Adewunmi, and Drs. Olubunmi Olusola, T. O. Osasanya, O. A. Adebiyi, Mabel Akinyemi, O. A. Olorunsomo, H. Osaiyuwu as well as Mr. O. Alaba for their various contributions towards successful completion of this study. I appreciate Professor A. O. K. Adesehinwa of the Institute of Agricultural Research and Training, Moor Plantation, Ibadan, Nigeria for his encouragement during the period of my study. I thank Mrs. T. T. Lawal and Messrs. S. Adelani, A. A. Fabowale and O. M. Omotoso for laboratory assistance. I shall forever remain grateful to Professor O. O. Oluwatosin of the Federal University of Agriculture,

Abeokuta, Ogun State, Nigeria, for supplying me with several published journals relating to the study. I say thank you sir.

My special thanks go to the Provost, Dr. S. O. Olusanya, Deputy Provost, Dr. K. Olojede and other members of the management of Federal College of Education (Technical), Akoka Lagos for granting me three years study leave to embark on this Ph.D programme. I thank the Federal Government of Nigeria for providing financial support through Tertiary Education Trust Fund (TETFUND). I appreciate Dr. W. A. Lamidi, Dean School of Vocational Education, Drs. E. O. Filani, A. W. Azeez, A.W. Olowa, Omowunmi Olowa, Olabisi Busari and Ruth Chigbu; Messrs. E. O. Ibiyemi, A. S. Ajibade, A. A. Falade and E. K. Ayeyemi; Mrs. Oluwatoyin Oyegunwa, Mrs. L. V. Ezechi of the Federal College of Education (Technical), Akoka Lagos. I specially thank Mr. J. A. Adedokun, for his financial and moral support during the course of the programme.

I am very grateful to the CEO of OOA Farms, Idi Osan, Balogun Village, Ibadan, Oyo State, Nigeria for providing me suitable poultry research farm site to carry out this work. I appreciate the Farm Manager and other workers for their assistance and support in the course of the research. Permit me also to appreciate the useful support and assistance of Mr. Lawrence Sule and family and Pastor Bejamin Ayodele. It is important to place on record the cooperation of the following undergraduate and post-graduate students whose involvements and participation enriched the quality of this research. First in this category are Miss Adedayo Bodunrin, Mr. I. K. Aikore, Miss Essien, Emem Aquaowo, Mr. Oyewo Muttiu, Miss Asuquo Christiana, Miss Lovette Dibia (Now Late RIP), Miss Kemi Akinleye, Miss Yemisi Oluremi, Mr.Dele Adedeji, Miss Ireti Oludoyi, Mr. Sabur Oladimeji, Mr. Ibrahim Akinfemi, Mr. Ahmed Lawal, Mrs. Folashade Jemiseye, Mrs. Aderonke Mosuro, Mrs. Titi Abokede, Miss Oluwagbemisola Mapayi and Mr. Peter Asiruwa. Also, I thank Mr. Afis and Mr. Lawrence Abegunde, both worked as research assistants in the course of the research. I appreciate the Director and entire staff of Lawrem Feed Mill where all feeds for the birds was milled

I thank Dr. S. O. Adeniyi, formerly in the Department of Educational Psychology, Federal College of Education (Technical), Akoka Lagos, and now in the Department of Educational Foundation, University of Lagos and Mr. Olabode of the Department of General Studies, Federal College of Education (Technical), Akoka Lagos for reading and making useful corrections on the first draft of this thesis.

I appreciate the moral support and encouragement of the following colleagues; Dr. I. O. Miller, Dr. S. A. Adebayo, Mr. K. T. Ijadunola, Mrs. Tolulope Aluko, Mrs. Ruth Lawal, Mr. D. Faleye and Dr. J. Owolabi of the Federal College of Education (Technical), Akoka Lagos. I acknowledge the wonderful and innumerable spiritual, financial and material support of Mr. and Mrs. Ogunmuditi and their children; Eniola and Lolade as well as Miss Rifkatu Anthony. May Almighty God continually bless your family and protect you always, Amen. Also, I appreciate the spiritual and financial assistance of Mr. and Mrs. Dotun Ologbon towards the successful completion of this study.

I am grateful to Pastor and Pastor (Mrs.) E. A. Adeboye, the General Overseer of The Redeemed Christian Church, all the Elders, Regional, Provincial, Zonal, Area and Parish Pastors as well as the entire body of Christ for their prayers for me. My thanks go to Olusegun Alawode, the Area Pastor of Sanctuary Dwellers and the entire congregation for their prayers for me. I thank the entire members of my extended family particularly, Mr. Adeshina Emmanuel Ojelade, who sponsored me right from secondary up to Master's Degree level in the university. I prayed that the Almighty God bless you, your children and grand-children. Let me express my appreciation to my niece, Miss Toyin Ojelade in Minnesota, USA for her financial support in the course of this study.

The home supports of my wife, Mrs. Temitope Yetunde Ojelade and my children: Oluwasegun Elijah Olusoji-Ojelade, Anuoluwapo Esther Olusoji-Ojelade, Oreoluwa Ruth Olusoji-Ojelade and Oluwadolapo Enoch Olusoji-Ojelade, are quite appr4eciated. I thank all for their prayers and endurance in periods I was far away from home at the University of Ibadan. May the good Lord bless all of you, amen.

AkinolaYinka Paul Ojelade

November, 2016

ABSTRACT

Housing Systems (HS), dietary vitamins and mineral supplements are obligatory components of poultry production. The composition of Proprietary Vitamin-mineral Premixes (PVmP) varies in forms and source which alongside HS could alter production and quality of eggs. There is dearth of information on effects of HS and different PVmP on production and quality indices of eggs. Therefore, effects of five PVmP and two HS on egg production and quality indices were investigated in Ibadan.

A basal diet was formulated without any PVmP (control diet, D1), while others were supplemented with 0.25% premixes K, L, M, N and P each to obtain diets D2, D3, D4, D5 and D6, respectively. In a completely randomised design, Bovan Nera pullets (n=576) aged 13 weeks were randomly allocated to two HS [Battery Cage (BC) and Deep Litter (DL)] and six treatments in a 2x6 factorial arrangement, and reared for ten months. Ambient temperature and Relative Humidity (RH) in HS were recorded. Hen Day Egg Production (HDEP) was assessed at peak and late-lay phases by standard procedure. Eggs collected at week 36 were stored for 28 days and assayed for Crude Protein (CP), Low Density Lipoprotein-cholesterol (LDLc), Lipid Oxidation (LO), Eggshell Weight (EW), Eggshell Thickness (ET) and Haugh Unit (HU) at 0, 7, 14, 21, 28 Days of Storage (DoS) under ambient conditions. Data were analysed using descriptive statistics, polynomial regression and ANOVA at $\alpha_{0.05}$.

Ambient temperature (^{o}C) and RH (%) ranged from 26.5±0.1 to 31.9±1.1and 40.6±1.0 to 90.5±8.7, respectively and were above thermoneutrality for chickens. Hens attained peak-lay at different periods during production irrespective of HS and PVmP type. The HDEP (%) in BC (64.1±26.4) and DL (82.0±13.8) at peak-lay reduced to 52.1±11.4 and 57.8±14.1, respectively in late-lay. The HDEP on D1 at peak-lay declined from 56.1±9.6 to zero at week 34. At week 34, HDEP in K (76.65) and M (76.60) were higher than 68.45, 68.59 and 67.72 obtained for birds on L, N and P respectively. At week 36, CP (%) of eggs from hens on D2 (11.6±0.17), D3 (11.55±0.23), D5 (11.55±0.23) and D6 (11.6±0.23) were higher than those on D4 (11.4±0.17). The LDLc (mg/dL) and LO (µmol/g) of egg from hens on DL (2.13±1.63 and 0.04±0.01, respectively) were higher than BC (0.74±0.15 and 0.028±0.01µmol/g, respectively). At zero DoS, LO (µmol/g) of egg from hens on D2 (0.028±0.009), D3 (0.031±0.008), D4 (0.033±0.008), D5 (0.032±0.008) and D6 (0.027±0.010) were significantly different and increased linearly with DoS. The EW and ET of eggs from BC (5.89±0.60 and 0.35±0.03) were significantly higher than in DL (5.58±0.48 and 0.34±0.03, respectively). Eggs from BC (48.7±24.6) had higher HU than DL (44.8±25.2). The HU of egg from hens on D5 (48.6±25.2) and D6 (48.0±25.0) were significantly higher than D2 (46.1±26.8), D3 (46.1±23.8) and D4 (44.8±25.1), and HU decreased significantly with DoS (R^2 = 0.98).

Birds raised on deep litter produced more eggs than battery cage system. Proprietary vitamin-mineral premix P reduced egg lipid oxidation, while interaction of proprietary vitamin-mineral premixes L and N with both housing systems enhanced bird laying capability.

Keywords: Deep litter, Battery cage, Laying chickens, Egg storage quality, Hen day egg production

Word count: **500**

TABLE OF CONTENT

TITLE PAGE ii

CERTIFICATION iii

DEDICATION iv

ACKNOWLEDGEMENT v

ABSTRACT viii

TABLE OF CONTENT ix

LIST OF TABLES xix

LIST OF FIGURES xxii

CHAPTER ONE

1.0**: INTRODUCTION** 1

1.1: Justification 5

1.2: Objectives of study 7

CHAPTER TWO

2.0: **LITERATURE REVIEW**

2.1: Housing systems in poultry production 8

2.2: Management of laying chickens 10

2.3: Energy requirement of laying chickens 12

2.4: Protein requirement of laying chickens 13

2.5: Vitamin and mineral nutrition in poultry production 16

2.6: Vitamin and mineral requirement of laying birds 21

2.7: Vitamin and mineral metabolism and immune systems 22

2.8: The use of vitamin-mineral premixes in poultry nutrition 24

2.9: Housing systems and performance of laying chickens 27

2.10: Composition, formation and structure of a chicken egg 29

2.11: Egg quality characteristics 32

2.12: External egg quality characteristics 33

2.12.1: Egg shape index (SI) 34

2.12.2: Egg weight (Ew) 36
2.12.3: Eggshell weight (EW) 37
2.12.4: Eggshell thickness (ET) 38
2.13: Internal egg quality characteristics 39
2.13.1: Albumen quality 40
2.13.2: Yolk quality 41
2.14: Relationship among quality characteristics 42
2.15: Effect of housing system on egg quality characteristics 44
2.16 Effect of housing system on egg lipid profile 47
2.17: Effect of nutrition on egg quality characteristics 48
2.18: Dietary influence on blood and egg-yolk cholesterol in poultry 53
2.19: Methods of chicken eggs storage 54
2.20: Changes in egg quality characteristics during storage 56
2.21: Lipid oxidation and biological implications in animals and products 61
2.22: Effect of dietary vitamins and minerals on lipid oxidation 63

CHAPTER THREE

3.0**:** **MATERIALS AND METHODS**

Study One

Effects of two housing systems on performance characteristics of pullets from 13 to 16 week of age

3.1.1: Experimental Site 66
3.1.2: Housing systems 66
3.1.3: Animals and Management 67
3.1.4: Gross composition of experimental diet 67
3.1.5: Data collection 69
3.1.7: Statistical analysis 69

Study Two

Effects of five different proprietary vitamin-mineral premixes and two housing systems on performance and egg production characteristics of pullets from 17 to 21 week of age

3.2.1: Experimental site 70

3.2.2: Housing systems 70

3.2.3: Experimental design and model 70

3.2.4: Animals and Management 70

3.2.5: Test proprietary vitamin-mineral premixes 70

3.2.6: Dietary layouts 72

3.2.7: Experimental diets 72

3.2.8: Data collection 74

3.2.9: Statistical analysis 74

Study Three

Effects of five different proprietary vitamin-mineral premixes and two housing systems on performance and hen egg production of laying chickens (22-70 weeks of age)

3.3.1: Experimental site 75

3.3.2: Housing systems 75

3.3.3: Experimental design and model 75

3.3.4: Animals and Management 75

3.3.5: Test proprietary vitamin-mineral premixes 75

3.3.6: Dietary layouts 77

3.3.7: Experimental diets 77

3.3.8: Data collection 79

3.3.9: Statistical analysis 79

Study Four

Effects of five different proprietary vitamin-mineral premixes, housing systems and duration of storage on external and internal quality indices of eggs

3.4.1: Experimental site 80

3.4.2: Housing systems 80

3.4.3: Experimental design and model 80

3.4.4: Animals and Management 80
3.4.5: Test proprietary vitamin-mineral premixes 80
3.4.6: Dietary layouts 80
3.4.7: Experimental diets 81
3.4.8: Data collection 81
3.4.9: Statistical analysis 82

Study Five

Effects of supplementing laying chickens feed with five different proprietary vitamin-mineral premixes, two housing systems and duration of storage on chemical compositions of eggs

3.5.1: Experimental site 83
3.5.2: Housing systems 83
3.5.3: Experimental design and model 83
3.5.4: Animals and Management 83
3.5.5: Test proprietary vitamin-mineral premixes 83
3.5.6: Dietary layouts 83
3.5.7: Experimental diets 83
3.5.8: Data collection 83
3.5.8.1: Determination of Moisture and Dry Matter content 84
3.5.8.2: Determination of Ash Content 84
3.5.8.3: Determination of Crude Protein 84
3.5.8.4: Determination of Ether Extract 85
3.5.8.5: Determination of Gross Energy 85
3.5.8.6: Determination of Calcium 86
3.5.8.7: Determination of Phosphorous 86
3.5.8.8: Determination of Nitrogen Free Extract (NFE) 86
3.5.9: Statistical analysis 86

Study Six

Effects of five different proprietary vitamin-mineral premixes and two housing systems on cholesterol profile of eggs

3.6.1: Experimental site 87

3.6.2: Housing systems 87
3.6.3: Experimental design and model 87
3.6.4: Animals and Management 87
3.6.5: Test proprietary vitamin-mineral premixes 87
3.6.6: Dietary layouts 87
3.6.7: Experimental diets 87
3.6.8: Data collection 87
3.6.9: Statistical analysis 88

Study Seven

Effects of supplementing five different dietary proprietary vitamin-mineral premixes, two housing systems and duration of storage on lipid oxidation of eggs

3.7.1: Experimental site 89
3.7.2: Housing systems 89
3.7.3: Experimental design and model 89
3.7.4: Animals and Management 89
3.7.5: Test proprietary vitamin-mineral premixes 89
3.7.6: Dietary layouts 89
3.7.7: Experimental diets 89
3.7.8: Data collection 89
3.7.9: Statistical analysis 90

CHAPTER FOUR

4.0: **RESULTS**

Study One

4.1: Performance characteristics of growing pullets from 13 to 16 week of age 91

Study Two

4.2.1: Performance characteristics of pullets fed diets supplemented with five different proprietary vitamin-mineral premixes in two housing systems from 17 to 21 week of age 93

4.2.2: Hen day egg production of pullets fed diets supplemented with five different proprietary vitamin-mineral premixes in two systems from 17 to 21 week of age 98

Study Three

4.3.1: Ambient temperature (^{o}C) and relative humidity (%) in the two housing systems 101

4.3.2: Performance characteristics of layers fed diets supplemented with five different proprietary vitamin-mineral premixes in two housing systems from 22 to 35 week of age 103

4.3.3: Egg production characteristics of layers fed diets supplemented with five different proprietary vitamin-mineral premixes in two housing systems from 22 to 35 week of age 107

4.3.4: Hen day egg production of layers fed diets supplemented with five different proprietary vitamin-mineral premixes in two housing systems 16 to 70 week of age 111

Study Four

4.4.1: External quality indices of eggs from layers fed diets supplemented with five different proprietary vitamin-mineral premixes in two housing systems from 22 to 35 week of age 114

4.4.2: Internal quality indices of eggs from layers fed diets supplemented with five different proprietary vitamin-mineral premixes in two housing systems from 22 to 35 week of age 118

4.4.3: External quality indices of eggs as affected by five different proprietary vitamin-mineral premixes and duration of storage under two housing systems from 36 to 52 week of age 122

4.4.4: Effect of duration of storage on internal quality indices of eggs from layers fed diets supplemented with five different proprietary vitamin-mineral premixes in two housing systems from 36 to 52 week of age 124

4.4.5: Effect of duration of storage on external quality indices of eggs from layers fed diets supplemented with five different proprietary vitamin-mineral premixes in two housing systems from 53 to 70 week of age 126

4.4.6: Internal quality indices of eggs from layers as affected by duration of storage, proprietary vitamin-mineral premixes and two housing systems in days of storage from 53 to 70 week of age 128

4.4.7: Relationship among external quality indices of eggs as affected by duration of storage 130

4.4.8: Relationship among internal quality indices of eggs as affected by duration of storage 134

Study Five

4.5.1: Chemical compositions of eggs from layers fed diets supplemented with five different proprietary vitamin-mineral premixes in two housing systems at week 22 to 35 weeks of age 138

4.5.2: Chemical compositions of eggs as affected by five different proprietary vitamin-mineral premixes, two housing systems and duration of storage from 36 to 52 week of age 143

4.5.3: Chemical compositions of eggs as affected by five different proprietary vitamin-mineral premixes, two housing systems and duration of storage from week 53 to 70 weeks of age 142

4.5.3: Relationship among parameters of chemical composition of eggs as affected by duration of storage from 53 to 70 weeks of age 148

Study Six

4.6.1: Cholesterol profile of whole-egg from layers fed diets supplemented with five different proprietary vitamin-mineral premixes in two housing systems from 36 to 52 week of age 151

4.6.2: Cholesterol profile of egg-yolk from layers fed diets supplemented with five different proprietary vitamin-mineral premixes in two housing systems from 36 to 52 week of age 149

Study Seven

4.7.1: Lipid oxidation of egg-yolk of layers fed diets supplemented with five different proprietary vitamin-mineral premixes under two housing systems in days of storage from 36 to 52 week of age 159

4.7.2: Lipid oxidation of egg albumen and whole-egg of chickens fed diets supplemented with five different proprietary vitamin-mineral premixes as affected by two housing systems and duration of storage from 52 to 70 week of age 161

4.7.3: Regression of lipid oxidation of egg albumen, yolk and whole-egg with duration of storage at late laying phase (52 to 70 week of age) 163

CHAPTER FIVE

5.0: **DISCUSSION**

Study One

Effects of two housing systems on performance characteristics of growing pullets from 13 to 16 weeks of age 165

Study Two

Effects of five different proprietary vitamin-mineral premixes and two housing systems on performance and egg production characteristics of pullets from 17 to 21 weeks of age 167

Study Three

Effects of five different proprietary vitamin-mineral premixes and two housing systems on the performance and hen egg production of laying chickens (22 to 70 weeks of age) 171

Study Four

Effects of five different proprietary vitamin-mineral premixes, two housing systems and duration of storage on external and internal quality indices of eggs 175

Study Five

Effects of supplementing laying chicken feed with five different proprietary vitamin-mineral premixes, two housing systems and duration of storage on chemical compositions of eggs 179

Study Six

Effects of five different proprietary vitamin-mineral premixes and two housing systems on cholesterol profile of laying chicken eggs 180

Study Seven

Effects of five different dietary proprietary vitamin-mineral premixes, two housing systems and duration of storage on lipid oxidation of eggs 182

CHAPTER SIX

5.0: **SUMMARY, CONCLUSION AND RECOMMENDATIONS**

5.1: Summary 183

5.2: Conclusion 182

5.3: Recommendations 185

REFERENCES 186

LIST OF TABLES

Table 1: Gross composition of experimental diet 68

Table 2: Gross compositions/2.5kg of test proprietary growers' vitamin-mineral premixes 71

Table 3: Gross composition (%) of experimental diets fed from 17 to 21 weeks of age 73

Table 4: Gross composition/2.5kg of test proprietary layers vitamin-mineral premixes 76

Table 5: Gross composition (%) of layers diets 78

Table 6: Performance characteristics of pullets in two housing systems from 13 to 16 weeks of age 92

Table 7: Performance characteristics of pullets fed five diets supplemented with five different proprietary vitamin-mineral premixes in two housing systems from 17 to 21 weeks of age 95

Table 8: Interaction effects of proprietary vitamin-mineral premixes and two housing systems on performance characteristics of pullets from 17 to 21 weeks of age 97

Table 9: Ambient temperature (^{o}C) and relative humidity (%) of two housing systems 102

Table 10: Performance characteristics of layers fed five diets supplemented with five proprietary vitamin-mineral premixes in two housing systems from 22 to 35 weeks of age 104

Table 11: Interaction effects of proprietary vitamin-mineral premixes and housing systems on performance characteristics of layers from 22 to 35 weeks of age 106

Table 12: Egg production characteristics of layers fed diets supplemented five different proprietary vitamin-mineral premixes in two housing systems from 22 to 35 weeks of age 108

Table 13: Interaction effects of proprietary vitamin-mineral premixes and two housing systems on egg production characteristics of layers from 22 to 35 weeks of age 110

Table 14: External quality indices of eggs from layers fed diets supplemented with five different proprietary vitamin-mineral premixes in two housing systems from 22 to 35 weeks of age 115

Table 15: Interaction effects of proprietary vitamin-mineral premixes and two housing systems on external quality indices of eggs from layers from 22 to 35 weeks of age 117

Table 16: Internal quality indices of eggs from layers fed diets supplemented with five different proprietary vitamin-mineral premixes in two housing in early-laying phase (22 to 35 weeks of age 119

Table 17: Interaction effects of proprietary vitamin-mineral premixes and two housing systems oninternal egg quality indices of eggs from layers at week 22 to 35 week of age 121

Table 18: Effect of duration of storage on external quality indices of eggs from layers fed diets supplemented with five different proprietary vitamin-mineral premixes in two housing systems from 36 to 52 weeks of age 123

Table 19: Effect of duration of storage on internal quality indices of eggs from layers fed diets supplemented with five different proprietary vitamin-mineral premixes in two housing systems from 36 to 52 weeks of age 125

Table 20: Effect of duration of storage on external quality indices of eggs from layers fed diets supplemented with five different proprietary vitamin-mineral premixes in two housing systems from 53 to 70 weeks of age 127

Table 21: Internal quality indices of egg as affected by duration of storage, proprietary vitamin-mineral premixes and two housing systems from 53 to 70 weeks of age 129

Table 22: Chemical compositions of eggs of layers fed diets supplemented with five different proprietary vitamin-mineral premixes in two housing systems from 22 to 35 weeks of age 140

Table 23: Interaction effects of proprietary vitamin-mineral premixes and two housing systems on chemical compositions of eggs from 22 to 35 weeks of age 142

Table 24: Chemical composition of eggs as affected by five different proprietary vitamin-mineral premixes, two housing systems and duration of storage from 36 to 52 weeks of age 145

Table 25: Chemical compositions of eggs as affected by five different proprietary vitamin-mineral premixes, two housing and duration of storage from 53 to 70 weeks of age 144

Table 26: Cholesterol profiles of whole-egg from layers fed diets supplemented with five different proprietary vitamin-mineral premixes in two housing systems from 36 to 52 weeks of age 152

Table 27: Interaction effects of proprietary vitamin-mineral premixes and housing systems on cholesterol profile of whole-eggs of layers from 36 to 52 weeks of age 154

Table 28: Cholesterol profile of egg-yolk from layers fed diets supplemented with five different proprietary vitamin-mineral premixes in two housing systems from 36 to 52 weeks of age 156

Table 29: Interaction effects of proprietary vitamin-mineral premixes and housing systems on cholesterol profile of egg-yolk of layers from 36 to 52 weeks of age 152

Table 30: Lipid oxidation of egg-yolk of layers fed diets supplemented with five different proprietary vitamin-minerals premixes as affected by two housing systems and duration of storage from 36 to 52 weeks of age 160

Table 31: Lipid oxidation of egg albumen and whole-egg of layers fed diets supplemented with five different proprietary vitamin-minerals premixes as affected by two housing systems and duration of storage from 53 to 70 weeks of age 156

LIST OF FIGURES

Figure 1: Hen Day Egg Production of pullets in two housing systems from weeks 16 to 21 99

Figure 2: Hen Day Egg Production of pullets of pullets fed diets supplemented with five different proprietary vitamin-mineral premixes from weeks 16 to 21. 100

Figure 3: Hen Day Egg Production of laying hens in battery cage and deep litter systems. 112

Figure 4: Hen Day Egg Production of laying chickens fed different PVmP 113

Figure 5: Relationship of eggshell weight on DoS from 36 to 52 and 53 to 70 weeks of age 131

Figure 6: Relationships between eggshell thickness and days of storage from 36 to 52 and 53 to 70 weeks of age 132

Figure 7: Relationships between egg weight loss and days of storage from 36 to 52 and 53 to 70 weeks of age 133

Figure 8: Regression of albumen quality (Haugh Unit) on days of storage of eggs from 36 to 52 and 53 to 70 weeks of age 135

Figure 9: Regression of egg-yolk quality on days of storage of eggs from 36 to 52 and 53 to 70 weeks of age 136

Figure 10: Regression of albumen and yolk quality on days of storage of eggs 137

Figure 11: Regression of eggs crude protein on days of storage at the early- and late-laying phases 149

Figure 12: Regression of egg fat on days of storage at the early- and late-laying phases 150

Figure 13: Regression of lipid oxidation on albumen, yolk and whole-egg in days of storage 164

CHAPTER ONE

1.0: INTRODUCTION

Poultry supply over 60% of the world's food (Brillard, 2004) which represents 25% animal protein production (FAO, 2000). Poultry industry has witnessed rapid expansion due to phenomenal improvement in animals' productivity through researches in breeding and genetics, nutrition and husbandry management (Hetland *et al*., 2004; Ogunwole, 2009) to meet the ever-increasing demand for animal protein consumption. Research into production of quality and health friendly eggs for human consumption is critical in commercial poultry industry. Production of quality eggs remains one of the determinants of economic sustainability in commercial egg industry (Ahmadi and Rahimi, 2011). The two main housing systems use in commercial egg production are battery cage and deep litter systems (Anderson and Adams, 1994a). Variations in housing systems relate to operating conditions, feeding and management practices which affect egg production and quality indices (Mahmoud *et al*., 1996; Ayo *et al*., 2007; Zemková *et al*., 2007; Lichovníková and Zeman, 2008; Obidi *et al*., 2008; Singh *et al*., 2009; Djukicstojcic *et al.*, 2009). Poultry farmers in developing countries are known to house more birds in deep litter than battery cage system (Njoya and Picard, 1994; Badubi and Ravindran, 2004). However, conventional battery cage system accommodates approximately 90% world population of laying chickens in commercial poultry industry (Awoniyi, 2003; Peterman, 2003). In temperate countries, housing laying chickens in conventional battery cage has comparative advantage over deep litter system (Abrahamsson *et al*., 1996; Pistikova *et al*., 2006; Voslářova *et al*., 2006; Banga-Mboko *et al*., 2010).

The emerging Animal Welfare Policy however tends to favour commercial egg production in deep litter system (Scientific Panel on Animal Health and Welfares, 2005). This is because birds generally retain natural behaviour of their wild counterparts (Price, 1984; Fraser and Bloom, 1990). Birds, therefore, prefer more space than is provided in conventional battery cage where feed trough and water line are provided in a restricted environment (Hughes, 1975; Dawkin, 1983). The natural behaviours such as nesting, perching, roosting, scratching, dust-bathing, wing flapping, preening and exercising are strongly motivated by internal factors such as hormones (Nicol, 1986).

These natural behaviours are important for well-being of birds but prevented when housed in conventional battery cage system. The wire floor in conventional battery cage deprives bird opportunity to express scratching behaviour. The domestic chickens spend more than 50% of their active time foraging and scratching as means of exploring the environment in search of food (Savory, *et al*., 1978; Dawkin, 1989). Although, birds in battery cage system are always provided with balanced diets*ad libitum* but still possess strong natural urge to scratch. Birds in deep litter system choose to scratch on littered floor rather than eating identical feed provided in feeder (Duncan and Hughes, 1972). Thus, birds in deep litter system are able to satisfy vitamins and minerals requirement by foraging on litter materials, faeces and other natural feed materials (Skinner, *et al.* 1992; Asadumzzaman *et al*., 2005). Lack of appropriate scratching substrate could result in abnormal behaviour like feather pecking (Blokhuis, 1989).

Nutrition is important for growth and production quality of eggs. All species of poultry require nutrients in balanced proportion for efficient growth, maintenance of healthy physiologic condition, reproduction and production. Birds respond differently to dietary nutrients (Morris, 2004). Vitamins and minerals are required for growth and egg production. Vitamins are complex organic nutrients present in small amounts in natural foodstuffs (McDowell, 2000) and participate in cellular metabolism (Marks, 1979). Feed ingredients do not normally contain all vitamins at the right amounts and proportion needed by Chickens. Vitamins; A, D, B_{12} and riboflavin are usually low in poultry feeds particularly in maize-soyabean diets where vitamins D and B_{12} are usually absent. Vitamin K is generally added to poultry feed because birds have short intestines and ingested feed pass through the intestine fast with less intestinal vitamin synthesis. Poultry species are more susceptible to vitamins deficiencies because microbial population in the intestinal tract synthesizes very little amount of vitamins and compete vigorously with the host dietary supply (Asaduzzman *et al*., 2005).

Mineral nutrients are inorganic elements required for efficient production. Calcium, phosphorus, copper, iodine, iron, manganese, sodium and zinc are essential for growth and efficient quality egg production (Ogunwole, 2009). Calcium and phosphorous are required for normal bone development, blood-clotting, muscle contraction, strong

eggshell and metabolic and energy functions. Chlorine in hydrochloric acid is required for digestion and maintenance of water and acid/base balance. Sodium and potassium are components of body electrolytes for metabolic, muscle and nerve functions as well as water and acid/base balance. Magnesium functions in metabolism and muscular contraction. Trace minerals are involved in metabolic functions. Iodine is needed for production of thyroid hormone for regulation of rate of energy metabolism. Zinc is involved in many enzymatic processes in body while iron serves as a component of blood haemoglobin and myoglobin necessary for oxygen transportation.

Effects of single vitamin and/or mineral premixes in poultry nutrition are well documented (Ogunmodede, 1974; 1975; 19977; 1978; 1981a and b; 1991; 1992). The metabolic responses of single vitamin and/or mineral premix are different compared with vitamin-mineral premixes which contain a condiment of vitamins and minerals. Also, there are interactions, interrelationship and interdependence among vitamins and/or minerals and other feed nutrients. Thus, single vitamin and/or mineral premixes are not solely responsible for metabolic process and productive performance in poultry. The effect of vitamins and minerals are largely interdependent in combination rather than individual vitamin and/or mineral. The variability and inconsistent supply of vitamins and minerals from feed ingredients as well as unreliability of commercial single vitamins and/or minerals premix necessitates the use of proprietary vitamin-mineral premixes. Hence, the discovery and use of vitamin-mineral premixes in poultry nutrition was a major breakthrough in poultry nutrition **(**Oduguwa and Ogunmodede, 1995; Oduguwa *et al*., 1996; Oduguwa *et al*., 2000**)**.

Proprietary vitamin-mineral premixes are marketed under different trade names and account for about 10% total feed cost (Singh and Panda, 1988). These are commercial micro-feed inputs that contain vitamins and/or trace minerals and antioxidants in different carrier media. The use of quality premixes is essential and indispensable for successful and sustainable commercial egg production and quality indices (Raven and Walker, 1980). Therefore, proprietary vitamin-mineral premixes are added in small amounts to feed to improve safety and reliability of productive performance as well as protect against deficiency diseases (Raven and Walker, 1980). Thus, any compromise or

neglect to include proprietary vitamin-mineral premixes in poultry feed as an attempt to minimize cost of feeding could make chickens to shut down all necessary metabolic processes, reduced or cease egg production, produce poor egg quality imdices, high mortality and farm economic losses (Suttle and Jones, 1989; Wuryastuti, *et al*., 1993). Also, sub-standard or adultrated vitamin-mineral premixes by proprietors could affect production and quality indices of eggs (Ogunwole *et al.*, 2012; Ogunwole *et al.* 2015 a and b) as optimum dietary vitamin and mineral requirements only allow for full expression of genetic potentials of birds (OVN, 2010).

Table-eggs produced by chickens are rich sources of high quality digestible proteins, carbohydrates, fats, minerals and vitamins. Egg quality is determined by standard procedure based on external and internal characteristics (Koelkebeck, 2003). The external quality indices of eggs influence consumers' acceptance or rejection and marketing (Natalie, 2009). The albumen and yolk quality indices as well as chemical compositions provide information on internal egg quality (Song *et al*., 2002). High internal egg quality is indicated by firm and thick albumen and yolks (Ihsan, 2012). Egg quality deteriorate depending on days of storage (Adeogun and Amole, 2004; Kul and Seeker, 2004). The physical changes that determine egg quality include thinning of albumen and flattening of yolk (Stadelman and Cotterill, 1995) which is cause by weakening of vitelline membrane (Fromm and Matrone, 1962). The changes in albumin quality are measured in Haugh Units (HU) and calculated from albumen height and weight (Haugh, 1937). Chemical oxidation in poultry products affects lipids, carbohydrates, proteins deoxyribonuclic acid (DNA) and vitamins (Kanner, 1994).

In animal muscle and eggs, chemical oxidation continues post-mortem and affects shelf-life quality of products. Chemical oxidation is inherent to metabolism since excessive formation of reactive species cause damage to some biological component (Halliwell *et al*., 1995). The oxidative damage in biological materials is due to imbalance between productions of free radicals and defense mechanism in response to oxidative stress. The rate of chemical oxidation increases with high intake of lipid or oxidation of polyunsaturated fatty acids (PUFA) or pro-oxidants and low intake of nutrients involved in antioxidant defense system. Lipid oxidation is one example of chemical oxidation

and responsible for deterioration of meat and egg quality indices during storage. Oxidative stability ofpoultry products may be maximised by dietary supplementation of vitamins and mineral especially vitamin A and E and selenium which possess antioxidants proprotey. Storage methods, length of storage days and temperature affect oxidative stability of poultry products (Coutts and Wilson, 1990; Jacob *et al*., 2000). The length of storage days and temperature affect albumin and yolk quality (Samli *et al*., 2005) becuase internal temperature of eggs above 7^{o}C degenerate albumen and vitelline membrane (Jones, 2006) making water move from albumen into the yolk and increase severity of mottling when eggs are stored (Jacob *et al*., 2000). There is therefore a dearth of information on the effect vitamin-mineral premixes by different proprietors on laying chickens egg production and quality indices. The present study was carried out to investigate effects of five different proprietary vitamin-mineral premixes and two housing systems on laying chickens egg production and quality indices.

1.1: Justification

Housing systems and vitamin-mineral nutrition greatly affect production and quality indices of eggs (Zemková *et al*., 2007; Lichovníková and Zeman, 2008; Singh *et al*., 2009; Djukic-Stojcic *et al*., 2009). To satisfy continuous demand for desirable quality eggs, there is need to investigate effects of different proprietary vitamin-mineral premixes and housing systems. Commercial egg industry in Nigeria is dominated by exotic strains of chicken that have been evaluated in the temperate region under optimal nutrition and housing systems. The productive performance of these strains is sub-optimal in developing countries due to sub-optimal housing systems and nutrition (Dingle and Henuk, 1999; Henuk and Dingle, 2000). Also, there are variations in performances among commercial strains of laying chickens under homogenous housing systems in controlled and natural environments (Duduyemi, 2005; Mmereole and Omeje.2005; Yakubu *et al*., 2007). Extensive studies (Oduguwa *et al*., 2000; Ogunwole *et al*., 2012) on effects of proprietary vitamin-mineral premixes on broiler chickens have been documented. However, fewer emhapsises have been on laying chickens egg production and quality indices (Asaduzzaman *et al*., 2005). Thus, there is dearth of information on effects of different propritarty vitamin-mineral premixes and housing sytems on laying chicken egg production and quality indices.

Generally, chickens reared in deep litter system are believed to satisfy their vitamins and minerals requirement by scratching litter materials and faeces (Skinner, *et al.* 1992). Also, speculations abounds that some proprietary vitamin-mineral premixes are of poor or sub-standard in quality. Low production and poor quality indices of table chicken eggs in developing countries like Nigeria could be attributed to the use of adulterated or sub-standard vitamin-mineral premixes. The use of poor or sub-standard quality propeitary vitamin-mineral premixes could reduce egg production and quality indices. Study (Anisuzzaman, 1993) indicated reduced production and low quality of eggs despite supplementation with well formulated balanced layer diets with proprietary vitamin-mineral premix.

Farmers, animal nutritionists and feed millers are therefore at cross road at determining the brand of propeitary vitamin-mineral premix to use in feed formulation. In addition, inadequacy of laboratory equipment for analyses and provision of needed information on vitamin and mineral profile remained a challenge. Thus, a slower but rational investigative approach of using live animals in feeding trials is explored to investigate effects of different proprietary vitamin-mineral premixes (Ogunwole *et al*., 2012). There is therefore the need for regular assessment of vitamin and mineral profile in different proprietary vitamin-mineral premixes by using live animals in feeding trials. This is important for quality control and regulation of products standard to ensure safety of poultry industry. Also, farmers, animal nutritionists and feed millers need to be well informed about the vitamin-mineral profile in different proprietary vitamin-mineral premixes so as to formulate and compound poultry feed that will have optimal productive performance and high profit returns on investment.

There is a general public misconception on eggs consumption as causative factor of heart disease (atherosclerosis) in human. Animal fat contains high content of poly-saturated fatty acids which encourages incidence of atherosclerosis. Also, dietary quantity of fat whichserves an indicator of egg-yolk cholesterol influences blood cholesterol (Olomu, 2011; Vasudevan *et al*., 2011). However, dietary supplementation with vitamins and minerals could elevate or reduced blood and egg-yolk cholesterol. Nicotinic acid, biotin, vitamin D, E, calcium, iron, vanadium, selenium and zinc affect blood and egg-yolk cholesterol. It is therefore hoped that results from the study will

provide baseline information on effects of different proprietary vitamin-mineral premixes and housing systems on laying chickens egg production and quality indices useful for quality control and monitoring by regulatory agenicies in Nigeria. By this, commercial poultry industries will be protected against proliferation of adulterated or sub-standard proprietary products. The information provided on egg-yolk cholesterol profile will possibly dispel public misconception and encourage consumption of table chicken eggs. This will reduce egg-glut and increase farm revenue through increase marketing and sales of table-eggs.

1.2: Objectives of study

The objectives of this study were to:

- investigate effects of two housing systems on performance of pullets from 13 to 16 weeks of age
- assess effects of five different proprietary vitamin-mineral premixes and two housing sytems on performance and egg production characteristics of pullets from 17 to 21 weeks of age
- assess effects of different proprietary vitamin-mineral premixes and housing systems on performance and hen day egg production of laying chickens;
- evaluate effects of different proprietary vitamin-mineral premixes, housing systems and duration of storage on externaland internal quality indices of eggs;
- assess effects of different proprietary vitamin-mineral premixes, housing systems and duration of storage on chemical composition of eggs;
- evaluate effects ofdifferent proprietary vitamin-mineral premixes and housing systems on cholesterol profile of eggs; and
- determineeffect of different proprietary vitamin-mineral premixes, housing systems and duration of storage on lipid oxidation of eggs

CHAPTER TWO

2.0: LITERATURE REVIEW

2.1: Housing systems in poultry production

The rapidly growing rate of human population is not commensurate with the increasing rate of demand for animal protein and its consequence attendance of food security challenges (Nworgu, 2006). Poultry production is one of the fastest ways of mitigating protein deficiency in human diet due to the relatively short maturity period and high feed conversion efficiency of birds (Ziggers, 2011). There are different housing systems for raising poultry which generally fall under intensive, semi-intensive or extensive housing depending on the purpose production. Housing systems significantly influence the performance characteristics of birds and the chemical composition of eggs (Zemková *et al*., 2007). Studies (Lichovníková and Zeman, 2008; Singh *et al*., 2009; Djukic-Stojcic *et al*., 2009) showed that housing systems affect egg quality in commercial flocks. Worldwide, housing systems for managing laying birds and producing eggs of good shell and internal quality is critical to the economic viability of commercial egg industry (Ahmadi and Rahimi, 2011). There are different housing systems used for management and production of commercial laying chickens (Anderson and Adams, 1994).

Housing systems vary in terms of facilities, husbandry operations, feeds and feeding management, therefore, the choice of housing system depends on available space, facility, man-power, technology and economy of production. Majority of laying chickens are reared in conventional battery cage system, although European Union Council Directive 1999/74 EC banned its use in EU States since January 2012. Animal welfare scientists are critical on the use of conventional battery cages for managing laying chickens because cages do not provide sufficient space for birds to stand, walk, flap wings, perch and make a nest. It is therefore widely considered that laying chickens suffer boredom and frustration (DEFRA, 2011) leading to a wide range of abnormal behaviours that are injurious. Conventional battery cage comprises small cages, usually made of metal in modern systems to accommodate 3 to 8 layers. The walls are made of either solid metal mesh with sloped wire mesh floor to allow the excreta to drop through

or eggs to roll onto an egg-collecting compartment or conveyor belt. Water is provided by overhead nipple systems and feed trough in front of cages at regular intervals manually or by automation.

The cages are arranged back-to-back in rows as multiple tiers hence the term battery cage. There may be several floors containing battery cages within a single shed meaning that a single shed may contain many tens of thousands of birds. The three-tier type of conventional battery cage is raised on a platform sheds with capacity up to 25,000 birds in 40ft wide of laying houses. Large laying houses of dimension 50ft wide with 5 blocks can accommodate 50,000 birds. The size of a cage is 12 inches deep and 15 inches fronts to accommodate three birds. The cage size of 15 inches front and 18 inches depth could accommodate four laying chickens, while cages with larger sizes accommodate more birds. The feeding is done by moving feed hopper and water by nipple drinkers (http://en.wilkipediaorg/w/ poultry_production). In the temperate countries, foggers are provided above cages during summer months. Automatic egg collection systems are installed in some specifications. Automatic feeding saves feed wastage and reduces the labour cost. Conventional battery cages and their installation are been improved to provide better ventilation, and avoid production of soiled eggs.

Light intensity is often kept low (e.g.10 lux) to reduce feather pecking and vent pecking. Floor space for laying chickens ranges upwards from 300 cm^2 per hen while EU standards stipulated at least 550 cm^2 per hen (United Egg Producer, 2003). In the U.S., the current recommendation is 67 to 86 square inches (430 to 560 cm^2) per bird (United Egg Producer, 2009).Some of the benefits of conventional battery cage system are easy management of the birds; reduced labour cost collection; clean eggs; capture at the end of lay is expedited; less feed requirement to produce eggs; broodiness is eliminated; high stocking capacity; easy treatment of internal parasites; and reduced labour requirement. In farms where cages are used for egg production, more chickens per unit area allow for greater productivity and lower feed costs (Appleby, 2001).

Deep litter system is not commonly used for egg production. It is most useful in production of meat-type chickens like broilers, cockerels and breeder stock. Chickens

are raised in large open structures known as brooding, rearing and breeding or breeder pens. These pens are equipped with manual or mechanical systems to deliver feed and water to birds. They have ventilation systems and heaters that function as the need arises. The floor of pen is covered with bedding material consisting of wood chips, rice hulls, or peanut shells. Dry bedding helps maintain flock health and such pens are provided with enclosed water systems ("nipple drinkers") to reduce water spillage (U.S Poultry and Egg Association, 2012). Deep litter house protects birds against predators such as hawks and foxes. Some deep litter houses are equipped with curtain walls, which can be rolled up in good weather to admit natural light and fresh air (http://en.wilkipediaorg/w/ poultry_production).

Traditionally, deep litter houses or pens may measure 400 feet long and 40 feet wide and provides about eight-tenths of a square foot per bird. The Council for Agricultural Science and Technology (CAST) provided minimum floor space requirement of one-half square foot per bird. Modern deep litter houses are often larger and contain more chickens with floor space allotment to meets the requirement per bird (U.S. Poultry and Egg Association, 2012). Recently, deep litters are equipped with "tunnel ventilation," in which a bank of fans draws fresh air into the house (U.S. Poultry and Egg Association, 2012). High stocking density in deep litter generates high concentration of ammonia gas from poultry dropping causing air pollution. This often results in ill-health damaging birds' eyes, respiratory systems and causing painful burns on the legs known as hock burns.

2.2: Management of laying chickens

The theoretical objectives of commercial egg production include attainment of standard and uniform body weights (1350-1375 g/bird) at 20 weeks and onset of egg lay at 18 weeks; 5% egg production at week 19, 50% egg production at the end of week 21 and 90% egg production at the end of weeks 25; attain an average egg weight (45gms) at weeks 20; and that mortality rate should not exceed 0.7% (htt://ag.ansc.purdue.edu/poultry). The period between 18th and 25th week of age can be referred to as early-laying period. A uniformly well grown flock starts to lay egg in time, and egg production increases steadily every day without records of mortality and

culling. The egg-lay initiation, daily rate of egg production, peak production, mortality rate, egg quality, and feed intake depend on the quality of birds, season and quality of nutrition (htt://ag.ansc.purdue.edu/poultry).

All operations like vaccinations are always completed and pullets in laying house before 18 weeks. Birds at onset of lay are expected to attain 1300 gm average body weight (htt://ag.ansc.purdue.edu/poultry). Birds usually have uniform size with well-built body without compromise for fat. The frame size can be judged by the shank length. The shank length of the pullets at 19 weeks is about 104 mm and remains same throughout the life. Smallest birds in among flock are usually not be below 1150 gm, while the heaviest should not be more than 1450 gm body weight (htt://ag.ansc.purdue.edu/poultry) with signs of maturity of feather shedding and re-growth of new feathers. The birds are usually docile having bright red combs and yellow shanks and beaks. Birds are normally fed standard layer diets from 18-22 weeks. The diet is changed from low protein-low calcium (1% calcium) to higher protein-higher calcium (4% calcium) at the onset of egg production (htt://ag.ansc.purdue.edu/poultry).

The change in diet may result in reduced feed intake for few days because the onset of egg production possesses stress on birds, hence the need for increase dietary calcium in order to reduce stress and help individual bird adjust to physical property of new diet. The extra quantity of calcium included is stored in the reserves pool for egg formation. The crude protein may be kept higher at 18% crude protein for flock below the standard weight. The quantity of feed consumed depends on the level of metabolisable energy in diet. Different levels of crude protein have been used for feeding birds before onset of lay. Birds are fed higher protein diets (20% CP) during the first six weeks but continuously decrease approximately 16 to 16.5% during egg production (htt://ag.ansc.purdue.edu/poultry). The amino acid composition of in diet decides the egg size. Higher levels of methionine up to 0.4% was recommended (htt://ag.ansc.purdue.edu/poultry) at the beginning of egg lay, while it quantity was reduced when eggs became over-sized.

2.3: Energy requirement of laying chickens

The energy requirement of laying chickens needs to be determined and managed in relation to other nutrients. Although chickens tend to adjust feed consumption to meet the energy need, this is not precisely enough to insure optimum performance. Additional energy in feed often results in better body weight gain, egg production and increase egg size particularly when nutrients such as protein and amino acids are proportionately balanced. A high energy ration reduces the daily feed consumption while low energy rations results in higher feed consumption with lesser protein intake. The range of recommended energy: protein ratio, calculated as C.P:M.E, is 1:150 to 1:160 (htt://ag.ansc.purdue.edu/poultry). Study (Hill and Dansky, 1954) obsereved 623 caloric per pound of diet as minimum productive energy required for maximum growth rate because feed intake increase as dietary energy concentration decrease. Fraps (1964) reported 800-850 caloric per pound of diet as minimum productive energy level required for maximum early growth rate. Total energy intake increases as dietary energy decrease progressively. Poultry and ruminant animals respond in opposite direction to variation in dietary energy concentration of diets. In ruminant animals, voluntary feed intake response to increase in dietary energy content, while voluntary feed intake reduces when poultry species are provided with more digestible diets (Morris, 2004).

Poultry species therefore reduce voluntary feed intake as dietary energy concentration increases. Voluntary feed intake in ruminant animals is limited by digestive capacity (Morris, 2004). Feed that are more digestible, pass through rumen more quickly to allow for more feed intake. In the case of poultry species, digestive capacity is often not limited so that feed that are rich in digestible energy are taken in smaller quantity. However, chickens reduce voluntary feed intake when diets contain high proportion of indigestible fibre. Conversely, ruminant animals tend to reduce feed intake when diet become enriched with digestible starch or fat. Poultry and ruminant animals have limitation for nutrient digestibility and utilization when fibre content increases in diets. Study with White Leghorn, (Hill, 1962) showed that chickens normally adjust their voluntary feed intake when fed different nutrient density. This adjustment was far being perfect in heavy breeds of laying chickens. A measurable relationship exists among poultry breed, appetite and there is a tendency to over-feed when supplus diets highly

rich in digestible energy are offered (Morris, 1968; Fisher and Wilson, 1974). Practical implication is that there is no definite dietary energy requirement for laying or broiler chickens without voluntary feed intake consideration.

It is therefore important to specify dietary energy requirement with voluntary feed intake at lowest feed cost (Morris, 1968; Fisher and Wilson, 1974). Jackson *et al*. (1969) reported an insignificant change in rates of egg lay and small increase in egg size with increased dietary metabolic energy (ME). Feeding high dietary energy concentration fattens pullets and provides extra income per bird at the end of production. This comparative advantage is often offset by high mortality rates cause by fat deposition, prolapse and haemorrhagic fatty liver syndrome (Manitoba Agriculture, Food and Rural Initiatives, 1945). For profitable egg production, laying birds are fed diets that minimize cost of dietary energy concentration per bird. The optimal dietary metabolic energy (ME) level is calculated by taking into account the changes in voluntary feed intake, feed cost, and live weight gain and egg production. Indigestible fibres have negative effect on the effective energy derive from diets fed to birds (Emman, 1994).

2.4: Protein requirement of laying chickens

Protein requirement in laying chickens follow egg production phases. It reduces with age and production phases. In a study (Reid *et al*., 1951) laying chickens fed 18% crude protein and high energy diets were superior in body weight to either to those fed 15% or 13% crude protein, while lesser body weight was obtained for 12% crude protein (Bray and Morrissey, 1962). In a similar study (Heywang *et al*., 1955) 15% crude protein diets at high energy level were required for maximum egg production in both hot and moderate weather and Haugh Unit score of eggs increased when dietary protein decreased (Deaton and Quisenberry, 1965). The eggshell thickness and specific gravity were not affected by dietary protein level (Aitken *et al*., 1977). Dietary protein requirement was affected by amino acids composition. Layer diets are usually formulated at least-cost by amino acid specification per minimum dietary protein levels. Minimum level of dietary protein intake for supply of non-essential amino nitrogen is allowed in poultry diets. Such level has not been defined because most diets formulated compose of natural feeding ingredients which supply more than enough of the non-

essential amino nitrogen. Hence, amino acids requirements are quoted at fixed proportion of feed intake for specific age, type of birds and energy content.

This is based on the requirement for reappraisal of changes in performance standards and environmental factors on voluntary feed intake. The requirement of dietary protein changes as a variation to voluntary feed intake occur. These changes define requirement of amino acids on daily basis. Feed intake is stated in per unit output or production and does not need revision because genetic selection does not change among species but improves level of performance. Fisher *et al*. (1973) proposed amino acids requirement model for laying chickens as follow.

R = a E + w b where, R = amino acid requirement (mg/bird/day)

E = egg output (g/bird day)

W= body weight (kg)

a = mg amino acid required per egg output

b = mg amino acid required per day to maintain 1 kg live weight

This model was used to formulate diets for laying birds at or near peak-lay phase and omits requirement for live weight gain. Empirical estimates of protein and amino acids requirements have been reported (Welhli and Morris, 1978; Huygheb *et al*., 1991). Laying chickens do not gain much weight so that coefficient of weight gain account for rate of protein deposition and not weight gain. Laying chickens deposit fat and not protein except for feather growth towards the end egg production phase. Thus, the coefficient of weight gain in adult chickens is probably zero. The presumption that laying chickens still grow during the early lay-phase by assessment of body weight is a misconception (Morris, 2004).

Pullets during first 7 weeks of lay fed uniformly well-balanced diet normally attain 50% egg production by laying one egg per day. Skeletal growth stops abruptly just before onset of lay and growth attained few weeks before onset of lay is due to increase in ovary, oviducts and combs, and storage of yolk precursors in liver and calcium phosphate in medullary bones. Pullets at onset of lay need higher supply of high protein diet in order to meet protein requirement and safely cover individual requirement for building organs and storage of materials for egg formation (Morris, 2004). At point of

lay, sufficient and quality feeds should be provided for egg production. Amino acid requirement do not increase as rate of egg increase or decrease as egg production decline during post-laying phase. This is because laying flock consists of individuals with diverse rates of egg production. When rate of egg production of most productive flock decline, egg size increases because feed intake increase to compensate for increase output (Banga-Mboko *et al*., 2010).

The body weight and egg output are normally distributed about their mean values. The expected response curve of essential amino acid is estimated from egg composition and potential egg output. Broiler breeders and aging laying flock do not exhibit normal distribution rates of egg production. The efficiency of amino acids utilization decline with age and does not indicate genuine ageing because moulting fully recovers efficiency of utilization. Diets containing surplus protein could lead to impaired utilization of first limiting essential amino acids (Hassan *et al*., 2013) Excessive dietary protein in laying chickens is catabolized and excreted via kidney in form of urea in excreta. This implies higher water intake. An increase in 1 per cent in protein level increases water consumption by 3 per cent (Larbier and Leclercq, 1997). Marks and Pesti (1984) reported that when diet of bird changed to increase protein content by increasing soyabean at the expense of maize, there was increase in water consumption and higher water: feed ratio.

Study (Alleman and Leclercq, 1997) that combined effect of temperature and dietary protein on water consumption of two diets (16% and 20% crude protein) at two temperatures (22^{o}C and 32^{o}C) from 23 to 44 day showed that water intake of birds at 22^{o}C increase linearly with age but remain constant at 32^{o}C. The increase in protein level increased water consumption at both temperatures. Water: feed ratio at 22^{o}C was 1.69 (16% crude protein) and 1.93 (20%) at 32^{o}C were 2.84 and 3.07 respectively. Soyabean meal-based diet was found to cause greater amount of water intake than an equal quantity of any animal protein-based diet (Wheeler and James, 1950). Soyabean contains some constituents such as fibre, fermentable sugar and potassium that are responsible for increase of water consumption in birds.

2.5: Vitamin and mineral nutrition in poultry production

Feed nutrients are found in cells and tissues of animals and important for various biological processes. Underwood (1981) reported that twenty-two (22) elements are found in animal feed which compose of seven elements (calcium, phosphorus, potassium, sodium, choline, magnesium and sulphur) and fifteen others (iron, iodine, zinc, copper, manganese, cobalt, molybdenum, chromium, tin, fluorine, nickel, and argon). Seven of these elements, usually referred to as macro-minerals and their requirement express as 100 part per million (ppm), and twenty-seven (27) micro- or trace minerals below 100ppm and requirement express in part per billion (ppb) are found in the body of animals (McDowell, 2005). Chickens require forty-three (43) nutrients for optimum productivity (Ogunwole, 2009) which include13 vitamins (A, B_1, B_2, B_3, B_6, B_{12}, Folic acid, E, K, Choline, D, Pantothenic and Biotin); and 13 minerals (Ca, P, Mg, Na. K, Fe, Cu, Cl, Mn, S, I, Mo and Zn). Mineral nutrients are inorganic compounds divided into two groups; macro-minerals and micro-minerals.

Macro-minerals are needed in relatively large amount. The macro-minerals include calcium, phosphorus, chlorine, magnesium, potassium and sodium. It has been reported (Chernick *et al*., 1948) that reduced availability of trace minerals and interference with enzymatic synthesis is among several growth-inhibitory factors in animals. Calcium is important for normal bone development, blood-clot formation, and muscle contraction and in maintaining good egg shell quality. Phosphorus also is important for normal bone development. It is a component of cellular membrane and a requirement for many metabolic functions. Chlorine is used in digestion as a component of hydrochloric acid found in the stomach. It is involved in water and acid/base balance in the body. Sodium and potassium are electrolytes that are important for metabolism, muscle and nerve functions. They are involved with water and acid/base balance. Magnesium assists with metabolism and muscle functions. The micro-or trace minerals are involved in metabolic functions and include copper, iron, iodine, manganese, selenium and zinc. Iodine is used to produce thyroid hormone that regulates the rate of energy metabolism. Zinc is involved with many enzymatic processes in the body. Iron aids in oxygen transportation but may be toxic at high level.

Ground limestone and oyster shell are the primary sources of calcium. Phosphorus and other calcium sources include mono-calcium phosphate, di-calcium phosphate, and de-fluorinated phosphate (Kershavarz and Nakajima, 1993). Common salt is the primary source of sodium and chlorine. The levels of magnesium, potassium and other minerals are supplied by dietary feed ingredients such as corn, soyabean meal and meat and bone meals. Nutritionists use traces minerals (micro-minerals) premixes when formulating ration to supply required amounts needed for production and maintenance (Larbier and Leclercq, 1997). Vitamins are a group of organic compounds found in feed in small amount. They constitute an essential parts of a good nutrition programme. Adequate intake levels of vitamin are necessary for normal body functions, growth and reproduction. Vitamin deficiencies can lead to a number of diseases, disorders or syndromes (Leeson, 2007). Vitamins can be divided into two classes base on their solubility in water and fat; fat-soluble and water-soluble. The fat-soluble vitamins include Vitamins A, D, E and K. Vitamin A is required for normal growth and development of epithelial tissues and reproduction in poultry (Leeson and Caston, 2003). Vitamin D is required for normal growth and development of bones and for egg shell formation (Leeson and Summers, 2001). Vitamin K is an essential part of blood-clot formation. Vitamin E is a powerful antioxidant (Mori *et al*., 2003).

The water-soluble vitamins include the B-complex (Vitamins B_{12}, biotin, choline, folic acid, niacin, pantothenic acid, pyridoxine, riboflavin and thiamine) and Vitamin C. The B-complex vitamins are involved in many metabolic functions including energy metabolism (McDowell, 2005). Birds can synthesize vitamin C and usually has no established requirement (Olomu, 2011; Majekodunmi, 2014). It may be beneficial in some circumstance, such as when birds are subjected to heat stress. Nutritionists usually add vitamin premixes to poultry diets to compensate for fluctuating levels found in natural animal feeds. This ensures that birds have required amounts necessary for normal productive efficiency (Majekodunmi, 2014). Vitamins are indispensable micro-nutrients that actively improve efficiency of Kreb or Citric cycle (Marks, 1979) and participate in body metabolism (Alahyari-Shahrab *et al*., 2011). Modern egg laying chickens often suffer from osteoporosis, a nutritional disorder of weakened skeletal system. During egg production, large amounts of calcium are transferred from bones for

formation of eggshell (Neijat *et al*., 2011). Although dietary calcium levels are adequate, absorption of dietary calcium is not always sufficient to fully replenish bone calcium given intensity of egg production. This can lead to increases in bone breakages, particularly when laying chickens are removed from cages at the end of lay.

Chickens are more susceptible to vitamins and mineral deficiency than any other species of poultry (Miles, 2001; McDowell, 2005).The gastro-intestinal tract in chickens is relatively short and permit faster rate of food passage. Also, microbial population in the gut of chickens provides very little synthesis of vitamins but competes with host for dietary supply (Leeson and Summers, 2001). Intensively managed laying chickens at high stocking density are quickly prone to vitamin deficiency. Vitamins A and D, riboflavin and B_{12} are usually found in low quantity in most poultry feed. Vitamins D and B_{12} are almost completely absent in maize-soyabean based-diets. Vitamin K is generally included in diets of chickens because their gastro-intestinal tract lacks synthetic ability for most vitamins (Rose *et al*., 1997). Tocopherol is a natural antioxidant, responsible for good keeping quality of animal products and improves utilization of vitamin A (Cerny *et al*., 1971). Vitamin E improves ovulation and reduces production stress. The concept of optimal input is used when formulating diets for vitamin and mineral requirements. The optimum input is an amount more requirements and satisfies all individual chicken in a laying flock (Optimum Vitamin Nutrition, 2010).

Minerals are essential for growth and egg production in laying chickens. Calcium and phosphorus are two important macro-minerals needed for egg production and good egg quality. Miller and Bearse (1934) found that approximately 0.8% phosphorous was required for optimum egg production when fixed calcium content of diet is 2.23 or 3.0%. Norris *et al*. (1934) found that 0.5% phosphorous was not sufficient for egg production but 0.75% was adequate. Schaible (1941) in a review concluded that 0.4% phosphorous was required but to allow for safety margin, 0.5% was recommended. The study by Evans and Carver (1942) reported that phosphorous requirement in diets is always considered alongside calcium requirements. When 1.5% calcium wass present, 0.6% phosphorous was adequate but if 2.5% calcium was added in diet, 0.8%

phosphorous was required. When 3.0% calcium was included in diet, 0.8% phosphorous was not satisfactory except 1.0%. Calcium requirement during egg production is an important mineral nutrient that determines eggshell quality.

Calbindin is a calcium-binding protein that improves eggshell quality (Heryanto *et al*., 1997). The mechanism for calcium transport to egg eggshell is related to vitamin D-dependent calcium absorption and a multifactor-dependent transfer of calcium to shell (Yosefi *et al*., 2003). These two steps are mediated by calbindin found in intestine and eggshell gland (Berry and Brake, 1991; Bar and Striem, 1999). Oestrogen is a reproductive hormone. This hormone is responsible for regulating calcium metabolism during eggshell formation (Etches, 1987). Calbindin concentration increase with onset of lay and decreases as egg production decline (Nys *et al*., 1989). There is a positive correlation between eggshell and shell gland calbindin (Nys *et al*., 1986). Park *et al*. (2004) found that feeding laying chickens with low-calcium diet less than 0.2 to 0.3% reduced rate of egg production to less than 5% within 10 to 14 days, and in some cases, a complete cessation of egg production within 21 days. Similarly, low-energy, low density and low-calcium diet was observed to paused egg production (Rolon *et al*., 1993). Structural bone losses due to poor calcium nutrition resulted in fragility and susceptibility to fracture during laying period (Whitehesd and Fleming, 2000). Gregory and Wilkins (1989) found that approximately 30% of laying chickens housed in batteries suffered at least one broken bone during their life time. Also, approximately one-third of broken bones occur in cages while remaining occurs during depopulation, transporting and processing.

The acid-base status of birds is determined primarily by amount of sodium, potassium chloridein diet under practical conditions. Excess dietary intake of sodium and/or potassium in relation to chloride leads to alkalosis, while excess intake of chloride results in acidosis. Sodium, chloride, and potassium are essential for maintenance of osmotic pressure, acid-base balance and fluid balance (Henry, 1995). Morgin (1981) reported an optimal growth performance in chicks fed purified diet using an electrolyte balance ($Na+ K^{+}- Cl^{-}$) of 250mEq/kg with a relation (K+Cl)/Na >1. Effect of dietary sodium level on water intake and droppings remained a controversial debate. There is a

controversy that excess dietary sodium in chicken increase excretion of moisture. Murakami *et al*. (1997) and Oviedon-Rondon *et al*. (2001) reported increased excretion of moisture which was linearly dependent on quantity of dietary sodium. Excess intake of sodium and potassium promote increase moisture in litter. However, increased water intake due to high dietary chloride seems unrelated to the wetness of poultry droppings (Oviedon-Rondon *et al*., 2001).

The dietary sodium requirement to achieve maximum growth in chickens was put at 0.20-0.28% (NRC, 1994; Murakami *et al*., 1997; Oviedon-Rondon *et al*., 2001). Smith *et al*. (2006) increased dietary sodium from 0.16 to 2.11% in layer diet and recorded a linear increase of 0.9% moisture excretion for 0.1% increase in dietary sodium. An increase of dietary sodium from 0.15 to 1.5% also resulted in linear moisture excretion with 10% moisture excretion and 0.52% dirty egg collected increase. Rolon *et al*. (1993) found that low-sodium diet less than 40ppm reduced rate of egg production to less than 5% within 14-21 days, and in some cases resulted in complete cessation of egg production within 4 hours. Damron *et al*. (1986) and Murakami *et al*. (1997) did not record any impairment in chickens fed sodium below 0.24 - 0.25% but observed a linear increase in water intake with increased dietary sodium supplementation. The water consumption of laying chickens increased by 2.9 folds and water: feed ratio by 6.7 folds increase when sodium supplementation increased from 0.16 to 2.11% (Smith *et al*., 2006). In broiler chickens and turkey, sodium bicarbonate is used as source of sodium to maintain body electrolyte, improve heat stress tolerance and keep litter dry. The same salt is used in laying chickens to mitigate heat stress and improve eggshell quality particularly in older layers.

Potassium is rapidly absorb from upper intestine and excreted from the body through urine. The mineral element is required for osmotic pressure regulation, maintenance of water and acid-base balance, nerve impulse conduction, muscle contraction and enzymatic reactions (Miller, 1995). An increase in dietary potassium causes corresponding increase in water consumption and moisture excretion. Study (Smith *et al*., 2008) showed that for every 0.1% increase in dietary potassium intake, there was increased excretion of moisture by 1.2% in laying chickens fed 0.23 to 2.0% potassium.

Addition of vitamin-mineral premixes to diets of poultry is a good insurance against nutritional deficiency and disorders.

2.6: Vitamin and mineral requirements of laying birds

Vitamins are organic substances needed in trace quantities for physiological and biochemical functions (Bolu, 2013). Balanced poultry feed requires feed additives for most vitamins. The effects of different dosage of feed additive were not only related to egg production, but also to their contents in liver and egg yolk as well as biochemical parameters (Whitehead, 1998). Laying hens requires vitamins A, D_3, E_3, K_3, B_1, B_2, B_6, B_{12}, Niacin, pantothenic acid, folic acid, biotin, and choline at levels of 2930, 295, 5, 0.5, 0.7, 2.5, 2.5, 0.004, 10, 2, 0.25, 0.1 and 1050IE/kg of feed, respectively (NRC, 1994). Leeson (2007) observed that NRC (1994) recommendations were not adequate for today's highly efficient layers. Pan (2005) and Leeson and Summers (2005) recommended a higher range of vitamin A level of 8000-11000 and 7000 – 12000IE/kg of feed, respectively compared to NRC (1994) recommendation. Other fat-soluble vitamins, vitamins D_3, E_2 and K_3were recommended for inclusion in feed at higher rate than recommendations by NRC (1994) for laying birds. Vitamins recommendations by Whitehead (1998) took into consideration B vitamins the contents in feed ingredients.

The mineral elements require by laying birds vary with body weight, rate of egg production, size of egg and breed and feed intake. Laying birds require calcium, non-phytine-P, available phosphorus, magnesium, sodium, chlorine. Magnesium, zinc, selenium, manganese, copper, iron and iodine are the most important trace element in for layers diet.The NRC (1994) recommendation for light strain layer weighing 1.8kg at 90% rate of egg production of egg were 32.5mg of calcium, 2.45mg of non-phytate phosphorus, 0.5mg of magnesium, 1.5mg of sodium and 1.3mg of chlorine per 100g feed intake per hen. Pan (2005) reported requirement of 35.0mg of calcium, 3.7mg of available phosphorus, 0.5mg of magnesium, 1.5mg of sodium and 1.6mg of chlorine for brown strain hen at above 85% egg production rate.

The addition of trace elements in feed supplements is carried out following specific recommendations. However, in designing feed supplements, trace elements in feed

ingredients are often ignored. This may lead to over-consumption and excessive levels in excreta. It has been demonstrated that some organic compounds of trace elements, especially selenium, had higher bio-availability than inorganic compounds in poultry (Bolu, 2013). Based on NRC (1994) nutrient requirement, laying birds require 446mg of iron, 34–44mg of zinc 20mg of manganese, 0.034-0.1mg of iodine and 0.06mg of selenium per kg of feed. Pan (2005) recommended a much higher level of these elements per kg of feed than NRC (1994) recommendation except for lower value of iron and absent of copper. Therefore selenium, cobalt, manganese, iron, zinc and copper are encouraged to be supplemented at varied levels of inclusion in diets of laying birds (Bolu, 2013).

2.7: Vitamin and mineral metabolism and immune systems

Nutrient metabolism provides information critical to performance and productivity of laying birds. The interactions between nutrition and immunity are important to growth and egg production. Nutrition modulates immune system of laying birds. The impacts of nutrients metabolism on immune-competence of birds are well documented (Cook, 1991; Koutsos and Klasing, 2001; Humphrey and Klasing, 2004). Immune responses to foreign bodies particularly pathogens influence nutrient requirement and metabolism in laying birds. Immune system is activated in order to play a major role in nutrient metabolism and production. Roura *et al*. (1992) reported that animals reared in germ-free conditions have higher growth and feed efficiency than those in a less sanitary environment. Therefore, exposing birds to high level of infection may result in slower growth and decreased accretion of many tissues (adipose tissues, liver, spleen and skeletal muscles) (Benson *et al*., 1993). Immune system produces regulatory factor which has systemic effects to alter nutrient partitioning or deter metabolic process associated with growth and egg production. The growth inhibiting effect of innate immunity on nutrient metabolism have been reported (Leshchinsky and Klasing, 2001; Humphrey and Klasing, 2004).

Earlier reports by Siegel *et al*. (1982), Martin *et al*. (1990), Qureshi and Havenstein (1994) and Parmentier *et al*. (1996) indicated that growth rate is inversely related to the level of adaptive immunity at genetic level. Klasing *et al*. (1987) observed that most

significant impact of infectious challenge on growth is the declines in feed intake which account for 70 per cent of decline in growth rate, and remaining 30 per cent due to inefficient nutritional metabolism induce by the immune system. Immune responses alter deposition of energy in form of lipid into adipose storage and fatty acid level in blood plasma. Lipoprotein lipases catalyze removal of fatty acids from plasma very-low density lipoprotein (VLDL) for tissues' usage. Study (Griffin and Butterwith, 1988) confirmed that lipopolysaccharides decrease lipoprotein lipase activity in chicken heart, adipose tissues, and skeletal muscles. The same effect was obtained on adipocytes by a chicken TNF-like cytokine (Butterwith and Griffin, 1989). Thus, there is increase in body fat level due to either innate or adaptive immune responses (Benson *et al*., 1993; Parmentier *et al*., 1996).

Vitamins and minerals are readily involved in body immune systems. Vitamin E is widely accepted for its effectiveness in inhibiting lipid peroxidation in biological systems (Kang *et al*., 1998; Lanari *et al*., 2004). Vitamin E increases humeral immunity in monogastric animals (Langweiler *et al*., 1983; Wuryastuti *et al*., 1993). Galobat *et al*. (2001) compared antioxidant activity from Rosemary extract (500 -1000 mg/kg) and vitamin E (200 mg/kg) and reported no significant difference in antioxidant activity on Thiobarbituric acid values. Vitamin E was highly transported to egg yolk in laying chickens (Grobass *et al*., 2002; Hayat *et al*., 2010). The metabolism of minerals is altered by immune systems. Selenium, copper, zinc and iron altered various components of immune system (Suttle and Jones, 1989). The interaction of mineral metabolism and immune system in animals is more profound with micro-mineral like copper, iron and zinc. Many alterations reflect hepatic production of their transport and storage protein during acute phase response. Plasma copper concentration increases during immune response along with copper containing protein, ceruloplasmin (Klasing *et al*., 1987; Tuff *et al*., 1988; Koh *et al*., 1996). Ceruloplasmin in positive acute phase is induced by IL-1β (Barber and Cousins, 1998).

Changes in ceruloplasmin are therefore related to dietary level of copper (Koh *et al*., 1996) so that higher dietary levels are required during innate immune response. Plasma concentrations of iron and zinc are known to decrease during immune response

(Klasing, 1984; Lauorin and Klasing, 1987; Tuff *et al*., 1988; Takahashi *et al*., 1997) by partitioning into the liver and other tissues for increase production of their protein storage forms, ferritin and metallothionein respectively (Klasing, 1984; Lauorin and Klasing, 1987). Iron is the first limiting mineral for bacterial growth and increases in susceptibility to disease (Knight *et al*., 1983). The severity of alteration in iron and zinc metabolism depends on the activity of antigens and the type of immune system. The activities of antigen that elicit innate immune response trigger the greatest decline in plasma iron and zinc concentration. Hence, repeated exposure of chickens to antigens promotes adaptive immune responses which cause reduction in plasma iron and zinc concentration too (Klasing, 1984). Thus, microbial immunogens produce larger changes in iron, zinc and copper metabolism than protein antigen which elicit innate responses (Klasing, 1984; Klasing *et al*., 1987).

2.8: The use of vitamin-mineral premixes in poultry nutrition

The use of vitamin-mineral premixes in poultry is well documented (Oduguwa and Ogunmodede, 1995; Oduguwa *et al*., 1996; Al-Nassar *et al*., 1998; Dingle and Henuk, 1999; Oduguwa *et al*., 2000; Asadumzzaman *et al*., 2005; Ogunwole, 2009; Ogunwole *et al*., 2012). Premix is a concentrated mixture of vitamins, trace minerals and diluents. It may contain other feed additives such as amino acids or medicaments. Vitamin-mineral premix is nutritional condiment that amongst others increase cost efficiency and laying ability of commercial chicken from an average of 150 eggs to about 330 eggs per lay cycle (Ogunwole. 2009). Vitamin-mineral premixes contain specific vitamins and minerals in amount and proportion recommended by the manufacturer for addition in poultry feed. Vitamin-mineral premix is required by animals due to the dynamics of unavailability from natural feed ingredients (Bolu, 2013). They come in different sizes, contents and composition as propounded by the proprietors and commercially sold in different locality.

Vitamin-mineral premix is a critical dietary input for improved safety, reliability and performance as well as successful poultry production (Raven and Walker, 1980). Although minerals and vitamins contribute only 10 per cent of the total cost of feed (Singh and Panda, 1988), the effects of using substandard or less potent vitamins on

production could easily be felt in poultry production. When formulating poultry diets, care and professional attention should be taken in the choice of vitamin-mineral premixes used (Ogunwole *et al*., 2013). Several proprietary vitamin-mineral premixes are sold in Nigeria with each manufacturer ascribing similar effectiveness and potency. The labeled composition on each proprietay vitamin-mineral premix claims high potency and efficacy claim without any cognate experimental evidence (Ogunwole *et al*., 2013). This situation is further compounded by the dearth or lack of suitable equipment and laboratory to undertake analyses of vitamin and mineral contents. The slower but rational approach is the use of live animals to assess the premixes. There have been studies on single use of vitamin-mineral premix in poultry nutrition (Oduguwa and Ogunmodede, 1995; Oduguwa *et al*., 1996) and several others on mix of vitamin-mineral premixes and their effects on specific parameters in poultry nutrition (Oduguwa *et al.*, 2000; Asaduzzman *et al*., 2005; Ogunwole *et al*., 2013). Inclusion of vitamin-mineral premix in formulated diet has become indispensable practice because feed ingredients do not contain all essential vitamins and minerals at the right amounts needed for chicken (Asaduzzaman *et al*., 2005). Diets formulated without vitamin-mineral premix may be nutrient deficient (McDonald, 1996).

Chickens managed under intensive systems of production are usually susceptible to vitamin-mineral deficiencies. Therefore, it is a general practice to include all supplemental vitamins-minerals premix at levels that provide margins of adequate safety under various stress conditions (Scott *et al*., 1982). For laying chickens, provision of adequate dietary minerals and vitamins is essential for good eggshell quality (Yoruk *et al*., 2004), while non-inclusion restrict performance of birds with heavy ecomomic losses. Birds in cages require more attention for supply of vitamin-mineral premix than those of floor housing because of more limited opportunity for natural behviours (Asaduzzaman *et al*., 2005). Vitamins are essential for growth, health, and survival. They are involved all cellular metabolism critical to efficiency of Krebs/Citric Acid cycle (Marks, 1979). For laying chickens,Optimum Vitamin Nutrient-diets (Optimun Vitamin Nutrition, 2010) increased egg weights, number of large eggs, lower percentage of broken eggs, higher percentage of lay and improved feed efficiency (McDowel, 1996).

The dietary water soluble vitamins affect vitamin egg white concentration (House, 2002). Riboflavin, folic acids, niacin, thiamine, pyridoxine, panthotenic acid, biotin, vitamin B12 are well transferred into egg white, and their concentrations depend on dietary consumption (Leeson and Caston, 2003). Ascorbic acid supplementation has beneficial effect on growth rate, egg production, egg shell strength, and thickness in stressed-poultry (Thornton, 1962; McDowell, 1989). Vitamin D, calcium, phosphorous, manganese, copper and zinc play a major role in maintaining eggshell integrity and quality, while excess or reduced concentration of phosphorous, chlorine, influence availability of calcium and vitamin D (Neospark, 2012). The inclusion of different vitamin D metabolites in diet enhances effect of vitamin D due to availability, sparing chain reactions required for synthesis of active metabolite (Nascimento *et al*., 2014). Critical vitamins like choline, folic acid, pantothenic acid, pyridoxine, riboflavin, Vit-A, Vit-D and Vit-E) and minerals e.g. calcium, phosphorus, copper, iodine, iron, manganese, sodium and zinc,are compulsorily added to diet (Asaduzzaman *et al*., 2005). Vitamin K plays an important role in blood clotting. Vitamin K deficiency can result in an increased occurrence of blood spots (Bains, 1999).

Trace mineral nutrition is a complex area of animal nutrition. A wide range of interactions and antagonisms occur in poorly absorbed or utilized essential minerals, particularly during shell formation (Burley and Vadehra, 1989). Trace elements affect eggshell quality. They serve as key enzymes involved in formation of membrane and eggshell or by direct interaction with calcite crystals during shell formation (Zamani *et al*., 2005). Mabel *et al*. (2003) reported that trace elements such as Mn, Zn, and Cu influence mechanical properties of eggshell. However, earlier studies (Mas and Arola, 1985; Miles, 2001) revealed that provision of adequate amounts of zinc, copper, iron and manganesein diets of laying chickens is key components of shell matrix and play an essential role as co-enzymes in shell and its associated membranes. Trace elements are transferred into egg white. Scatolini (2007) evaluatedquality of eggs produced by laying chickens fed supplemental inorganic and organic Mn, Zn, Se, Fe and Cu and stored for 14 days at environmental temperature. The results of this study indicated that organic trace minerals allowed maintenance of egg weight during the experimental period.

Eggs from layers fed a combination of organic Mn and Zn lost less weight than eggs of layers fed organic Zn and Se, while treatment with organic Mn presented the lowest Haugh Unit. These results were different from treatment containing combination of organic Mn and Se and there was no influence of treatments on yolk index. Correia *et al.* (2000) fed layers with feeds supplemented with or without organic selenium observed no effect on external or internal egg quality of eggs stored at environmental temperature for up to 21 days. However, Zamani *et al*. (2005) reported that addition of organic Mn and Zn influenced internal egg quality when eggs were stored up to 20 days independently of storage temperature. This suggested that combined supplementation of organic Se and Zn improved internal egg quality and extend egg shelf-life. Zamani *et al*. (2005) indicated that Zinc is commonly supplemented in diets of laying chickens and other livestock because most feed ingredients are marginally Zn-deficient. Organic complexes of zinc are readily available sources of zinc for laying chickens. They are metabolized differently than inorganic forms (Aliarabi *et al*., 2007). Tabidi (2011) reported that diets of brown parent stock layers should include 180 mg zinc /kg for optimal performance and hatchability traits.

2.9: Housing systems and performance of laying chickens

The effects of different housing systems on egg production indices of poultry abound in literature (Al-Rawi and Abu-Ashour, 1983; Anderson and Adams, 1994a; Abrahamsson *et al*., 1996; Pistikova *et al*., 2006; Voslářova *et al*., 2006; Banga-Mboko *et al*., 2010). The evidences from these studies show comparative advantage for birds in conventional battery cage to include; increase spatial density of birds, easier control of microclimate, simplified waste disposal, reduced labour costs and easier supervision of individual birds for production level and health status. The egg production of laying birds in battery cage was significantly higher than in deep litter. The eggshell thicknesses of birds in cages were also greater than those in the deep litter. Earlier reports by Al-Rawi and Abu-Ashour (1983) showed that laying birds in deep litter had higher laying rate and consumed more feed than those in cages. In a study, Voslářová *et al*. (2006) compared performance of laying Isa Brown hybrid kept in cages and deep litter and fed diets that contained meat and bone meal replaced by vegetable feeds (lupin) in hot climates. The number of eggs laid, egg weight, shell quality, clinical state of birds and

mortality daily over a period of nine months were recorded. The authors reported that birds in cages had higher number of eggs; lower mean egg weight ($p<0.01$); higher number of eggs per bird per day ($p<0.01$); and higher egg mass weight per bird per day ($p<0.01$). The number of cracked eggs ($p<0.01$) was reported higher ($p<0.05$), while number of membranous eggs laid was not different ($p>0.05$). The mortality was lower ($p<0.05$) in in deep litter system. These authors concluded that differences in the egg production indices of laying birds in deep litter and battery cage system and deep litter met animal welfare requirements despite lower egg production.

The responses of laying birds in battery cage and deep litter under tropical climate in Congo Brazzaville using a sample of 3,620 laying birds in two groups of 1,660 each were evaluated by Bannga-Mboko *et al*. (2010). Each group of birds was replicated four times (415 hens x 4) and separately transferred into battery cages (first group). The second group of birds was raised in deep litter. Feed and water were supplied *ad libitum*. The two groups were compared on data collected during 70 days on egg production indices, egg and shell quality and food efficiency. This study showed that birds in battery cage improved significantly ($p<0.05$) in egg number (+55%), egg-laying rate (+ 25.3%), mass egg (+59.6%) and egg weight (+2.3%). Also, the feed consumption (199.2 versus 155.7g/hen/day) and feed efficiency (2.7 versus 3.42) were better ($p<0.05$) in caged birds than those raised in deep litter. However, the caged birds were observed to produce more broken eggs (+1.08%) and there was no difference in egg shell quality. Battery cage system was better preferred because birds recorded higher egg production and better feed efficiency as these indices are major determinants of revenue in commercial egg production. The authors suggested the need to evaluate and validate economic profitability in each housing system because of the high percentage of broken eggs in cages, and high cost of battery cage.

The diet of laying birds consists primarily of corn and soybean meal with addition of essential vitamins and minerals. Laying birds are not fed on too high protein diets which may results in high growth rate and fat accumulation in the body. This could make laying birds suffer high leg deformities because of development of large breast muscles which could cause distortions in developing legs and pelvis. Laying birds, in particular

those in cagescannot support increase in body weight since additional weight puts strain on the hearts and lungs to cause Ascites. In deep litter, birds are kept indoors with more floor space requirement which allow for feeding, exercise, perching, mating and nesting boxes. Birds in deep litter are expose to richer natural environment in terms of provision of natural nutrients that encourages foraging on litter and excreta materials to meet some nutrient requirements (http://en.wilkipediaorg/w/ poultry_production; Asaduzzaman *et al*., 2005). Birds in deep litter therefore tend to grow more slowly and live longer than those in cages.

2.10: Composition, formation and structure of a chicken egg

Eggs are important part of human food since ancient times (McGee, 2004) and one of nature's nearly perfect supplies of protein foods. They contain readily digested nutrients required daily for growth and maintenance of body tissues and utilized in many ways in food industry. Chicken eggs are more importance than eggs of other poultry species like geese, ducks, plovers, and seagull's and quail (McGee, 2004). Chicken eggs provide valuable nutrients such as proteins of outstanding biological value, phospholipids, minerals and vitamins (De Ketelaere *et al*., 2004). The average composition of 60 grams chicken egg by USDA (2000) is given as 29% yolk, 61.5% albumen and 9.5% shell. The chemical composition of a 58g chicken egg was recently quoted as; water (~74%), protein; (~12%), and lipids; (~11%); 56-61% egg white; and 27-32% egg yolk (www.egghealth.com, 2014). In a related studies, Li-Chin *et al*. (1995) and Kiosseoglou (2004) reported that egg white constitute 67-89% water and 9-11% of protein, whereas egg-yolk contain 50% water, 32-35% lipid and 16% protein.

The eggshell contains 95-97% calcium carbonate crystals, 0.3% phosphorous and magnesium and traces of sodium, potassium, zinc, manganese, iron and copper and organic matter (Arias *et al*., 2001; Nys *et al*., 2004; Neospark, 2012) which makes it a rich source of calcium. An egg is formed gradually over a period of about 26 hours in birds' reproductive system. Many organs help to convert raw materials from the feed eaten by the hen into the various substances that become part of the egg. The hen, unlike most animals, has only one functional ovary situated in the body cavity near the backbone. At maturity, a female chick has up to 4000 tiny ova (reproductive cells) from

which full-sized yolks developed to form eggs. Each yolk (ovum) is enclosed in a thin-walled sac, or follicle attached to the ovary. This sac is richly supplied with blood. The mature yolk is released when the sac ruptures and received by the funnel at the left oviduct (the right oviduct is not functional).

The left oviduct is a coiled or folded tube about 80 cm in length. It is divided into five distinct sections, each with a specific function. An egg is surrounded by 0.2–0.4 mm thick calcareous and porous shell. The structure and composition of the eggshell are designed to naturally protect eggs against damage and microbial contamination, loss of moisture, regulate exchange of gases for the growing embryo, and provide calcium for embryogenesis (Hunton, 2005).The eggshell consists of calcite crystals embedded in an organic matrix or framework of interwoven protein fibers and spherical masses. The shell structure is divided into four parts: the cuticle or bloom, spongy layer, mammillary layer and pores (Belitz *et al*., 2009). Eggshells in chicken are white-yellow to brown, greenish to white in ducks and characteristically spotted in most wild birds. The inner structure of shell is lined with two (inner and outer) closely adhering membranes (McGee, 2004). The two membranes are separate at the large end of egg to form an air space called air cell. The inside of eggshell has two membranes; the outer membrane is attached to the shell while the inner membrane is attached to the albumen or egg white.

These two membranes provide a protective barrier against bacterial penetration. An air space or air cell is a pocket of air usually found at the interior large end of eggs between the outer and inner membranes. Air cell is created by contraction of inner contents it cools and evaporates moisture after laid. Air cell increases with days of storage (Belitz *et al*., 2009). Air cell is approximately 5mm in diameter in fresh eggs. The air cell can be used to determine age of eggs. Egg albumen is an aqueous, faintly straw-tinted, gel-like liquid, consisting of three fractions that differ in viscosity (www.egghealth.com, 2014).The thin and thick albumen is build-up from four layers that surround yolk. The first layer is the thick albumen close to yolk and adjacent to vitelline membrane, while the second layer is composed of thin albumen, followed by another layer of thick albumen and finally a layer of thin albumen closest to shell membranes (Stadelman and Cotterill, 1995). There is another type of albumen formed as long twisted fibres called

chalazae, a structure that keep yolk in central position in eggs. The chalazae resemble two twisted rope-like cords, twisted clockwise at the large end of the egg and counter clockwise at the small end. They serve as anchors to keep the yolk in the centre of eggs.

Chalazae is positioned at each side of the yolk, attached with one end to the surface of vitelline membrane and other interlaced with fibres in thick albumen layer closest to yolk (Rose *et al.*, 1997). The proteins albumen includes ovalbumin, conalbumin (ovotransferin), ovomucoid, lysozyme and ovomucin (Parkinson, 1966). The pH of albumen ranged from 8.2 to 9.0 (Toney and Bergquist, 1983). Egg albumen is water storage depot containing approximately 88% water (Farooq *et al.*, 2001). Moreover, albumen supplies some nutrients approximately 11% protein, 1% carbohydrates and minimal amount of fat (Rose, *et al.*, 1997). Egg-yolk is surrounded by a thin but very firm layer of albumen (chalaziferous layer) which branches on opposite sides into two chalazae and extends into thick albumen (McGee, 2004). In an opened egg, the chalazae remain with the yolk. The germinal disc (blastoderm) is located at the top of a club-shaped latebra on one side of the yolk. Egg yolk is almost spherical and surrounded by a colourless membrane. It is a mixture of particles and plasma of low density globules rich in fat (Parkinson, 1966). It contains high capacity for pigmentation of yellow yolk globules. The yolk colour is determined by amount of xanthophyll, a yellow colouring pigment present in maize which does not affect nutritional quality of eggs. The yolk consists of alternate layers of dark- and light-coloured material arranged concentrically (www.egghealth.com/2014).

2.11: Egg quality characteristics

Eggs are vehicle for reproduction and a staple food in human diets because of their balanced nutrient composition. They are fragile poultry products which can be subjected to quality loss with age. Eggs quality characteristics have natural variability and often fail to meet the requirement for consistency and consumers' demand. Egg quality characteristics are influenced by a variety of factors including genetics, hen age, body weight, feed quality, length of holding period and environment (Silversides, 1994; Monria *et al*., 2003; Silversides and Budgell, 2004; Van den Brand *et al*., 2004). The age of laying chicken is most important factor that determines egg quality because

young pullets produce smaller eggs with strong egg shell and albumen that stand high. As laying flock aged, eggshell thins and albumen begins to weaken and run. The flock can be moulted to induce egg cycle which improve egg quality or by replacement with young pullets. In the humid tropics, natural environment are characterized by daily and seasonal fluctuations in temperature and relative humidity.

Temperature and relative humidity are two main indices of stress. Arima *et al.* (1976) reported that egg qualities of older birds were more severely affected by increased temperature than younger ones. A planned nutrition and good quality control procedure could help to reduce variation in egg quality. Egg quality encompasses a number of aspects that relate to shell, the albumen and the yolk and usually classify as external and internal egg quality, respectively. External quality of eggs is the index that appeal to consumers' patronage and influenced by degree of defects. Stadelman (1977) stated that egg quality is composed of characteristics that affect consumers' acceptability. Egg shell is therefore assessed on the basis of shape, texture and soundness. The internal quality is based on air cell size, albumen quality, yolk quality and presences of blood or meat spots. Albumen quality is a major indicator of overall interior egg quality. Thinning of albumen is a sign of quality loss. When a fresh egg is carefully broken on a smooth flat surface, egg-yolk remains in central position surrounded by thick albumen. When a stale egg is broken, egg-yolk become flattened and displaced to one side with thick albumen becoming thinner resulting in large area of albumen which collapse and flatten to produce a wide arc of liquid.

This principle is used in measuring Haugh Unit, an indicator of albumen quality (Haugh, 1937). Egg-yolk quality is related to its appearance, texture, firmness and smell. Egg quality evaluation information is fundamental for successful handling and transporting during marketing of eggs (Altuntas and Sekkeroglu, 2007). Egg quality measurements have application in eggs grading for enhancement of safety and quality assurance as well as need for farmers and feed millers to monitor outcome of feed and feeding, animal health status and ambient condition in housing system. Many egg quality characteristics can be quantified to determine physical (internal and external)

and chemical qualities. Egg value is determined by standards based on interior and exterior characteristics

2.12: External egg quality characteristics

Shell quality appeal to consumers' patronage. It is a major factor for consideration during handling, packaging, storage, transporting and hatchery operation (Rogue and Soares, 1994; Kemps *et al*., 2006; King'ori, 2011). Hamilton, (1982) stated some of the external eggs quality traits as egg shell colour, shell thickness, shell weight, egg weight, egg shape index which are highly affected by breed and age of chicken, molting, level of nutrition, prevalence of disease and type production system. Egg colour is one of the external characteristics that influence grading, price, consumer preference and hatchability (King'ori, 2011). Egg colour is considered as external quality characteristic that protects egg from harmful solar radiation (Lahti, 2008), reinforce eggshell structure (Gosler *et al*., 2005) and protect developing embryos from thermal deterioration (Bakken *et al*., 1978). Although shell colour have genetic component, there are several other factors that influence intensity of eggshell colour. Obadaşi *et al*. (2007) reported that size of egg affected colour of eggshell with assumption that older laying birds lay lighter coloured eggs due to increase in egg size associated with proportionate change in quantity of pigment deposited over shell surface.

Butcher and Miles (1995) studied relationship between stress and eggshell colour in laying birds. The authors reported that loss of shell pigment may provide a basis for a non-invasive method of assessing stress in laying birds. The strength, texture, porosity, shape, cleanliness, soundness, and colour are used in determining shell quality (Natalie, 2009). Large sized eggs break more easily than small ones as laying birds are genetically could be prone to depositing finite amount of calcium in shell (Neospark, 2012). Poor eggshell quality has been of major economic concern in commercial egg industry (Washburn, 1982). Reduction in shell quality lowers egg shelf-life, hatchability and increases breakage. Shell thickness and porosity regulate exchange of carbon dioxide and oxygen between developing embryo and extental environment during incubation (Rogue and Soares, 1994). Thin shelled eggs loose more moisture than do thick shelled eggs; possess serious difficulty of hatching (Rogue *et al.*, 1994) and

deteriorates in quality (Bennett, 1992). The shell of table-eggs must be strong enough to prevent failure during packing and/ transportation (Pavlovski *et al*., 2012), while shells of hatching eggs must be initially thick and strong to preserve embryo but become thin and weak later during incubation in order to allow gaseous exchange and easy chick hatching (Roland, 2000).

Some category of eggshell defects that make eggs loss integrity include gross cracks, hairline cracks, star cracks and thin shelled or shell-less eggs. Cracked eggs attract lower patronage and lower monetary value (Natalie, 2009). Cracking of eggs could be due to mechanical damage by birds and poor management practices such as infrequent collection of eggs, rough handling and poor design and/ or maintenance of cage floor (Natalie, 2009). The strength of eggshell affects soundness of shell, with weaker shelled-eggs more prone to breakages and microbial contamination (King'ori, 2011). Eggshell strength is affected by age of birds, egg size and stress (Coutts and Wilson, 1990; Butcher and Miles, 2003). Butcher and Miles (2003) reported that birds lay bigger eggs as they grow older with an implication on shell strength (Butcher and Miles, 2003). Coutts and Wilson (1990) reported that young birds have immature shell glands that produce shell-less eggs or eggs with very thin shells but when onset of sexual maturity was delayed by one to two weeks, incidence of shell-less eggs or eggs with very thin shells was insignificant. The authors observed that smaller eggs have stronger shells than larger ones because birds have finite capacity to deposit calcium in shell so that same amount of calcium are spread over a larger area of shell. Stress de-synchronise process of egg formation e.g. oviposition prior to completion of shell deposition which results in soft or thin-shelled eggs (Coutts and Wilson, 1990).

2.12.1: Egg shape index (SI)

The determination of egg shape index is a matter of natural convenience rather than aesthetic consideration. The overall shape of an egg should be smooth in order to assist laying birds (Abanikannda *et al.*, 2007). Panda (1996) defined egg shape index as ratio of short border relative to long border. Egg length, which is also referred to as height, is the longest portion observed on external egg surface. Egg width is shortest portion of the egg and referred to as breadth or short border where the dense mass of yolk is

situated (Gunlu *et al*., 2003). Abanikannda *et al*. (2007) also reported relationship between egg weight, egg length and egg width. The shell shape and weight are dependent on heredity, age of bird, season of the year and diet (Izat *et al*., 1985). Burtov *et al*. (1990) reported that eggs of normal shape hatch more successfully than those with shaped abnormally. Shape index is a measurement of overall shape of an egg. There are three classification of egg shape: sharp (SI of<72), normal (SI of 72-76) and round (SI>76), most prevalent in commercial egg production. Eggs outside normal range do not fit into pre-made packaging while sharp eggs are not as resistant to handling and transporting processes (Altuntas and Sekkeroglu, 2007).

To calculate shape index, the egg diameter or width (ED) and egg length (EL) are measured in mm using callipers. The egg diameter (EW) is then divided by egg length (EL) and multiplied by 100 (Van den Brand *et al*., 2004). These authors evaluated egg shape index of 776 eggs from layers aged 25 to 59 weeks at 4-week interval (25, 29, 33, 37, 41, 45, 49, 55 and 59 weeks) within 3 hours of egg lay and observed that shape index decreased steadily from 77.02 to 72.85±0.29 ($p<0.05$) and were classified as normal shaped eggs. Anderson *et al*. (2004) using 6 hens per cage in a tri-deck system at 18 week collected and measured egg shape index starting at 28 week and continuing through second production cycle with a moult occurring at 62 week reported an overall long-term shape index with a base population from 1950; SI of 71.54 classified as sharp and for strain derivatives from 1959 SI of 72.48; 1970 SI of 73.59; 1993 SI of 74.76 ($p<0.05$), indicating possible selection for larger normal round eggs.

Popva-Ralcheva *et al*. (2009) examined effect of age and genotype on egg quality characteristics using eggs from hens at 32 and 50 weeks and observed that shape index increase with age of hens. The shape index ranged from 75.88±0.7 to 78.45±0.7 in eggs from the hens at 32 week and 73.46±0.84 to 76.29±0.52 in eggs from hens at 50 week. There was a numerical decrease in shape index from 32 to 50 week suggesting that as laying flock aged, egg shape become more normal. Shape index is an important consideration during processing and marketing procedure particularly for pre-made packaging. Normal shaped eggs are ideal for fitting into pre-made package containers and provide more strength to eggshell compared to sharp eggs, making them more

resistant to breakage during handling and transportation (Altuntas and Sekkeroglu, 2007). Additionally, uniformity in egg shape is important as market for further processing continues to grow. The efficiency of market is based on use of automatic breakers and conformity in egg shape to machine specification.

2.12.2: Egg weight (Ew)

Egg weight is an important egg trait which influences quality as well as grading (Farooq *et al.,* 2001). It is one of egg quality parameters determined without breaking the eggs (Wilson, 1991). Egg weight has direct relation with weight of albumen, yolk and shell **(**Pandey *et al*., 1986). It varies significantly with strains (Brake *et al*., 1997). Tixier-Boichard *et al.* (2006) recorded weight of 42.8g for Fayoumi eggs and 58.8g Isa Brown eggs. The hens' age affects proportion of yolk, albumen and shell and egg weight increases with hens' age, reaching a plateau at the end of lay cycle (Scott and Silverside, 2000). Hocking *et al.,* (2003) reported that higher weight of egg from commercial strains is not a surprise since such strains submitted to important breeding pressure for egg weight improvement. Egg weight is measured simply by placing an unbroken egg on sensitive weighing scale. It is greatly influenced by genetic, nutrition and other environmental factors (Silversides, 1994; Monria *et al*., 2003; Silversides and Budgell, 2004; Van den Brand *et al*., 2004). Laying flock with heavier body weight produce smaller eggs relative to their body size while those with lighter body weight produce larger eggs.

There are six different egg weight categories; jumbo (68.6g), extra-large (61.5g), large (54.4g), medium (47.3g), small (40.3g) and peewee (no minimum requirement). Egg weight, unlike all other quality characteristics does not decrease with age of laying flock (Silversides, 1994; Tharrington *et al*., 1999; Ledur *et al.*, 2002; Van den Brand *et al*., 2004; Altuntas and Sekkeroglu, 2007). Silversides (1994) in a study of sex-linked gene for imperfect albinism on egg production collected eggs at 30, 45, 60 and 75 weeks of age and stored overnight at 4^{o}C. The authors reported steady increase in egg weight with age of laying chickens. The overall egg weight at 30 week was 52.56±0.25g which increased ($p<0.05$) over four measurements to 60.13±0.25g at 75 week. The results clearly indicate that egg weight numerically increased across four measurements,

though the increase was not significantly different between last two measurements to signal a leveling off. Tharrington *et al*. (1999) assessed the quality and composition of eggs as influenced by genetic selection using the same strains used by Anderson *et al*. (2004) with initial measurement at 28 week and taken over a period of 60 weeks. Eggs were collected within 24 hours of lay tested for Specific Density (SG), dried and stored overnight at 5^{o}C; on the next day, 10 egg samples from each strain were weighed and broken for further analysis. The observed values were not reported but egg weight increased progressively with strains ($p<0.05$) and moulting at 63 week caused increase in egg weight to level off. Van den Brand *et al*. (2004) in a study found that an average egg weight from laying flock at 25 week was 49.21±0.43g which steadily increased to 61.01±0.43g ($p<0.05$) at 59 week indicating that egg weight increased with age. In all the studies x-ray, egg weight increased at decreasing rate with age of laying flock. The increase in egg weight in recently developed strains shows that larger egg weight is desirable and could be selected genetically.

2.12.3: Eggshell Weight (EW)

Eggshell weight is the weight of shell portion of egg alone, although procedure varies. Eggs are rinsed and set upside down to drain. The membrane inside of eggshell may be removed during rinsing or included prior to weighing. The eggshell is dried either by air, fume hood (Anderson *et al*., 2004) or oven at 100^{o}C (Silversides, 1994). Eggshell weight increased with age of laying flock (Silversides, 1994; Silversides and Budgell, 2004; Popova-Ralcheva *et al*., 2009). In a study of comparison of albino and non-albino strains of layers with commercial layers at 30 week, Silversides (1994) reported average eggshell weight of 5.44g with an increase ($p<0.05$) of 15g at 45 week ($p<0.05$) and decreases ($p<0.05$) with average values of 5.50g and 5.35g at weeks 60 and 75, respectively. Commercial strains of layer had significantly ($p<0.05$) heavier eggshells than selected strains with eggshell weight measuring 5.88±0.04 and 6.13±0.04g, respectively. The increase weight of eggshells in commercial strains was consistent with increase in egg weight. Anderson *et al*. (2004) in a study of four strains of White Leghorn measured eggshell weight and found heavier eggshell in recently developed strains, while older strains had average eggshell weight of 5.28g.

The second strains recorded an average value slightly higher though insignificantly different from the oldest strains.The younger strains recorded significantly ($p<0.05$) increase average eggshell weight 0.35 and 0.52g when compared to the oldest strains. These increases were due in part to the selection for birds of heavier egg weight.

Popva-Ralcheva *et al*. (2009) in agreement with the findings of Silversides (1994) reported that eggshell weight varied in eggs produced from week 32 to week 50. These authors in agreement with Silversides (1994) submitted that eggshell weight numerically increased with age of laying flock from 0.32 to 1.18±0.02g. However, two groups of birds were observed to decrease in eggshell weight (0.04 and 0.23±0.18 g) with age of layers. The values of eggshell weight were inconsistent because newly developed strains of laying flock produced eggs which were heavier in shells than older strains. Silversides (1994) reported that eggshell varied with age of laying flock. The eggshell weight was observed to increase at first with age but later decreased after 60 week. Popva-Ralcheva *et al*. (2009) reported that there was no specific pattern of variation but commercial strains produced eggs that were heavier than those selected for or against albinism. The variations were reported to be due to differences in egg weight, strains and age of laying flock (Silversides, 1994).

2.12.4: Eggshell Thickness (ET)

Eggshell thickness measurement is taken along mid-line of an egg using micrometer. It is done after egg has been broken. Eggshell strength is highly dependent on egg thickness (Zeidler, 2002). The values of egg thickness vary slightly across similar breeds of chicken (Potts *et al*., 1974; Anderson *et al*., 2004) and can either decrease (Anderson *et al*., 2004) or remain constant as laying flock increases in age (Van de Brand *et al*., 2004). Eggshell thickness is affected by temperatures above 32^{o}C, age of laying chickens and dietary calcium below 3% (Zeidler, 2002). An eggshell thickness of at least 0.33 mm has been estimated to be necessary for at least 50% chance of withstanding normal handling conditions without breakage (Stadelman, 1995). Van de Brand *et al*. (2004) reported that eggshell thickness did not vary with age of laying flock ($p>0.05$) but differences ($p<0.05$) was observed among strains with eggshell thickest of

0.344±0.003mm and thinnest eggshell of 0.295±0.003mm. This distribution corresponds with values obtained for eggshell percentage.

Eggshell percentage is weight of eggshell as a percentage of total egg weight. The authors reported 12.87-12.36±0.11% ($p<0.05$) as ranged value obtained for thicker eggs. Anderson *et al*. (2004) in a study to determine significance of eggshell selection in breeding programme, dried eggshells under fume hood to constant weight and measured eggshell thickness at two different locations near mid-line. There were no significant differences in eggshell thickness. Potts *et al*. (1974) evaluated breaking strength eggshell thickness and specific gravity among brown and white eggs. They reported that the thickness brown shelled eggs were not significantly ($p>0.05$) different and ranged from 0.322 mm to 0.330 mm. The thickness of white shelled-eggs ranged from 0.330 mm to 0.353 mm. This finding showed little variation in eggshell thickness differences between white and brown eggs of similar breeds of chickens. The little variation could be attributed to factors that affect eggshell thickness including temperatures over 32^0 C and low dietary calcium levels.

2.13: Internal egg quality characteristics

The interior egg quality is based on albumen quality, yolk quality and the presence of blood or meat spots (Jacob *et al*., 2000). Sinha and Giri (1989) described internal egg quality as a measured of factors like yolk colour, albumen height, yolk height, Haugh unit, yolk width and nutritive values. High quality egg contents are indicated by firm and thick albumen and yolks which contains no blood or meat spots (Ihsan, 2012). Study conducted by Jones (2006) showed that internal egg quality traits are functions of albumen height and weight and yolk index. However, Jacob *et al.* (2000) reported that interior egg quality could also be based on air cell size, albumen quality and yolk quality. Kul and Seeker (2004) reported internal egg quality traits based on the albumen weight, height, ratio and Haugh Unit, and yolk diameter, height, weight, index and ratio. The best indicators of internal egg quality traits were yolk index and Haugh Unit (Isikwenu *et al.*, 1999). The higher the yolk index and Haugh Unit, the more desirable is egg internal quality (Jones, 2006).

2.13.1: Albumen quality

The albumen quality is influenced by factors like genetic, environmental (such as temperature, relative humidity and the presence of CO_2), bird's age, nutrition status, egg storage condition and storage time (Jones, 2006). A good quality egg is free from internal blemishes such as blood spots, pigment spots and meat spots (Robert, 2004). High foam-forming quality in eggs correlates with high albumen viscosity which is measured in terms of albumen height and thick of egg on flat surface (Silversides and Budgell, 2004). Albumen refers to as egg 'white' and consists of thick and thin portion. The thick albumen is portion immediately surrounding egg yolk, where thin albumen comprises the rest of white portion. Albumen quality is measured in terms of Haugh Units (HU) and calculated from albumen height and egg weight (Haugh, 1937; Coutts and Wilson, 1990). This is calculated as logarithm of thick albumen with an adjustment for differences in egg weight (Haugh, 1937, Silversides, 1994). The albumen height indicates egg freshness and is measured using tripod micrometer. Once an egg is broken onto a flat surface, tripod micrometer is placed over thick albumen. The center pin is lowered until it ''kisses'' albumen which measured albumen height in cm or mm. The thicker the albumen; the better the egg quality Eggs with albumen height ranging from 8 to 10mm are considered to have superior interior quality (Zeidler, 2002). It is also possible to theoretically calculate albumen height from Haugh Unit (Haugh, 1937; William, 1992) using the formula below.

$$\mathbf{Hu = 100 \log_{10} (AH - 1.7\, EW^{0.37} + 7.57)}$$

where AH is the albumen height in mm and EW is the egg weight in g.

The higher is value of Haugh Unit; the better is quality of eggs (Chukwuka *et al.*, 2011). Haugh Unit of eggs is classified by United States Department of Agriculture (USDA, 2000) as AA (100-72), A (71-60), B (59-30) and C (below 29). Most eggs have 75-80 HU with a minimum value of 60 (Chukwuka *et al.*, 2011). Haugh Unit and albumen height decreases with storage time (Scott and Silversides, 2000). Albumen height is greatly influenced by age of laying flock and length of time laying flock is in continuous lay without moulting. Albumen quality decreases with age of laying flock (Doyon *et al*., 1986; William, 1992; Silversides, 1994; Ledur *et al*., 2002; van de Brand *et al*., 2004) and storage period (Silversides, 1994; Silversides and Budgell, 2004).

On the other hand, Silversides (1994) measured albumen height of eggs laid at 30, 45, 60 and 75 weeks of age and found that the average albumen height steadily decreased ($p<0.05$) (average range values at 30 and 75 weeks were 6.70±0.06 mm and 5.50±0.06 mm, respectively) as laying chickens increased in age. Monria *et al*. (2003) examined internal and external quality of Barred Plymouth Rock, White Leghorn, Rhode Island Red and White Rock chickens from day 220 to 260 in storage time 1, 7, 14 21 days and ambient temperature 27.40±1.25^{o}C and relative humidity 80.50±1.90%. The authors found that average albumen height of breeds decreased as egg holding period increased ($p<0.05$), indicating reduction in egg freshness. Van de Brand *et al*. (2004) reported average value of albumen height as 7.27±0.18mm for young laying flock which decreased ($p<0.05$) in value to 1.78±0.18mm for old laying flock.

Silversides and Budgell (2004) in a study to determine significance of genetics, age and storage time using ISA Brown and Babcock B300 commercial strain of laying chickens, collected eggs at 32, 50 and 68 weeks of age to represent early, middle and late phases of production and measured albumen height within 24 hours of lay and 5 and 10 days of storage time at 21^{o}C.The authors reported albumen height values of 6.47±0.06mm at 32 week which decreased ($p<0.05$) to 0.71±0.06 at 50week and 1.71±0.06mm at 68week. The average albumen height of eggs laid within 2 hours was 8.45±0.06mm. A decrease of 3.49 and 4.35±0.06mm ($p<0.05$) was reported for eggs stored for 5 and 10 days respectively. The decrease in albumen height with increase storage time indicated freshness or staleness in eggs.These studies suggested that albumen height of eggs decreases with age of birds and length days of egg storage.

2.13.2: Yolk quality

Egg-yolk is enclosed in tender and elastic membrane called vitelline membrane. Vitelline membrane keeps yolk together and separates from albumen content (Rose, 1997). Yolk quality is determined by colour, texture, firmness and odour (Jacob *et al.*, 2000). Yolk colour is a key factor in determining egg quality (Jacob *et al.*, 2000; Okeudo *et al.,* 2003). Consumers' preference for egg-yolk colour is highly subjective and varies widely. The colour of egg-yolk is affected by carotenoid pigments (xanthophylls) present in feedstuffs like maize, lucerne, grasses, tomatoes, carrots, algae

among others (Hasin *et al*., 2006). Yellow maize, in addition as energy source, supplies xanthophyll pigment to animals. It contains 20-25 mg xanthophyll/kg. Egg producers make efforts to produce eggs whose yolk would have rich yellow colour using yellow maize. There are approved synthetic pigments used to replace natural pigments as components of feed additives. Mottled yolks occur when contents of albumen and yolk mix as a result of degeneration and increase permeability of vitelline membrane (Jacob *et al*., 2000).

Yolk colour is variable and subject to easy change ranging from light to medium colour of yellow (Galobart *et al*., 2004). It is subjectively determined using Roche Colour Fan Score (Vulleumier, 1968; Stadelman, 1995). Food processing and other industries often prefer darker yolk colour that impart yellow colouration of products (Zeidler, 2002). The diets of laying chickens greatly affect egg yolk colour (Galobart *et al*., 2004). The yolk colour can be manipulated by inclusion of synthetic additive or natural feeding ingredients for example xanthophyll in diets (Zeidler, 2002; Galobart *et al*., 2004). Galobart *et al*. (2004) investigated effect of saponification of paprika products, marigold products and both on xanthophyll levels in yolk colour at days 19, 20, 21, 26, 27 and 28 using a Roche Yolk Fan Score. The authors reported that yolk colour darkened as levels of red xanthophyll increased in diets. The concentration of products in diets varied significantly ($p<0.05$) with dark yolk colour. Popova-Ralcheva *et al*., (2009) determined egg yolk colour from laying chickens at 32 and 50 week of age using Roche Colour Fan Score and found that younger layers had yolk colour score ranging from 8.20±0.43 to 8.87±0.26, while older laying chickens recorded 8.21±0.12 to 8.60±0.28. When laying chickens were fed the same diets, there were no differences in yolk colour because diet predominantly influence egg yolk colour.

2.14 Relationship among egg quality characteristics

Some egg quality characteristics are either moderately or strongly related. The different correlational studies enable egg quality characteristics to be evaluated when resources are limited in supply. Egg quality encompasses entire egg mass and characterized by eggshell thickness, weight and specific gravity. Thus, correlation among specific egg specific gravity, eggshell thickness and eggshell weight help to determine shell quality.

Specific gravity of eggs is determined from egg shell thickness and weight without breakage. There is strong positive correlation between eggshell and specific gravity of eggs. This relationship is plausible since specific gravity gives a method of measuring eggshell quality. Stadelman (1995) reported correlation value of 0.78 between eggshell thickness and specific gravity. As eggshell thickness increase, specific gravity of egg increases. Zhang *et al.*, (2005) reported moderately strong and positive genetic correlation between eggshell thickness and weight in dwarf brown-egg layers developed from pure line.

In a post-moult study using using white and brown layers at 57 week for 40 weeks fed four different diets, Aygun and Yatisir (2010) reported a strong positive correlation between eggshell thickness and specific gravity. The relationship between eggshell thickness and specific gravity (0.06) was lower than 0.78 obtained by Stadelman (1995). However, stronger correlation was obtained by Aygun and Yatisir (2010) in barley-based diets (0.78±0.03) and oat-based diets (0.53±0.03). The two diets had lower correlations values of 0.06±0.04 and 0.03±0.04 in wheat- and bran-based diets, respectively. The diets contained 1% calcium but wheat- and bran-based diet had lower level of di-calcium phosphate which may affect overall shell quality. The reduced level of di-calcium phosphate may have affected either eggshell thickness or specific gravity of eggs more than the other to cause reduction in correlation values between the two characteristics.

Relationship among egg equality extends beyond eggshell characteristics. Zhang *et al.* (2005) examined a number of egg quality characteristics and reported that genetic correlation exist between internal and external quality of eggs. The positive correlation values between albumen and egg weight and shell index and eggshell weight were 0.32, 0.33 and 0.36, respectively. These correlation values though weaker than values recorded for eggshell, these values remain significant for determining egg quality since high quality eggs are expected to have high multiple egg quality characteristics. Aygun and Yatisir (2010) investigated phenotypic correlation across several egg quality characteristics. The overall strongest relationship between albumen height and Haugh Unit reported was 0.95±0.01 because Haugh Unit is related with directly albumen

height. The correlation values of egg weight and egg width, egg length and specific gravity were 0.70±0.01, 0.60±0.02 and -0.32±0.02, respectively. Shell percentage was correlated with specific gravity and eggshell thickness to obtain 0.39 and 0.37±0.02, respectively

2.15: Effect of housing system on egg quality characteristics

Many studies have shown that housing systems affect quality of eggs in commercial flocks (Zemková *et al*., 2007; Lichovníková and Zeman, 2008; Djukicstojcic *et al*., 2009) but fewer reports (Casagrande *et al*., 2001; Minelli *et al*., 2007; Rossi, 2007) and review (Pavlovski *et al*., 2012) did not establish comparative advantages or disadvantages of housing systems on egg quality. Egg quality is influenced by housing system (Vits *et al*., 2005). Birds in non-cage systems spend more energy on movement which may result in production of either smaller eggs or reduced egg-yolk content (Van Niekerk, 2014). In a study to compared egg quality of laying birds in cage, cage-free, organic and free-range systems, Hidalgo *et al*. (2008) reported that eggs from organic management system had greatest whipping capacity, foam consistency and lowest Haugh Unit scores which indicated poorer egg quality. In a similar study, Abrahamsson and Tauson (1995) reported that there were no clear trends in interior egg quality characteristics produced by birds in conventional cage and aviary production.

The reports by Pavlovski *et al*. (1981) and Shawkat (2002) showed better albumen height and Haugh Unit for eggs collected from free-range than caged birds. This observation was corroborated by the study carried out by Djukic-Stojcic *et al*. (2009), that eggs from free-range system were significantly greater in albumen height and Haugh Unit than eggs from cages. Van den Brand *et al*. (2004) compared egg quality of birds individually in cages with those that co-habited with male counterparts on free-range. These authors reported inconsistency in external and internal egg quality characteristics but observed darker egg-yolk colour in eggs obtained free-range system. Minelli *et al*. (2007) reported higher values of egg-yolk colour in eggs from conventional systems. In another study, Simčič *et al*. (2009) reported that egg-yolk weight of native breed of chicken kept under free-range system was higher than caged birds. Silversides and Scott (2001) and Pavlovski *et al*. (2012) reported that albumen

percentage and yolk-albumen ratio decreased with age for birds in cages with no variable differences in albumen height. The frequency of blood and meat spots was less than 1% in eggs produced from commercial lines (Smith *et al*., 2008).

The incidence of blood and meat spots increased with age of bird (Silverside and Scott, 2001). Other environmental factors that are likely to affect egg quality include noises, temperature changes, infections and incidence of blood and meat spots (Campo, *et al*., 1998). A greater incidence of meat spots has been found in aviary versus conventional cage eggs (Abrahamsson *et al*., 1996). The study conducted by Hidalgo *et al*. (2008), which did not include aviary production, showed lowest incidence of meat spots in free-range eggs when compared with conventional cages, cage-free, and organic production. The effect of housing systems on egg quality was investigated (Williams, 1992). A number of studies also showed that housing systems affect egg quality characteristics of chickens. Wang *et al*. (2009) and Silversides and Scott (2001) observed higher internal egg quality traits in deep litter than in battery cage. Jin and Craig (1994) reported that housing conditions affected growth, egg production and qualities in laying hens. Ojedapo (2013) reported that egg quality trait e.g. egg weight, length, breadth, shell weight and thickness (external egg quality traits) and yolk weight and colour, albumen weight and height (internal egg quality traits) were better in eggs from deep litter system than cages.

Egg production, weight and shell quality parameters like specific gravity, weight, thickness and percentage shell were not significantly affected by different housing systems (Neijat *et al*., 2011). However, Usturoi *et al*. (2010) reported that laying birds in deep litter produced lower proportion of eggs with shell faults when compared to those managed in other housing systems. Mohan *et al*. (1991) observed that egg weight and shell thickness of laying birds in cages were higher than in deep litter. The production practices and physiological stress have direct impact on egg size (Morris, 1985; Keshavarz and Nakajima, 1995). Eggs from free-range production systems weighed more than those from battery and conventional cages (Hughes *et al*., 1985; Hidalgo *et al*., 2008). There was however no difference in egg weight from furnished cages compared with those from conventional cages (Guesdon and Faure, 2004).

Tanaka and Hurnik (1992) compared egg size of laying birds in conventional cages and aviary production between 27 and 63 weeks of age and found no differences in egg size between the two housing systems. Abrahamsson *et al*. (1996) reported that eggs from conventional cages had significantly greater weight compared with those from aviary systems.

Hughes *et al*. (1985) observed that variation in egg weight could be due to differences in environmental temperature in free-range and cage systems of egg production. In a similar study, Anderson and Adams (1994) reported that laying birds in cages always produce heavier eggs and birds were less fearful at end of production cycle than in deep litter. High environmental temperatures are known to affect voluntary feed intake of birds which may result in decreased availability of calcium for shell deposition (Okoli *et al.*, 2006). High atmospheric temperature therefore adversely effects oviposition and oviposition interval leading to drop in egg production and weak eggshell (Oguntunji and Alabi, 2010). Although, laying birds mitigate heat stress by panting (Koelkebeck, 1999), heat stress causes a decrease in amount of carbon dioxide (CO_2) in blood leading to condition known as respiratory alkalosis (Koelkebeck, 1999; Nys *et la*., 1999). Since egg shells are made up of 95% calcium carbonate (CaCO3), a decrease in blood CO_2 level combined with increased blood pH and subsequently decrease in Ca^{2+} ions for shell formation could lead to increase production of thin or soft shelled eggs (Koelkebeck, 1999; Okoli *et al.*, 2006).

Laying birds under stress often retain eggs in oviduct for longer period of time leading to deposition of amorphous calcium carbonate and eggs laid are described as ‘whiter eggs’ (Walker and Hughes, 1998). Egg quality traits such as egg cracking and dirtiness are affected by design of housing systems (Abrahamsson and Tauson, 1995). The effects of design of housing systems on egg cracking and dirtiness is pronounced in deep litter system than battery cage because of higher frequency of nesting and perching behaviour (Elston, 2000). Guesdon and Faure (2004) reported no differences in shell breaking strength of eggs from furnished and conventional cages. Eggs collected from free-range system were observed to have greater shell thickness and stronger shells than those from conventional cages (Hughes *et al*., 1985). However, when eggs collected

from aviaries, conventional cages and floor pens were compared, Tauson *et al*. (1999) reported greater percentages of cracked eggs for eggs from aviaries and conventional cages than those from floor pens. Victor *et al.* (2013) opined that laying birds in free range systems generate higher occurrence of dirty and shell cracked eggs as well as decrease shell quality parameters such as eggshell density, thickness and mass, especially toward the end of laying period.

On the contrary, Mertens *et al*. (2006) reported highest percentage of cracked eggs at point of lay in conventional and furnished cages, while lower percentage was observed in aviary and free-range production. Short (2001) explained that competition among laying birds for dust bathing might produce increased stress which in turn reduces eggshell density. In a study by Hidalgo *et al*. (2008), eggshell thickness was lowest for eggs from cages but varied for those from litter-floor and free-range systems. Pavlovski *et al*. (2001) however reported thicker eggshell for eggs from deep litter than those from free-range system.

2.16: Effect of housing systems on egg lipid profile

The effect of housing systems on egg quality and its chemical composition have been investigated (Williams, 1992). Several studies (Lopez-Bote *et al*., 1998; Silversides and Scott, 2001; Cherian *et al*., 2002; Rizzi *et al.*, 2006; Rossi, 2007; Zemková *et al*., 2007; Minelli *et al.*, 2007; Stefano *et al.*, 2008; Krawczyk and Gornowicz, 2009; Wang *et al*., 2009; Józefa *et al*., 2011; Kamil *et a*l., 2012) have shown that housing systems affect egg qualities of hens in cages and deep litter. Wang *et al*. (2009) and Silversides and Scott (2001) observed higher internal egg quality traits in deep litter floor than in battery cage. Zemková *et al*. (2007) reported lower cholesterol level in yolks of eggs from caged compared with litter-kept hens. Also, Józefa *et al*. (2011) recorded lower yolk cholesterol in eggs laid by hen reared under free range system than those in litter floor. Simčič *et al.* (2009) found that the yolks of eggs raised with outdoor access contained more fat and cholesterol than yolks of eggs from hens raised indoors.

Krawczyk and Gornowicz, (2009) observed less fat in egg-yolk from free range compare to litter floor and no effect of housing system on yolk cholesterol. Minelli *et al.*(2007) reported lower cholesterol from egg yolk of hen from conventional system. The study by Kamil *et a*l. (2012) showed that housing systems (battery cage and deep litter) had no influence on yolk cholesterol. Reported studies (Lopez-Bote *et al.*, 1998; Cherian *et al*., 2002; Rizzi *et al.*, 2006; Rossi, 2007; Stefano *et al.*, 2008; Józefa *et al.*, 2011; Kamil *et al.*, 2012) have shown that eggs from free range contain two thirds the amount of cholesterol compared to conventional cage. These authors compared composition of fatty acids of eggs from conventional battery cage system and found no clear effect of housing system on yolk lipid composition.Józefa *et al.* (2011) reported a lower level of saturated fatty acids, higher levels of monounsaturated fatty acids, lower level of polyunsaturated fatty acids, higher level of n-3 fatty acid and a lower level of n-6: n-3 fatty acid ratio in the yolk of egg from eggs from hens reared under free range system.

Cherian *et al*. (2002) and Rizzi *et al.* (2006) declared that fatty acid compositions of eggs from organic system were not significantly different from conventional system. Rossi (2007) also found no difference in fatty acid composition of eggs from organic and conventional systems except total saturated fatty acids. Kamil *et al.* (2012) observed a lower level of omega 3 fatty acid in egg yolk from litter floor compared with conventional cage. Lopez-Bote *et al.* (1998) reported that eggs from free range system had higher omega-3 and lower omega-6 contents compared with those from conventional system while Stefano *et al.* (2008) reported no influence of total lipid in yolk but significant changes in yolk fatty acid profile of eggs from hens reared on different housing systems.

2.17: Effect of nutrition on egg quality characteristics

Nutrition has direct effect on egg quality. When laying birds are nutritionally compromised, their body shut down necessary biochemical processes (Jones, 2006). A lack of appropriate level of required nutrients in diets of laying birds will not only impair efficiency of production but also inferior with egg quality (Jones, 2006). Egg mass is important for maintenance of good shell quality (Pavlovski *et al*., 2012).

Smaller eggs have stronger shells than larger ones because laying birds have finite capacity to deposit calcium in shell since the same amount of calcium is spread over a larger shell area (Butcher and Miles, 2003). Pavlovski *et al*. (2012) reported that egg mass can be reduced by lowering the total amount of dietary protein or methionine levels in diet. These authors observed adverse effect on egg production and reported that 0.25-0.5% supplementation of amino ethyl-sulfonic acid in powder form in layer diet will not decreases egg mass nor has effect on production traits. Also, Grobas *et al*. (1999) reported that addition of 4% fat affected egg mass at early laying phase and should be avoided in order to prevent decrease in egg production.

It has however been observed that addition of more saturated fatty acids (palm oil) and linoleic acid in quantities that meet physiological function of laying birds are alternatives to control increase in weight with age especially when egg mass reduction does not reflect changes in mass of eggshell (Harms *et al*., 2000). Thus, any delay in introducing required calcium in diet of laying birds has serious negative effect on eggshell quality during early laying phase and subsequent eggs produced (Roland, 2000). The optimal eggshell quality was determined in eggs from laying birds fed diets containing more than 3.5% calcium brown shell eggs (Vitorović *et al*., 1995; Safaa *et al*., 2008) and 4 to 4.5g per day. Evidence from these studies and others (NRC, 1994; Vitorović *et al*., 1995; Safaa *et al*., 2008) showed that feeding laying birds high levels of calcium may interfere with availability of other minerals causing negative impact on ability of birds to utilize calcium, particularly when calcium levels in diets is sub-optimal in quantity. Coetzee (2002) reported in South Africa that laying birds supplied 200mg of calcium per litre in drinking water laid eggs with higher mean shell strength compared to those fed un-supplemented water. However, Kershavarz and Nakajima (1993) reported that feeding laying birds with calcium levels above requirement did not improve shell quality.

Similarly, feeding high levels of dietary phosphorous has been shown to have negative effect on eggshell quality (Taylor, 1965; Boorman, *et al*., 1989; Kershavarz and Austic, 1990). Miles *et al*. (1983) reported negative correlation between phosphorus content in diets and eggshell as high dietary phosphorus increases blood phosphorus content which

in turns inhibits bone calcium mobilization and cause poor eggshell quality. Pavlovski *et al*., (2012) observed that laying birds often require increase phosphorus during warm period and where quantity fall below 0.25% in order prevent mortality, eggshell breakage increases. Low level of phosphorus in feed reduces the need for calcium but lead to bone problems and poor quality of eggshell. This condition can be improved partially by adding large particles of marble (Nys 1995). Mas and Arola (1985) and Miles (2001) showed that provision of adequate amounts of zinc, copper, iron and manganese, key components of eggshell matrix and shell integrity, play essential role as co-enzymes in shell formation and its associated membranes. The deficiency of these microelements in diet reduce shell mass (Zamani *et al*., 2005).

Magnesium deficiency affects number of egg laid and shell quality. The diets of laying birds often contain four times more than its requirement (Vogt *et al*., 1984). There is no definite magnesium requirement since plant feedstuffs such as bran, sunflower, rapeseed, contain sufficient quantity of magnesium. However, excess amount of magnesium in diets increases water consumption leading to increase in number of dirty eggs (Pavolvski *et al*., 2012). Laying birds fed manganese deficit diet produced thinner shell partly due to deterioration of see-through spots arising from worsening of ultra-shell structure and reduction of concentration of polysaccharides which are precursors of protein matrix (Pavlovski *et al*., 2012). Faria *et al*. (1999) reported that 70-100mgMn/kg diet is needed for good quality eggshell strength and thickness. Chowdhury (1990) reported that inadequate supply of copper in diets of laying birds affects biochemical and mechanical properties of eggshell membrane negatively to caused egg shape deformation. Meluzzi *et al*. (2000) also observed that metals such as nickel, chromium and lead reduce eggshell mass.

The report of study on selenium supplementation in diets of laying birds up to 0.8 mg/kg by Pavlovski *et al*. (2012) indicated no negative effect on eggshell quality. Vitamin D3 up to 400 IU increased number of egg laid and improves shell quality (Whitehead, 1996). Seven (2008) reported that addition of vitamin C to layer diets in order to mitigate heat stress conditions had positive effects on mass and eggshell thickness. This observation contradicts report by Supić *et al*. (1997). However, Çiftçi *et*

al. (2005) reported that egg production and egg weight, egg specific gravity and shell thickness were significantly increased when laying birds were fed vitamin C and E after exposure to heat stress. Mori *et al.* (2003) also reported that specific gravity, shell index, and shell thickness of eggs from laying birds fed diets supplemented with vitamins A and E were not different from those fed basal diet. Sodium and chloride deficit diets adversely affect egg production and shell quality. Excess chloride has detrimental effect on eggshell quality (Gezen *et al*., 2005). The study by Belnave *et al.* (2000) revealed that shell quality decreases as concentration of NaCl in drinking water increases.

A significant linear relationship exited between shell quality and NaCl concentration in drinking water. The concentration of 600 mgNaCl/L in water increased incidence of damaged shell and reduced shell breaking strength, thickness, weight, weight/egg ratio, weight and shell weight/unit surface in domestic fowl by three-folds (Balnave and Yoselewitz, 1987). Keshavarz and Austic (1990) examined interaction of phosphorus and chloride on egg shell integrity and reported that elevated dietary levels of chloride resulted in decreased eggshell quality and blood acid-base indicators. In contrast, Hess and Britton (1989) fed diets lower in chloride to laying birds and found virtually no effect on shell quality. Water quality affects eggshell quality. Water containing high levels of electrolytes has long-term negative effects on eggshell quality (Balnave and Yoselewitz, 1987). Water temperature is important, especially during hot weather when birds reduce water intake or even cease to drink if water gets too hot. Provision of cool drinking water improves eggshell quality in heat stressed hens (Glatz, 1993). A number factor has been reported (Robert, 2004) to affect albumen quality although Williams (1992) concluded that albumen quality is not greatly influenced by nutrition.

Nevertheless, albumen quality decrease with increasing dietary protein and amino acid content (Hammershoj and Kjaer, 1999); increases with higher amount of dietary lysine (Belnave *et al*., 2000); decreases with dietary addition of neem kernel meal (Verma *et al*., 1998); increases with ascorbic acid supplementation (Franchini *et al*., 2002); and increases with vitamin E supplementation, especially at high ambient temperatures (Kirunda *et al*., 2001; Puthpongsiriporn *et al*., 2001). At temperatures above or below thermo-neutral zone, corticosteroid secretion increases in response to stress (Brown and

Nestor, 1973). By decreasing synthesis and secretion of corticosteroids, vitamin C alleviates negative effects of stress such as cold stress-related depression in poultry performance (McDowell, 1989; Kutlu and Forbes, 1993). Ajakaiye *et al.* (2011) examined impact of supplementing L-ascorbic acid and DL-tocopherol acetate in diets of laying birds under heat stress and observed that egg-yolk was higher in group fed combination of vitamin C and E compared to those fed vitamin E and vitamin C treated groups and control respectively. They also reported that Haugh Unit was higher in group fed combination of vitamin C and E compared with those fed vitamin C and E treated groups and control.

Mori *et al.,* (2003) reported that albumen quality of eggs from hens fed diets with supplemental vitamins A and E did not differ from those fed basal diet. This observation is in agreement with findings of Qi and Sim (1998) who supplied laying birds with 800 mg vitamin E/kg of diet without changes in internal egg quality. The primary determinant of yolk colour is xanthophyll (plant pigment) content in diets (Silversides *et al.,* 2006).The omission of xanthophyll in diets led to pale egg-yolk (Esonu, 2006). It is therefore possible to manipulate the colour of egg yolk by the addition of natural or synthetic xanthophyll in poultry diets. However, the ease with which yolk colour can be manipulated can lead to unwanted colour changes. The inclusion of more than 5% cottonseed meal in layer diets resulted in olive or salmon coloured yolks (Beyer, 2005; Esonu, 2006), while inclusion of certain weeds or weed seeds produced green yolks (Beyer, 2005; Coutts and Wilson, 1990). The alteration in yolk colour can result due to any factor which alters or prevents absorption of pigments from diet or deposition of pigments in yolk. Such factors include worm infections (Coutts and Wilson, 1990); mycotoxicosis caused by aflatoxin B1 (Zaghini *et al.,* 2005); coccidiosis; and any other factor that inhibits liver function and lipids metabolism.

Study (Coutts and Wilson, 1990) on diphenyl-para-phenylenediamine (DPPD), an antioxidant, was found to cause excessive deposition of pigments in egg-yolk. Mottled yolks in egg occur when albumen and yolk mix as a result of degeneration and increase permeability of the vitelline membrane (Jacob *et al.,* 2000). Other factors that may cause mottled egg-yolks include presence of nicarbazin (an anticoccidal agent) in feed

(Cunningham and Sanford, 1974; Jones *et al.*, 1990), deworming drugs such as phenothiazine (Coutts and Wilson, 1990), dibutyltin dialaurate (Coutts and Wilson, 1990;Jacob *et al.*, 2000) or Piperazine (Jacob *et al.*, 2000; Coutts and Wilson, 1990); gossypol from cotton seed meal (Jacob *et al.*, 2000) and presence of tannin and tannic acid (Coutts and Wilson, 1990; Esonu, 2006) in feed. Miles (2001) reviewed effects of vanadium on poultry performance and noted poorer albumin quality from laying hens that consumed as little as 6ppm. This finding agreed with earlier reports by Sell *et al.* (1982), that there was decrease in interior egg quality from two strains of laying birds fed 3 or 6 ppm vanadium. The report of study by Duyck *et al.* (1990) of feeding laying birds 10ppm of vanadium for 30 days recorded 71 and 64HU after first and seventh day of storage at 16.6^{o}C temperature and 60% relative humidity after seven days of storage respectively.

Miles (2001) reported that negative effects of vanadium may be overcome by feeding cotton seed meal, ascorbic acid, vitamin E or carotene. Karmel *et al.* (2010) reported higher albumen height and improvement in Haugh Unit but lower albumen and yolk pH in eggs from birds fed garlic juice when compared to those on control diet. Senkoylu *et al.*, (2005) examined effect of inclusions of poultry by-products in layer diets and found significant effect on egg breaking strength, shell weight, albumen weight, and yolk weight. They observed significant reduction in egg weight when birds were fed 4% feather meal and 4% poultry by-product and decrease in Haugh Unit was more pronounced when compared with those on control diet. Dietary treatments indicated differences in the amino acid contents in poultry by-products (Senkoylu *et al.*, 2005).

2.18: Dietary influence on blood serum egg yolk cholesterol in poultry

Animal fats encouraged incidence of atherosclerosis because they contain larger proportion of poly-saturated fatty acids. There is positive correlation between animal fats and cholesterol content in egg-yolk but maintained inverse relationship with plant fats. Thus, animal fats enhance synthesis of cholesterol while plant fats reduce its level (Maynard *et al*., 1979). Dietary factors elevate blood cholesterol which is indicated by egg-yolk cholesterol. Reports of studies (Kokantnur *et al.*, 1958) stated that dietary components have little or no alteration on egg-yolk cholesterol content. The type and

quantity of dietary fat appear to have no effect on total lipid content (Edwards *et al*., 1962), however, dietary cholesterol largely and uniformly increases the egg-yolk cholesterol which strongly suggests that hens have ability to control serum cholesterol and prevent hypercholesterolemia by excretion through the egg-yolk (Harris and Wilcox, 1963). Blood serum or plasma cholesterol level of hen were not significantly affected by different dietary fats (Edward *et al*., 1962), though a reduction in blood cholesterol level was recorded in a study where laying hens were fed melted animal fats.

The total blood cholesterol level increase when hens were fed soyabean oil (Daghir *et al*., 1960) while corn oil caused decrease the total liver lipid and total cholesterol (Marion and Edward, 1962). The egg-yolk cholesterol did not respond to soyabean oil as serum cholesterol and yolk cholesterol remains relatively independent of serum cholesterol in *in-vitro* experiment with membrane surrounding growing ovum in chickens (theca interna and granulosa) which are equally active as liver slice in cholesterol biosynthesis. Dietary protein has some part to play on serum and egg-yolk cholesterol content. There were significantly higher cholesterol values in hens fed lower dietary protein irrespective of amount or type of fat (Mone *et al*., 1959). Serum cholesterol level was not affected by increasing dietary fat from 4-10%, but reduction in dietary protein with sucrose gave higher blood cholesterol and more aortic and coronary atherogenesis which were prevented when protein uptake was restored by supplement of soyabean protein (Stamler *et al*., 1958). The excess fat and cholesterol combined with inadequate protein may be of primary importance in production of high blood cholesterol and atherosclerosis.

2.19: Methods of egg storage

Egg production is on the increase in Nigeria but poor storage conditions may result in quality deterioration and consequently causing loss in farm revenue (Raji *et al*., 2009). Most egg quality characteristics aside shape index and shell thickness are affected by type and storage time (Dudusola, 2009). Egg loss could be due to accumulation of carbon dioxide, ammonia, nitrogen, hydrogen sulphide gas and water in eggs when poorly stored or stored for long period of time (Dudusola, 2009; Alsobayel and Albadry, 2011). In Nigeria, eggs in most cases are stored under ambient condition due to irregular

electric power supply (Okeudo, 2005; Raji *et al*, 2009). Retailers usually display eggs for sales on open paper or plastic egg trays while housewives store eggs in kitchens. The most profound factor that causes deterioration in egg quality is storage temperature (Stadelman and Coterill, 1995). The deterioration of interior egg quality can be delayed significantly by maintaining storage temperature near freezing point (Zeidler 2002).

Several studies (Dudusola, 2009; Scott and Silverside, 2000) have shown that refrigeration method of egg storage effectively reduce egg weight loss by half and maintained quality grade for at least 4 weeks compared to storage under room temperature (Biladeau and Keener, 2009). Storage of eggs at temperatures of 7–13^{o}C and humidity of 50-60% reduced rate of degeneration of thick albumen proteins and consequently maintained albumen quality for longer period (Jones, 2006). This report agreed with the finding of Silversides and Scott (2001) that Haugh units were not significantly decreased by storing for 3-14 days at 4ºC, while albumen pH of refrigerated eggs (5°C) decreased and these qualities increased at 21°C or 29°C (Samli *et al*., 2005). The decrease in albumen pH during storage may be due to continuing breakdown of major constituents in egg white and/or changes in bicarbonate buffer system (Obanu and Mpieri, 1984; Biladeau and Keener 2009). Samli *et al*. (2005) reported that yolk indices of eggs from old laying hens decreased with increase storage time but decrease at slower rate at 5°C than at 21°C or 29°C.

In some developing counties where refrigeration method of storage is seldom practiced, egg-coating method is effectively used to preserve the egg quality from microbial deterioration. The different food-grade coating materials have been proven to be efficient in reducing quality deterioration. These materials include chitosan, whey protein, waxes, mineral and vegetable oils (Obanu and Mpieri 1984; Wong *et al.*, 1996). Oil-coating eggs reduce CO_2 losses and help maintain egg quality (Coutts and Wilson, 1990; Koelkebeck, 1999; Beyer, 2005) but cannot be a substitute for refrigeration method (Jacob *et al.*, 2000). Williams (1992) and ACIAR (1998) observed that oil-coating of eggs within 24 hours of lay effectively retarded albumen deterioration but does not replace the need for refrigeration method.

Mineral oil used for coating egg must be odourless and colourless, and free of fluorescent materials (Stadelman and Coterill, 1995). Waimaleongora-Ek *et al*. (2009) in a study using mineral oil with different viscosities as egg-coating materials reported that mineral oils with highest viscosity were more effective in preventing weight loss and preserving albumen quality deterioration. The authors observed that coating with mineral oils reduced egg weight loss by more than 10 times and extended keeping quality by at least 3 weeks compared with non-coated eggs during 4 weeks of storage at 25°C. However, shell colour and visual appearance of eggs were altered after storage (Stadelman and Coterill, 1995). According to FAO (2003), weight loss of 2-3% was common among commercial eggs which are hardly noticeable by consumers. This indicates that non-coated eggs may not be suitable for market after approximately 3 weeks (if stored at 25 °C) and 5 weeks (if stored at 4°C) of storage (Bhale *et al*., 2003).

2.20: Changes in egg quality characteristics during storage

Storage condition like temperature, relative humidity and storage time affect egg quality characteristics. As egg storage condition of changes, egg weight, shell weight and eggshell percentage are affected (Jin *et al*., 2011). The changes in egg quality characteristics vary among species of poultry (Tebesi *et al.*, 2012; Tilki and Inal, 2004). The effects of storage time and temperature on albumen quality have been documented (Stadelman and Cotterill, 1995, Scott and Silverside, 2000). Egg weight decrease with storage time as a result of loss of moisture through eggshell pores (Brake *et al*., 1997). The decrease in egg weight with storage time was also reported by Samli *et al*. (2005). The authors observed decrease in egg weight within 10days of storage at 29^{o}C. However, eggs stored in refrigerator and by oil-coating had lower egg weight loss due to less moisture loss (ACIAR, 1998). Alade *et al*. (2009) reported that egg qualities are affected by storage time except shell weight, shape index, egg length and egg width and shell thickness. This report was in line with observations of Hamilton (1982) and Tilki and Inal (2004) that shell thickness did not change with days of storage in geese eggs although, specific gravity and compression fracture strength of eggs were altered by storage time.

The report of Tebesi *et al.* (2012) on guinea fowl eggs showed that storage time significantly affected shell thickness while egg weight, egg dimensions (width and length), egg shape index, shell weight and shell percentage were not affected by storage time. Egg weights decreases due to increase in weight losses with increase in days of storage. The losses were accounted for by losses in carbon dioxide, ammonia, nitrogen, hydrogen sulphide gas and water from eggs (Dudusola, 2009; Alsobayel and Albadry, 2011). Weight losses were not the same for all storage methods. Eggs refrigerated did not lose as much solvent as those under room temperature. Thus, reduction in egg quality characteristics was not as higher in refrigerated eggs compared with those stored at room temperature. Albumen height and Haugh Units decreased with storage time. The decrease in albumen height occurred more quickly at higher storage temperatures (Li-Chan and Nakai 1989; Dudusola, 2009). The rapid cooling of with carbon dioxide was found to improve Haugh Units of stored eggs (Keener *et al*., 2000). During storage of eggs, pH of albumen increases which accounts for deterioration. After three days of storage, pH of albumen rose to 9.3 or more thereby rendering eggs less susceptible to bacterial infection (Scott an Sliverside, 1987).

The changes in albumen quality during egg storage are related to changes in ovomucin, particularly thick albumen (Kato *et al*., 1994; Toussant and Latshaw, 1999). As egg ages and carbon dioxide (CO_2) is lost through shell pores, the contents in eggs become more alkaline making albumin more transparent and increasingly watery (Okeudo *et al.*, 2003). At higher temperatures, loss of carbon dioxide (CO_2) become faster and albumin quality deteriorates faster, while eggs stored at ambient temperatures and humidity lower than 70% lost 10–15 HU in few days from point of lay and at 35 days eggs lost up to 30HU (Natalie 2009). Ihsan (2012) in his study reported that storage time significantly affected albumin index. This report agree with findings of Scott and Silversides (2000), who observed significant decrease from 9.16-4.75mm in albumin height for stored eggs at 10 days. Storage time and temperature affect degree of egg yolk mottling (Coutts and Wilson, 1990; Jacob *et al.*, 2000). Jones (2006) stated that when internal temperature of eggs increases above $7^{o}C$, protein structures of thick albumen and vitelline membrane breakdown fast.

As vitelline membrane degenerates, water from albumen moves into yolk resulting in enlarged and decreased viscosity and consequently gives yolk a flattened shape when broken (Fromm and Matrone, 1962; Okoli and Udedibe, 2003; Jones, 2006,). The report by Obanu and Mpieri (1984) and Stadelman and Coterill (1995) showed that yolk index, an indicator of spherical nature of egg yolk, decreases as a result of progressive weakening of vitelline membranes, reduction in total solid and liquefaction of yolk due to osmotic diffusion of water from albumen during storage. Brake *et al*. (1997) reported that yolk index of non-coated eggs decreased from an initial value of 0.45 to 0.25 and 0.16 after 2 and 4weeks of storage at 25 °C, respectively. In addition, Hidalgo *et al.* (1996) observed decreased yolk index, increased water content, pH, furosine, pyroglutamic acid and urdine as well as progressive transition of egg yolk rheological properties from pseudo-plastic to Newtonian behaviour and decrease in apparent viscosity of egg yolk during storage. However, storage of eggs at temperatures of 7–13^{o}C and humidity of 5 -60% reduced rate of degeneration of thick albumen proteins and consequently maintained egg albumin quality for longer period (Jones, 2006). This finding agreed with reports by Silversides and Scott (2001); Gavril and Usturoi (2012), who observed that Haugh Units were not significantly affected when eggs were stored for 3-14 days at 4ºC.

Oiling method of egg storage reduced CO_2 losses and maintained internal egg quality (Coutts and Wilson, 1990; Koelkebeck, 1999; Beyer, 2005) but was not a substitute for cold storage (Jacob *et al.*, 2000). Pasquoal *et al.* (2012) reported that increased storage time, regardless of the temperature, caused loss in albumen quality. Refrigeration method of egg storage did not significantly alter proximate composition in eggs (Ihisan, 2012). This finding was in consonance with the report by Dudusola (2009) on Japanese quail eggs that control and refrigeration methods did not alter the proximate composition significantly except for egg protein content. Some researchers (Simopoulos, 2000; Kovács *et al*., 2000; Meluzzi *et al*., 2000; Gonzales-Esquerra and Leeson, 2000; Kralik *et al*., 2006; Škrtić *et al.*, 2007) have shown that fatty acid in eggs can be modified through the use of plant and animal oil. Kovács *et al*. (2000) reported that dietary supplementation with oil from linseed resulted increased α-linolenic acid (LNA, C18:3n-3), while Meluzzi *et al.* (2000) reported increase in Eicosapentaenoic

(EPA, C20:5n-3) and Docosahexaenoic (DHA, C22:6n-3) acids with fish oil. These were omega n-3 fatty acids and their content in egg reflected higher concentration of low density lipoprotein (LDL).

Simopoulos (2000) reported that EPA and DHA were of higher biological value than LNA in egg-yolk. Kralik *et al*. (2006) reported that replacement of one part of sunflower oil in hens' diets with a combination of fish and rapeseed oil significantly altered lipid profile in egg-yolk. In a study, Škrti*ćet al.* (2007) observed that laying hens fed mixture of rapeseed and fish oil supplemented diets produced egg-yolk that were significantly higher in favorable fatty acids (linolenic, C18:3n-3; Eicosapentaenoic, C20:5n-3 and Docosahexaenoic, C22:6n-3) compared with those fed sunflower oil alone. The study showed higher content of yolk PUFA, n-3 PUFA and favorable ratio of n-3 PUFA and n-6 PUFA. Meluzzi *et al*. (2000) stated that addition of 3% of fish oil in hens' diets positively affected the content of EPA and DHA of egg-yolk Gonzales-Esquerra and Leeson (2000) observed an increase in n-3 PUFA in the egg-yolk of hens fed diet enriched with 6% fish oil. Galobart *et al*. (2002) reported a more favorable ratio of SFA, MUFA and EPA plus DHA in egg-yolk of hens fed diets supplemented with linseed oil compared with diets supplemented with sunflower oil.

Cherian (2007) reported 5.4% increase of total lipids in egg-yolk with addition of yellow grease (3.0%) in diet compared with rations that contained 2.5%yellow grease plus 0.25% conjugated linoleic acid and0.25% fish oil. Cashew nut meal impacted significant influence on total lipid in egg-yolk (Vidal *et al*., 2013). Reduction in Palmitic acid, PUFA and increase in the level of oleic acid (MUFA) were observed in egg-yolk of hen fed cashew nut meal (Vidal *et al.*, 2013). High level of MUFA is a reflection of relatively lower level of LDL and higher level of high density lipoprotein (HDL) (Lima, 2000). Filardi (2005) reported lower palmitic acid in egg-yolk of hens fed diets containing canola oil compared with those fed diets containing cotton seed oil and lard. These differences were attributed to the low palmitic acid in canola oil. Palmitic acid increases serum total cholesterol and low density lipoproteins (Ponnampalam *et al*., 2011). Milinsk (2003) fed five different diets to laying hens (four diets containing either canola, linseed, soybean or sunflower meals and oils and a control diet containing

maize, soybean meal, and soybean oil) and observed decrease in palmitic and stearic acid contents in e eggs compared with control diet.

Nam (1997) reported high MUFA/SFA ratio in yolks of hens fed linseed diet containing animal fat. Grobas (2001) also observed increase in the level of fatty acids in yolks of hens fed diets containing olive oil. Yolk cholesterol content was reduced when cashew nut meal was added to layer's feed (Vidal *et al*., 2013). The reduction in cholesterol content of egg-yolk was due to increase in oleic acid found in the diets and MUFA cause reduction in cholesterol levels during lipid metabolism in birds. Freitas (2000) also reported reduction in cholesterol content of abdominal fat of broiler chickens fed diets containing cashew nut meal. Dietary supplementation of fish oil rich in n-3 PUFA reduced triacylglycerol and cholesterol level in egg and meat products of chickens (Ruxton *et al*., 2007). The addition of PUFA-rich oils in diet reduced blood and egg cholesterol (Holland *et al*., 1980) however, other studies (Santos, 1998; Brandão, 2005) showed that yolk cholesterol cannot be changed because it is independent of dietary factors. Studies on dietary manipulation to influence egg cholesterol content have reported conflicting results since some authors claimed reduction in blood and yolk cholesterol with diet enriched with polyunsaturated fatty acids (Mori *et al*., 1999) and others did not observe any effect (Grobas *et al*., 1997; Santos, 1998).

Grobas *et al*. (1997) did not observe any differences in egg cholesterol compared with wheat and soybean-based diet without fat supplementation (control) and 7.5% supplemental tallow, olive oil, soybean oil, rapeseed oil or fish oil. Santos (1998) also found no effect on egg-yolk cholesterol when diet containing soybean (2 and 4%), canola (2 and 4%), or polyunsaturated marine (0.1 and 0.2%) were fed to commercial layers. The amount of cholesterol ingested did not automatically increase blood and egg cholesterol (Brandão, 2005). Chickens are capable of producing 10 times more cholesterol per kg of liver than humans. Therefore, manipulating layer diets to reduce egg cholesterol levels may not be effective because chickens maintain egg cholesterol levels essential for egg composition and embryo development (Shafey and Cham, 1994). However, hens change egg-yolk polyunsaturated fatty acid content in response to

dietary lipid source through absorption of dietary fat in portal system as portomicrons into blood and transport them into liver for lipogenesis (Van-Elswyk *et al.*, 1994).

2.21: Lipid oxidation and biological implications in animals and products

Lipids are diverse group of naturally occurring organic compounds classified based on solubility in non-polar organic solvents such as ether, chloroform, acetone, benzene, and are general insolubility in water. Lipid include fats, waxes, sterols, fat soluble vitamins (such as vitamins A, D, E, and K), mono-glycerides, di-glycerides, triglycerides, phospholipids among others. The main biological functions of lipids include storing energy, signalling, and acting as structural components of cell membranes (Fahy *et al.*, 2009). Lipids have applications in cosmetic and food industries as well as nano-technology (Mashaghi*et al.*, 2013). The different lipid include: fatty acids, glycerolipids, glycerophosphholipids, sphingolipids, saccharolipid, polyketides (derived from condensation of ketoacyl subunits), sterol lipids and prenol lipids (derived from condensation of isoprene subunits) (Fahy *et al*., 2009. Lipid oxidation is considered as a main molecular mechanism involved in oxidative damage to cell structures and toxicity process leading to cell death. It is oxidative degradation of lipids. Free radicals and variety of metabolites like alcohols, ketones, alkanes, aldehydes and ethers are formed in cells to destroy membrane lipids (Dianzani and Barrera, 2008).

This process is preceded by free radical chain reaction mechanism. It affects polyunsaturated fatty acids because they contain multiple double bonds in between which lie methylene bridges (-CH_2-) that possess especially reactive hydrogen. The reaction consists of three major steps: initiation, propagation, and termination. During initiation, fatty acid radical is produced and involves hydrogen abstraction or addition of oxygen radical resulting in oxidative damage of polyunsaturated fatty acids (PUFA). The most notable initiators in living cells are reactive oxygen species (ROS) such as OH and HO_2, which combines with hydrogen atom to produce water and fatty acid radical (www.lipis.com/2014). The fatty acid radical is not a stable molecule, so it reacts readily with molecular oxygen, thereby creating a peroxyl-fatty acid radical.The formation of peroxyl radicals leads to production of organic hydroperoxides, which in turn subtract hydrogen from another PUFA. This reaction is termed propagation which

implies that one initiating reaction result in conversion of numerous PUFA to lipid (www.lipids.com/2014). The radical reaction stops when two radicals react produce non-radical species.

This happens only when concentration of radical species is high enough for high probability of collision of two radicals. Living organisms have different molecules that speed up termination by catching free radicals and therefore, protecting cell membrane. An important antioxidant is Vitamin E. Other anti-oxidants within the body include superoxide dismutase, catalase and peroxide. As a result of lipid peroxidation, great varieties of aldehydes like hexanal, malondialdehyde (MDA) and 4- hydroxyl-nonenal are produced (Catala, 2006). Production of reactive oxygen species primarily superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) are capable of damaging molecules of biochemical classes including nucleic acids and amino acids as well as cell membrane. The exposure of reactive oxygen to proteins produces denaturation, loss of function, cross-linking, aggregation and fragmentation of connective tissues as collagen (Chance *et al*., 1979). Toxicity from lipid peroxidation affects liver lipid metabolism where cytochrome P-450 is an efficient catalyst in oxidative transformation of lipid derived aldehydes to carboxylic acids.

The toxicity of lipid peroxidation products in mammals generally involves neurotoxicity, hepatotoxicity and nephrotoxicity (Boveris and Navarro, 2008).Lipid peroxidation has a role in pathogenesis of several pathologies as neurodegenerative (Dominguez *et al.*, 2008); inflammatory (Farooqui and Farooqui, 2011); infectious gastric and nutritional diseases (Repetto *et al*., 2010). Oxidised lipids have a signalling function in pathological situations which are pro-inflammatory agonists and contribute to neuronal death under conditions in which membrane lipid peroxidation occurs. The end-products of lipid peroxidation may becomen mutagenic and carcinogenic (Marnett, 1999). The degree of lipid oxidation is measured by several products of the damage such as Malondialdehyde/Thiobarbituric Acid Reactive Substances (TBARS) (Pryo, 1991). Malondialdehyde is one of several low-molecular-weight end products formed via decomposition of certain primary and secondary lipid oxidation products but not a substance generated exclusively through lipid oxidation. The more utilized

determination of lipid oxidation product is MDA which is determined with great efficiency by simple and useful assay of Thiobarbituric Acid Reactive Substances (TBARS). The degree of oxidation is indicated by level of end product ofof lipid oxidation. As these products increases the level of damage also increases (www.TBARS.com/2014**).**

2.22: Effect of dietary vitamins and minerals on lipid oxidation

Poultry diets are supplemented with vegetable oils to increase energy density and concentrations of polyunsaturated fatty acids particularly n-3 PUFAs in final product (Grashorn, 2005; Bou *et al*., 2006). Numerous experiments have shown that increased PUFA concentrations in eggs enhance lipid oxidation (Grashorn, 2005; Mohiti-Asli *et al.* 2008). Therefore, PUFA-rich poultry diets should contain increased levels of antioxidants. Vitamins and mineral serve as antioxidants when oils are added to poultry diet. Vitamin C and E are primary antioxidants in biological systems and break chain of lipid oxidation in cells. Kucuk *et al*. (2003) reported that vitamin E and C improved overall egg quality traits, reduced serum cholesterol and triglyceride, increased serum calcium and phosphorus and reduced serum malondialdehyde (MDA) concentrations in laying hens. The antioxidant effect of these vitamins increased when in combined supplementation (Kucuk *et a*l., 2003). Morrissey *et al*. (1997) reported that dietary supplementation of α-tocopherol in chicken diets increased tissues α-tocopherol concentrations, while it markedly decreased MDA concentration.

Vitamin E, natural antioxidant in biological systems, functions as free radical scavenger and inhibits lipid oxidation within membranes (McDowell, 1989; Halliwell and Gutteridge, 1989). Vitamin C and E act synergistically such that vitamin E functions mainly as chain-breaking antioxidant in lipid phases at cellular membrane, while vitamin C serve as terminal reductant by oxidizing free radical chain reactions in aqueous compartments (Tappel, 1968; Gey, 1998). Dietary *α*-tocopheryl acetate supplementation has been shown to protect fatty acids (Botsoglou *et al*., 2005; Bou *et al*., 2006) and cholesterol (Grau *et al*., 2001; Galobart *et al*., 2002) from oxidation in eggs. Hens fed dietary supplements of *α*-tocopherol recorded reduced primary and secondary oxidation compounds in fresh and spray-dried eggs (Galobart *et al*., 2001).

Also, raw and cooked dark meat from chickens fed longer periods of supplementation of *α*-tocopherol showed decreased lipid hydro peroxides and lower TBARS values (Bou *et al*., 2006). Research reoports by Zduńczyk *et al.* (2011) showed that eg-yolk laid by hens fed diets with increased levels of selenium had higher n-3 PUFA content, a lower n-6 PUFA content and a lower n-6/n-3 fatty acid ratio, while vitamin E had no influence.

Meluzzi *et al.* (2000) found that different doses of dietary vitamin E (0, 50, 100 and 200 mg/kg) slightly affected fatty acid composition of yolk whereas Cherian *et al.* (1996) reported a significant increase in egg-yolk content of C20:5n-3 and C22:6n-3 in with dietary tocopherols. This was attributed to beneficial effect of tocopherols on n-3 fatty acid synthesis via denaturation of n-6 PUFA. Carrillo-Domínguez*et al.* (2012) recorded higher contents of Eicosapentaenoic (C20:5 EPA n-3) and Docosapentaenoic (C22:5 DPA n-3) with 100 mg/kg vitamin E and lower content of palmitic acid (C16:0), palmitoleic (C16:1), n-6 fatty acid (C18:2 and C20:4) and n-3 fatty acid (C18:3, C20:5, C22:5, C22:6) with 200mg/kg of vitamin E in egg of hens fed diets supplemented with sardine oil. The report by Cherian *et al.* (1996) indicated that diets containing 3.5% Fish oil and 367 to 423 µg/g vitamin E did not affect fatty acid composition in eggs. Qi and Sim (1998) discovered no effect on fatty acid content of eggs when high concentrations of vitamin E (200, 400, and 800 mg/kg) were supplemented with 15% linseed oil + 0.5% Fish oil. There was no effect on fatty acid composition of eggs when 50 or 100 mg/kg of Vitamin E was supplemented with 3% fish oil (Meluzzi *et al*., 2000).

Cortinas *et al*. (2004) and Zduńczyk *et al*. (2011) found no influence of vitamin E on fatty acid profile of broiler meat. There are empirical evidences (Gutteridge, 1995; Meluzzi *et al.*, 2000; Leeson and Summers, 2001; Surai, 2003, Mabe *et al*., 2003; Franco and Sakamoto, 2005; Fernandez e*t al*,. 2011) to confirm that vitamins and minerals function primarily as antioxidants in stabilizing lipid component by reducing lipid oxidation and increase shelf-life rather than altering lipid profile in biological systems. Vargas and Naber (1984) correlated yolk cholesterol content with dietary energy balance and reported that excessive energy intake beyond maintenance and production requirements increased body weight and cholesterol synthesis. Therefore,

excessive cholesterol in blood would be transferred into egg-yolk. Hassan *et al*.(2013) reported insignificant decreased in saturated fatty acid and increase of unsaturated fatty acid in eg-yolk with increasing levels of ME (2750 kcal/kg) and decreasing level of CP (17%). Thus, as dietary energy levels increase to 2800 kcal/kg ME while protein dropped to 16%, egg-yolk UFA/SFA and n-6/n-3 ratio linearly increase and no effect found on yolk PUFA and MUFA.

Quirino *et al.,* (2009) reported that energy had no effect on yolk cholesterol and fatty acid profile. Mohammed *et al.* (2013) reported reduction in plasma and yolk cholesterol and triglyceride when 3% and 6% of brown marine algae (*Sargasum dentifebium*) supplemented diet was fed to layers. The reduction was attributed to effect of high fibre content in algae. Furthermore, algae supplementation in human and animal diets significantly improved lipid profile. Since nutrition, vaccination, hygiene and other management practices affect lipid profile in eggs, management system could be manipulated for production of good egg quality and enhancement of eggs shelf-life.

CHAPTER THREE

3.0: MATERIALS AND METHODS

Study One

Effects of two housng systems on performance characteristics of growing pullets from 13 to 16 weeks of age

3.1.1: Experimental Site

The experiment was carried out at the Rearing Unit of OOA Farms, Idi Osan, Balogun Village, Ibadan, in the tropical rainforest of Nigeria on latitude 7^{o} 39" N and longitude 3^{o} 89"W at altitude above 255 m above sea levels with mean minimum and maximum temperature of 24^{o}C and 35^{o}C, respectively and average relative humidity of 53% (2012-2017-www.latlog.net)

3.1.2: Housing systems

A conventional 3-tier battery cage (BC) was used for the study. The BC was placed inside a standard laying housing unit built with 3 to 4 cemented blocks from the foundation. Perimeter of the BC housing unit was covered with steel wire mesh and supported by steel and wood poles to allow cross ventilation. The floor was cemented with a deep bathe below the BC for collection of faecal deoppings and asbestors roofing sheet. The housing unit was not provided with light at night during the period of the study. The BC was partitioned into individual cage that measured 50 x 45 x 40 cm^3 with a floor space of 450cm^2/ bird. Each cage accommodated four pullets. A standard open-sided deep litter (DL) system was used for the study. The DL housing unit was constructed with 3 or 4 cemented blocks from foundation. Perimeter of the DL housing unit was covered with steel wire mesh and supported by steel and wood poles to allow cross ventilation were the two housing types. The DL was partitioned into 36 smaller cubicles using steel wire mesh and wooden poles with a door and floor space of 450 cm^2/ bird. Each cubicle accommodated eight pullets.

3.1.3: Animals and Management

Bovan Nera pullets (n=576) at week 13 (point-of-cage) weighing 1.06±0.01-1.08±0.03kg/bird purchased from a reputable poultry farm with proven track records of vaccination and medication schedules in Ibadan were used for the study. They were randomly allocated into two equal parts of 288 pullets and housed in BC and DL systems. Each housing type had six tretments and a treatment was replicated six times. Each replicate comprised 8 pullets both in the BC and DL systems. Birds in BC and DL were provided feed manually three times daily in the morning (7.00-8.00 hrs), noon (12.00-13.00 hrs) and evening (16.00-17.00 hrs). In the BC, birds were provided fresh drinking water through automatic water pipe with nipples while in the DL, water bowls with iron gaurds were used. Feed were provided in the feed through in front of the cage while steel hyanging feeders were used in the DL housing unit. Pullets in BC and DL systems were offered experimental diet and fresh clean water *ad libitum* throughout the course of study which lasted 21 days.

3.1.4: Gross composition of experimental diet

The gross composition of experimental diet fed from weeks 13 to 16 is shown in Table 1.

Table 1: Gross composition of experimental growers diet

Ingredients	(%)
Maize	54.89
Soybean meal	7.98
Groundnut cake	7.98
Palm kernel cake	13.97
Wheat bran	9.98
Bone meal	2.00
Oyster shell	2.00
Common salt	0.30
DL-Methionine	0.15
L-Lysine	0.10
*Grower premix	0.15
Biotronic	0.30
Mycofix	0.14
Avatec	0.06
Total	100.00
Calculated nutrients	
ME (KCal/kg)	2,781.38
Crude protein (%)	16.18
Crude fibre (%)	4.84
Fat (%)	4.70
Calcium (%)	1.58
Phosphorus (%)	0.82
Lysine (%)	0.77
Methionine + cysteine (%)	0.69

*Growers premix: Vitamin A-10,000,000 IU, Vitamin D3-2,000,000 IU, Vitamin E-12,000 IU, Vitamin K3-2,000IU, Vitamin B1-1,500 mg, Vitamin B2-5,000mg, Vitamin B6-1,500mg, Vitamin B12-10mg, Niacin-15,000mg, Calpan-5,000 mg, Folic acid-600 mg, Biotin-20mg, Choline Chloride-150,000mg, Antioxidants-100,000mg, Manganese-80,000mg, Iron 40,000mg, Zinc-60,000mg, Copper-8,000mg, Iodine-1,000mg, Cobalt-250mg, Selenium-150mg

3.1.5: Data collection

Feed consumption per replicate was obtained by subtracting the leftover from the quantity of feed offered to the birds on weekly basis. Total feed intake per bird was determined by dividing the total feed intake per replicate by number of birds while the daily feed intake per bird was obtained by dividing by seven. Pullets in each replicate were weighed individually and the mean live weights of pullets in each replicate were used to determine live weight changes. Live weight changes were determined by subtracting initial from the final live weight. Feed conversion ratio was obtained by dividing feed intake (kg) by the live weight gain (kg). Feed cost per gain was obtained by dividing the amount of feed consumed by live weight gain. The number of deadbirds was expressed as percentage mortality.

3.1.6: Statistical analysis

Data were subjected to descriptive statistics and t-test at $\alpha_{0.05}$ and means separated by LSD procedure of SAS (2012)

Study Two

Effects of five different proprietary vitamin-mineral premixes and two housing systems on performance and egg production characteristics of pullets from 17 to 21 weeks of age

3.2.1: Experimental site

As described in 3.1.1.

3.2.2: Housing systems

As described in 3.1.2

3.2.3: Experimental design and model

The experimental birds were randomly allocated to two HS (BC and DL systems) and six treatments in acompletely randomised design of 2 x 6 factorial arrangements.The experimental model is given below.

$$X_{ijk} = \mu + "i + \$_j + "\$_{ij} + e_{ijk}$$

Where: X_{ijk} = the observed values of each of the response variables

μ = the overall population mean
"i = Observed effect of the i^{th} dietary treatment
$\$_j$ = Effect of the j^{th} week of performance characteristics
"$\$_{ij}$ = Effect of the interaction between dietary treatments and time in weeks
e_{ijk} = Random residual error due to the experimentation

3.2.4: Animals and nanagement

Bovan Nera pullets (n=576) at point-of-lay (16 weeks of age) from study one in BC and DL systems were used for this study. Management was as described in 3.1.3 above and study lasted 35 days.

3.2.5: Test proprietary vitamin-mineral premixes

Five commonly used brands of proprietary growers vitamin-mineral premixes in poultry tolls feed milling in Ibadan were sampled for investigation. The test proprietary vitamin-mineral premixes (PVmP) were Nutripoult, Hi-Nutrient, Agrited, Daram vita-mix and Micro-mix which were designated as premix K, L, M, N and P, respectively. The gross compositions of the test vitamin-mineral premixes as shown on their respective labels are shown in Table 2

Table 2: Gross compositions / 2.5 kg of test proprietary growers vitamin-mineral premixes

	Proprietary vitamin-mineral premix				
Ingredients	K	L	M	N	P
Vitamin A(IU)	10,000,000	8,000,000	7,000,000	8,000,000	10,000,000
Vitamin D3(IU)	2,000,000	2,000,000	1,400,000	1,600,000	2,000,000
Vitamin E(mg)	12,000	8,000	5,000	5,000	20,000
Vitamin K3 mg	2,000	2,000	2,200	1,500	2,000
Vitamin B1(mg)	1,500	1,500	1,500	4,000	3,000
Vitamin B2(mg)	5,000	4,000	4,800	1,500	5,000
Vitamin B6(mg)	1,500	1,500	1,500	10	4,000
Vitamin B12(mg)	10	10	10	15	20
Niacin(mg)	15,000	15,000	15,000	5,000	45,000
Folic Acid (mg)	600	500	500	300	1,000
Biotin (mg)	20	20	20	20	50
Ca pantothenate (mg)	5,000	5,000	5000	5,000	10,000
Choline chloride (mg)	150,000	100,000	100,000	200,000	300,000
Antioxidants (mg)	100,000	125,000	125,000	125,000	120,000
Manganese (mg)	80,000	75,000	75,000	80,000	300,000
Iron (mg)	40,000	20,000	20,000	20,000	120,000
Zinc (mg)	60,000	45,000	45,000	50,000	80,000
Copper (mg)	8,000	4,000	5,000	5,000	8,500
Iodine (mg)	1,000	1,000	1000	1,200	1,500
Cobalt (mg)	250	500	200	200	300
Selenium (mg)	150	200	100	200	120
Prce/kg (₦)	200.00	160.00	175.00	175.00	200.
Mixing instruction(kg/ton)	25.0	25.0	25.0	25.0	25.0

K-Nutripoult, L-Hi-Nutrient, M-Agrited, N-Daram vita-mix, P-Micro-mix

3.2.6: Dietary layout

The dietary layout is schematically shown as follows;

D_1 - Diet without premix

D_2 - Diet with 0.25% Premix K

D_3 - Diet with 0.25% Premix L

D_4 - Diet with 0.25% Premix M

D_5 - Diet with 0.25% Premix N

D_6 - Diet with 0.25% Premix P

3.2.7: Experimental diets

A basal diet was formulated without any PVmPwhich served control diet (D1). Five other diets were each supplemented with 0.25% of premixesK, L, M, N and P to obtaindiets D2, D3, D4, D5 and D6, respectively. The experimental diets were fed from weeks 17 to 21. The gross compositions of experimental diets are shown in Table 3.

Table 3: Gross composition (%) of diets fed from 17 to 21 weeks of age

Ingredients	D_1	D_2	D_3	D_4	D_5	D_6
Maize	50.00	50.00	50.00	50.00	50.00	50.00
Soybean meal	20.00	20.00	20.00	20.00	20.00	20.00
Wheat bran	15.00	15.00	15.00	15.00	15.00	15.00
Palm kernel cake	11.33	11.08	11.08	11.00	11.08	11.08
Salt	0.30	0.30	0.30	0.30	0.30	0.30
Di-calcium phosphate	1.20	1.20	1.20	1.20	1.20	1.20
Limestone	1.50	1.50	1.50	1.50	1.50	1.50
Biotronics	0.30	0.30	0.30	0.30	0.30	0.30
Mycofix	0.10	0.10	0.10	0.10	0.10	0.10
Methionine	0.15	0.15	0.15	0.15	0.15	0.15
Lysine	0.12	0.12	0.12	0.12	0.12	0.12
Premix K	-	0.25	-	-	-	-
Premix L	-	-	0.25	-	-	-
Premix M	-	-	-	0.25	-	-
2Premix N	-	-	-	-	0.25	-
Premix P	-	-	-	-	-	0.25
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Calculated nutrient values						
ME (Kcal/kg)	2,694.31	2,687.56	2,687.56	2,687.56	2,687.56	2,687.56
Crude protein (%)	17.72	17.67	17.67	17.67	17.67	17.67
Crude fibre (%)	5.40	5.37	5.37	5.37	5.37	5.37
Fat (%)	4.26	4.25	4.25	4.25	4.25	4.25
Lysine (%)	0.98	0.98	0.98	0.98	0.98	0.98
Meth+Cyst (%)	0.74	0.74	0.74	0.74	0.74	0.74
Calcium (%)	1.11	1.11	1.11	1.11	1.11	1.11
Phosphorus (%)	0.76	0.76	0.76	0.76	0.76	0.76

Meth+Cyst-Methionine plus Cystiene, K-Nutripoult, L-Hi-Nutrient, M-Agrited,N-Daram vita-mix, P-Micro-mix, Diet without proprietary vitamin-minerals-D1, Diet with premixK-D2, Diet with prmix L-D3, Diet with premix M-D4, Diet with premix N-D5, Diet with premix P-D6

3.2.8: Data collection

Feed consumptions per replicates were obtained by subtracting leftover from the quantities of feed offered to birds on weekly basis. Total feed intake per bird was determined by dividing total feed intake per replicate by number of birds while daily feed intake per bird was obtained by dividing by seven. Pullets in each replicate were weighed individually and mean live weights of pullets in each replicate were used to determine live weight changes. Live weight changes were determined by subtracting initial from final live weight. Feed conversion ratio was obtained by dividing feed intake (kg) by the weight gain (kg). Feed cost per gain was obtained by dividing the amount of feed consumed by body weight gain. The number of birds dead was expressed to percentage. The number and weight of eggs produced per replicate, treatment and housing system were recorded on daily basis. The Hen Day Egg Production (HDEP) was determined as follows:

$$\text{Hen Day Egg Production (HDEP)} = \frac{\text{Total number of eggs produced per week}}{\text{Total number of hen-day per week}}$$

Eggs were weighed using an electronic top loading scale (JS-B LCD® Display Scale).Egg mass was calculated by first determining the average weight of representative samples of eggs produced and then using mathematical relation as shown;

Average egg mass (g/hen/day) = Per cent HDEP X Average egg weight in grams

Feed conversion ratio per egg mass (FCR/EM) was determined by taking into consideration feed intake, egg weight and egg production and calculated as a ratio between the feed consumed and the egg mass thus;

$$\text{FCR/EM} = \frac{\text{Feed consumed}}{\text{HDEP x Average egg weight}}$$

3.2.9: Statistical analysis

Data were analysed using descriptive statistics and GLM procedure of analysis of variance (ANOVA) at $\alpha_{0.05}$ (SAS, 2012). Means were separated using LSD option of the same software.

Study Three

Effects of five different proprietary vitamin-mineral premixes and two housing systems on performance and hen day egg production of laying chickens (22 to 70 weeks of age)

3.3.1: Experimental site

As described in 3.1.1.

3.3.2: Housing systems

As described in 3.1.2.

3.3.3: Experimental design and model

As described in 3.2.4 above.

3.3.4: Animals and management

Bovan Nera pullets (n=571) at early-lay (22 weeks of age) from study two in BC and DL systems were used for this study. Management was as described in 3.1.3 above and the study lasted 43 days.

3.3.5: Test proprietary vitamin-mineral premixes

Fivecommonly used brands of proprietary layers vitamin-mineral premixes inpoultry tolls feed milling in Ibadan were sampled for investigation. The test PVmP wereNutripoult, Hi-Nutrient, Agrited, Daram vita-mix and Micro-mix which were designated as premix K, L, M, N and P, respectively. The gross compositions of the test PVmP as shown on their respective labels are in Table 4.

Table 4: Gross composition / 2.5 kg of test proprietary layers vitamin-mineral premixes

	Proprietary vitamin-mineral premix				
Vitamins & Minerals	K	L	M	N	P
Vit. A (IU)	10,000,000	10,000,000	10,000,000	12,000,000	10,000,000
Vit. D3 (IU)	2,000,000	2,000,000	2,000,000	2,400,000	2,000,000
Vit. E (IU)	12,000	12,000	12,000	12,000	23,000
Vit. K (mg)	2,000	2,000	2,000	2,000	2,000
Vit. B1 (mg)	1,500	1,500	1,500	1,500	3,000
Vit. B2 (mg)	5,000	4,000	5,000	4,000	6,000
Vit. B6 (mg)	1,500	1,500	1,500	1,800	5,000
Vit. B12 (mg)	10	10	10	10	25
Niacin (mg)	15,000	15,000	15,000	25,000	50,000
Pantothenic acid (mg)	5,000	5,000	5,000	5,000	10,000
Folic acid (mg)	600	500	600	500	1,000
Biotin (mg)	20	20	20	25	50
Choline chloride (mg)	150,000	100,000	150,000	240	400,000
Manganese (mg)	80,000	75,000	75,000	80,000	120,000
Zinc (mg)	60,000	50,000	50,000	50,000	80,000
Iron (mg)	40,000	20,000	25,000	20,000	100,000
Copper (mg)	8,000	5,000	5,000	5,000	8,500
Iodine (mg)	1,000	1,000	1,000	1,200	1,500
Selenium (mg)	150	200	100	200	120
Cobalt (mg)	250	500	400	200	300
Antioxidant (mg)	100,000	125,000	125,000	125,000	120,000
Price/kg (₦)	200.00	165.00	175.00	175.00	200.00
Miing instruction (kg/ton)	25.0	25.0	25.0	25.0	25.0

K-Nutripoult, L-Hi-Nutrient, M-Agrited, N-Daram vita-mix, P-Micro-mix

3.3.6: Dietary layout

D_1 - Diet without premix

D_2 - Diet with 0.25% Premix K

D_3 - Diet with 0.25% Premix L

D_4 - Diet with 0.25% Premix M

D_5 - Diet with 0.25% Premix N

D_6 - Diet with 0.25% Premix P

3.3.7: Experimental diets

A basal diet was formulated without any PVmP which served ascontrol diet (D1). Five other diets were each supplemented with 0.25% of premixesK, L, M, N and P to obtaindiets D2, D3, D4, D5 and D6, respectively. The experimental diets were fed from weeks 22 to 71. The gross compositions of experimental diets are shown in Table 5.

Table 5: Gross compositions (%) of layers diets

Ingredients	D1	D2	D3	D4	D5	D6
Maize	59.00	59.00	59.00	59.00	59.00	59.00
Soybean meal	24.37	24.37	24.37	24.37	24.37	24.37
Wheat bran	3.00	3.00	3.00	3.00	3.00	3.00
Palm kernel cake	3.25	3.00	3.00	3.00	3.00	3.00
Common salt	0.30	0.30	0.30	0.30	0.30	0.30
Di-calcium phosphate	0.11	0.11	0.11	0.11	0.11	0.11
Limestone	9.30	9.30	9.30	9.30	9.30	9.30
Biotronics	0.30	0.30	0.30	0.30	0.30	0.30
Mycofix	0.10	0.10	0.10	0.10	0.10	0.10
DL-Methionine	0.15	0.15	0.15	0.15	0.15	0.15
L-Lysine	0.12	0.12	0.12	0.12	0.12	0.12
Premix K	-	0.25	-	-	-	-
Premix L	-	-	0.25	-	-	-
Premix M	-	-	-	0.25	-	-
Premix N	-	-	-	-	0.25	-
Premix P	-	-	-	-	-	0.25
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Calculated nutrients						
ME (Kcal/kg)	2,692.94	2,687.56	2,687.56	2,687.56	2,687.5	2,687.56
Crude protein (%)	17.05	17.00	17.00	17.00	17.00	17.00
Crude fibre (%)	3.83	3.80	3.80	3.80	3.80	3.80
Fat (%)	3.61	3.59	3.59	3.59	3.59	3.59
Lysine (%)	0.97	0.97	0.97	0.97	0.97	0.97
Meth + Cyst (%)	0.71	0.71	0.71	0.71	0.71	0.71
Calcium (%)	3.68	3.68	3.68	3.68	3.68	3.68
Ave. Phosphorus (%)	0.40	0.40	0.40	0.40	0.40	0.40

Meth + Cyst - Methionine plus Cystiene, K-Nutripoult, L-Hi-Nutrient, M-Agrited,N-Daram vita-mix, P-Micro-mix, Diet without proprietary vitamin-minerals-D1, Diet with premixK-D2, Diet with prmix L-D3, Diet with premix M-D4, Diet with premix N-D5, Diet with premix P-D6

3.3.8: Data collection

Thermo-hydrometers were strategically positioned at different locations in the two housing systems to measure ambient temperature (^{o}C) and relative humidity (%) daily between 7.00-8.00, 12.00-1.00 and 17-18 hours. The average values of ambient temperature (^{o}C) and relative humidity (%) were then determined. The number of eggs produced per replicate, treatment and housing system was recorded on daily basis. The Hen Day Egg Production (HDEP) was determined as follows:

$$\text{Hen Day Egg Production (HDEP)} = \frac{\text{Total number of eggs produced per week}}{\text{Total number of hen-day per week}}$$

3.3.9: Statistical analysis

Data were analysed using descriptive statistics and GLM procedure of analysis of variance (ANOVA) at $\alpha_{0.05}$ (SAS, 2012). Means were separated using LSD option of the same software.

Study Four

Effects of five different proprietary vitamin-mineral premixes, two housing systems and duration of storage on external and internal quality indices of eggs

3.4.1: Experimental site

As described in 3.1.1.

3.4.2: Housing systems

As described in 3.1.2 above

3.4.3: Experimental design and model

The experimental birds were randomly allocated to two HS (BC and DL systems) and six treatments in acompletely randomised design of 2x6 factorial arrangements. Eggs were stored for 0, 7, 14, 21 and 28 days. The experimental model is given below.

$Xijk = u+S_i+S_j+S_k+ S_{ij}+S_{jk}+S_{ijk}+e_{ijkl,}$ where:

u = overall population mean
S_i = effect of housing system (deep litter and battery cage)
S_j = effect of proprietary vitamin-mineral premixes (k-n)
S_k= effect of days of egg storage (0, 7, 14, 21, 28)
S_{ij} = interaction between housing systems and proprietary vitamin-mineral premixes
S_{jk} = interaction between housing systems and days of egg storage
S_{ijk} = interaction between housing systems, proprietary vitamin-mineral premixes and days of egg storage
e_{ijkl}= random residual error

3.4.4: Animals and management

Bovan Nera pullets (n=563) at early-lay (22 weeks of age) from study two in BC and DL systems were used for this study. Management was as described in 3.1.3 above. The experimental diets were fed from 22 to 70 weeks.

3.4.5: Test proprietary layers vitamin-mineral premixes

As described in 3.3.5 above.

3.4.6: Dietary layout

As described in 3.3.6 above.

3.4.7: Experimental diets

As described in 3.3.7

3.4.8: Data collection

At week 36, 180 fresh eggs representing fifteen eggs per treatment and90 eggs per housing system were randomly sampled. Fifteen fresh eggs, three eggs per treatment, were immediately evaluated for external and internal quality indices, while the remaining eggs were stored in trays with the broad ends containing air cells upward on the shelf at avaerage ambient temperature of 26 ^{o}C for 7, 14, 21 and 28 days, respectively. The ambient temperature of egg storage was determined using Thermo-hygrometers. Stored eggs were then evaluated for external and internal quality indices at different days of storage using standard procedures. Egg and shell weights were measured using electronic top loading scale (JS-B LCD® Display Scale). Egg length and diameter were measured using electronic venier caliper, while shell thickness was measured using micrometer screw gauge after drying at room temperature (Scott and Silverside, 2000). Egg diameter and shell thickness were measured in three places (at the narrow, middle and broad ends) and the average taken (Tyler, 1961). Egg weight loss was determined as difference between successive weights at different days of storage (Bhale *et al*., 2003).

Each egg was broken on a flat plate to measure internal egg quality indices. Albumen pH was measured using pH meter. Albumen height was measured using tripod micrometer. Yolk was carefully separated from albumen to measure yolk height and diameter using electronic venier caliper. Yolk weight was measured using electronic top loading scale (JS-B LCD® Display Scale). The weight and diameter of the petri dish bottom used for holding egg-yolk was noted. Yolk weight and height were determined by difference. Albumen weight was determined by difference of egg weight, yolk weight and shell weight. Yolk index was estimated as ratio of yolk height to width. The DSM Roche Yolk Colour Fan (RYCF) was used to determine the yolk colour. Haugh Units were determined from albumen height and egg weight as described by Haugh (1937) from the equation;

$$HU = 100 \log_{10} (h - 1.7W^{0.37} + 7.6); \text{ where}$$

HU=Haugh Unit; h=observed height of the albumen in millimeters and W= egg weight in grams

3.4.9: Statistical analysis

Data were analysed using descriptive statistics and GLM procedure of analysis of variance (ANOVA) at $\alpha_{0.05}$ (SAS, 2012). Means were separated using LSD option of the same software.

Study Five

Effect of supplementing laying chicken feed with five different proprietary vitamin-mineral premixes, two housing systems and duration of storage on chemical compositions of eggs

3.5.1: Experimental site

As described in 3.1.1 above

3.5.2: Housing systems

As described in 3.1.2 above.

3.5.3: Experimental design and model

As described in 3.4.3 above.

3.4.4: Animals and management

Bovan Nera pullets (n=558) at early-lay (22 weeks of age) from study two in BC and DL systems were used for this study. Management was as described in 3.1.3 above. The experimental diets were fed from 22 to 71 weeks.

3.4.5: Test proprietary layers vitamin-mineral premixes

As described in 3.3.5 above.

3.4.6: Dietary layout

As described in 3.3.6 above.

3.5.7: Experimental diets

As described in 3.3.7

3.5.8: Data collection

At week 36,180 fresh eggs representing 15 eggs per treatment and 90 eggs per housing system were randomly sampled. Fifteen fresh eggs representing 3 eggs per treatment were immediately evaluated chemically, while the remaining eggs were stored as described in 3.4.7 Egg were broken and homogenized for determination of chemical composition at different days of storage using standard procedures (AOAC, 2000).

3.5.8.1: Determination of moisture and dry matter (AOAC Offical Method 934.01)

Moisture and dry matter determinations in eggs were carried out using air-oven dry method. Crucibles were washed and dried in an oven. They were allowed to cool in desiccators and the weight noted. Ten grams of homogenized eggs were then transferred into the crucibles and dried at a temperature between 103-105^{o}C. The dried samples were cooled in desiccators and weighed. They were later returned to the oven and the process continued until constant dry weights were obtained.

Moisture content (%) = (weight loss ÷ initial weight) x 100
Dry Matter content (%) = (Dry weight ÷ initial weight) x 100

3.5.8.2: Determination of ash (AOAC Offical Method 942.05)

Five grams homogenized samples of eggs dried at 55^{o}C were weighed into clean and previously dried weighed crucibles. The samples were ignited over a low flame to char organic matter. The crucibles were then placed in muffle furnace at 600^{o}C for 6 h until it ash completely. They were then transferred directly to desiccators, cooled and weighed immediately.

Ash (%) = {(Initial weight – ash weight)/initial weight} x 100.

3.5.8.3: Determination of crude protein (AOAC Offical Method 2001.11)

The crude protein content was determined using micro Kjeldahl method. Two grams of homogenized eggs was weighed into a long necked Kjeldahl flask. One tablet of Kjeldahl catalyst was added to the sample in flask with 25cm^3 of conc. H_2SO_4. The flask was swirled, gently clamped in an inclined position and heated electrically in a fume cupboard. The heating continue until a clear solution was obtained. The clear solution was cooled, poured into 100cm^3 volumetric flasks and made up to mark with distilled water. Ten milliliter of the resulting mixture was measured into distillation set through a funnel.

Five cubic centimeters of boric acid was pipetted into a 100 cm^3 conical flask and placed at the receiving end of the distillatory. The conical flask was placed such that the delivery tube dipped completely into the boric acid inside the flask. 40% NaOH was used to liberate ammonia from the digest under alkaline condition during distillation. Two drops of methyl orange was added to the round bottom flask containing the digested sample before 40% NaOH added. As soon as the contents became alkaline, the

red colour changed to yellow showing excess NaOH. Steam was then generated into the distillation set using a steam chest. The liberated ammonia was trapped in the boric acid solution and about 50 cm^3 of the solution collected into a conical flask. The solution in the flask was titrated against 0.1M HC1 until the first permanent colour change observed. A blank sample was allowed to go through the same procedure to obtain blank titre value. The titre value for the blank was used to correct for the titre values of samples.

$$N\ (\%) = \frac{\text{Molarity of HC1 X (Sample titre – Blank titre) X 0.014 X DF X 100}}{\text{Weight of sample used.}}$$

N (%) was converted to the percentage crude protein by multiplying by 6.25.

3.5.8.4: Determination of ether extracts (EE) (AOAC Offical Method 960.09)

Soxhlets extraction method was used to extract ether. A known weight of homogenized eggs (dried at 55^oC) was weighed into a weighted filter paper and folded neatly and placed inside pre-weighed thimble. The thimble was inserted into the Soxhlets apparatus and extraction under reflux was carried out with petroleum ether (40–60^oC boiling range) for 6 hrs. At the end of extraction, the thimble was dried in the oven for about 30 minutes at 100^oC to evaporate solvent and thimble cooled in desiccator and later weighed. The ether extracted from a given quantity of sample was then calculated:

$$\text{Ether Extract (\%)} = \frac{\text{Loss in weight of sample X 100}}{\text{Original weight of sample}}$$

3.5.8.5: Determination of gross energy (AOAC, 1995)

Gross energy (GE) is the amount of heat produced from sample when it is completely burnt down to its ultimate oxidation products; carbon dioxide (CO_2) and water (H_2O). Samples of freeze-dried eggs were burnt in Bomb calorimeter and heat produced measured to determine Gross Energy according to the procedure of AOAC (1995) using the formula;

$$\text{Gross heat of combustion (cal/g)} = \frac{T \times W \times [C_1 + C_2 + C_3)}{M}$$

where T =Rise in temperature,W = Water equivalent, C_1 and C_2 = Heat of combustion (cal) of H_2SO_4 and HNO_3, C_3 = Heat of combustion (cal) of used wire, paper and thread, M = Weight of freeze-dried eggs

3.5.8.6: Determination of calcium (AOAC Offical Method 927.02)

The ash sample obtained was digested by adding 5 mL of 2M HCL to the ash in the crucible and heat to dryness on a heating mantle, 5 mL of 2M HCL was added again, heat to boil and filtered through a Whatman No.1 filter paper into a 100 mL volumetric flask. The filtrate was made up to mark with distilled water stopper and made ready for reading of concentration of calcium on the Jenway Digital Flame Photometer (PFP7 Model) using the filter corresponding to each mineral element. The concentration of each of the element was calculated using the formula:

Calcium (%) = Meter reading (MR) X Slope X Dilution factor/1000

NB: MR x Slope x dilution factor gave the concentration in part per million (ppm or mg/kg) and when divided by 10000 concentration in % was derived.

3.5.8.7: Determination of phosphorous (AOAC Offical Method 964.06)

Phosphorus was determined routinely by the Vanado-molybdate colorimeter or spectrophotometric method. The ash sample obtained was treated with 2M HCL solution as described for calcium determination above, 10 mL of the filtrate solution was pipetted into 50 mL standard flask and 10 mL of vanadate yellow solution was added and the flask was made up to mark with distilled water, stoppered and left for 10 minutes for full yellow development. The concentration of the phosphorus was obtained by taking the optical density (OD) or absorbence of the solution on a spectronic-20 at 470 nm wavelengths. The percentage phosphorus was calculated using the formula:

Phosphorus (%) = Absorbance x Slope x Dilution factor/10000

3.5.8.8: Determination of nitrogen free extracts (NFE) (AOAC Offical Method 978.1)

The nitrogen free extract (NFE) was calculated by the difference of crude protein, ash, fat and moisture content from 100.

NFE (%) = 100-(crude protein + fat + ash+ moisture)

3.5.9: Statistical analysis

Data were analysed using descriptive statistics and GLM procedure of analysis of variance (ANOVA) at $\alpha_{0.05}$ (SAS, 2012). Means were separated using LSD option of the same software.

Study Six

Effects of five different proprietary vitamin-mineral premixes and two housing systems on cholesterol profile of chicken eggs

3.6.1: Experimental site

As described in 3.1.1 above

3.6.2: Housing systems

As described in 3.1.2 above.

3.6.3: Experimental design and model

As described in 3.4.3 above.

3.6.4: Animals and management

Bovan Nera pullets (n=558) at early-lay (22 weeks of age) from study two in BC and DL systems were used for this study. Management was as described in 3.1.3 above. The experimental diets were fed from 22 to 71 weeks.

3.6.5: Test proprietary layers vitamin-mineral premixes

As described in 3.3.5 above.

3.6.6: Dietary layout

As described in 3.3.6 above.

3.6.7: Experimental diets

As described in 3.3.7

3.6.8: Data collection

At week 36, 72 fresh eggs representing one per replicate, 6 per treatment and 36 per housing system were randomly sampled and labeled appropriately and analysed for cholesterolprofile in egg-yolk. Fresh eggs were broken and the yolks separatedfrom albumen using egg-yolk separator. The yolks were beaten and mixed together to obtain a clear and homogenous mixture. Five milliliter of homogenized samples were put in K_3EDTA bottles with anticoagulant EDTA for cholesterol profile analyses. The samples

were centrifuged at 1800 r/m and then analyzed using Hitachi 902: Auto Analyzer for total cholesterol, triglycerides, high density lipoprotein-cholesterol (HDLc) and low density lipoprotein-cholesterol (LDLc) as described (Friedwald *et al.*, 1972; Bauer, 1982). The value of very low density lipoprotein (VLDL) was calculated by division of triglycerides values by 5.

3.6.9: Statistical analysis

Data were analysed using descriptive statistics and GLM procedure of analysis of variance (ANOVA) at $\alpha_{0.05}$ (SAS, 2012). Means were separated using LSD option of the same software.

Study Seven

Effect of supplementing five different proprietary vitamin-mineral premixes, two housing systems and duration of storage on lipid oxidation of eggs

3.7.1: Experimental site

As described in 3.1.1 above

3.7.2: Housing systems

As described in 3.1.2 above.

3.7.3: Experimental design and model

As described in 3.4.3 above.

3.7.4: Animals and Management

Bovan Nera pullets (n=558) at early-lay (22 weeks of age) from study two in BC and DL systems were used for this study. Management was as described in 3.1.3 above. The experimental diets were fed from 22 to 71 weeks.

3.7.5: Test proprietary layers vitamin-mineral premixes

As described in 3.3.5 above.

3.7.6: Dietary layout

As described in 3.3.6 above.

3.7.7: Experimental diets

As described in 3.3.7

3.7.8: Data collection

At week 36, 180 fresh eggs representing 15 eggs per treatment and 90 eggs per housing system were randomly sampled. Fifteen fresh whole eggs, 3 eggs per treatment, were immediately evaluated for lipid oxidationmeasured as secondary product, while the remaining eggs were stored on the shelf at ambient temperature of 26 ^{o}C for 7, 14, 21 and 28 days. The ambient temperature of egg storage was determined using Thermo-

hygrometers. Stored eggs were then evaluated for Thiobarbituric Acid Reactive Substance (TBARS, µm/g) at different days of storage (Kang *et al*., 2001)

MDA (TBARS mg /100g) = K x A; where: K = -9.242; A = Absorbance

3.7.9: Statistical analysis

Data were analysed using descriptive statistics and GLM procedure of analysis of variance (ANOVA) at $\alpha_{0.05}$(SAS, 2012). Means were separated using LSD option of the same software.

CHAPTER FOUR

4.0: RESULTS

Study One

4.1.1: Performance characteristics of growing pullets from 13- 16 weeks of age

Performance characteristics of growing pullets in the two housing systems (HS) [Battery cage (BC) and Deep litter (DL)] from 13 to 16 weeks of age are presented in Table 6. The daily feed intake (g/bird/day) of pullets in BC (100.00±0.06) was higher ($p<0.05$) than 80.00±0.08 in DL. The initial live weight (kg/bird) of pullets at week 13 in BC (1.08±0.08) and DL (1.06±0.07) were not different ($p>0.05$). Pullets in BC (1.32±0.10) and DL (1.33±0.11) increased in live weight to gain 0.28±0.02 and 0.24±0.03 respectively. There were no differences ($p>0.05$) in the live weight and live weight gain. However, regression of live weight and age in weeks of pullets in the two HS were strong and positive as shown by equations 1 and 2 below:

BC: $y = 0.097x + 0.95$…… ($R^2 = 0.96$)…….... 1

DL: $y = 0.08x + 0.985$…… ($R^2 = 0.97$) …….. 2

Feed conversion ratio of pullets in BC (9.86±0.02) was similar to 9.71±0.03 in DL. Feed cost per gain of pullets in BC (₦701.71) was higher ($p<0.05$) than ₦691.13 in DL. There was no mortality of pullets in the two housing systems during the period of study.

Table 6: Performance characteristics of pullets in two housing systems from 13- 16 weeks of age

Parameters	BC±SD	DL±SD
Initial live weight/bird at week 13 (kg)	1.08 ± 0.08	1.06 ± 0.07
Final live weight/bird at week 16 (kg)	1.32 ± 0.10	1.33 ± 0.11
Live weight change (kg/bird)	0.28 ± 0.02	0.24 ± 0.03
Live weight gain (g/bird/day)	13.33±0.03	11.43 ± 0.04
Daily Feed intake (g/bird/day)	100.00 ± 0.06[a]	80.00 ± 0.08[b]
Feed conversion ratio	9.86± 0.02	9.71± 0.03
Feed cost/live weight gain (₦/kg)	701.71[a]	691.13[b]
Age at point of lay (days)	122	122
Weight of egg at point of lay (g)	32.00	31.83
Mortality (%)	0.00	0.00

[a-b]Means with different superscripts within the same row are significantly different ($p<0.05$). BC- Battery cage, DL-Deep litter, SD- Standard Deviation

Study Two

4.2.1: Performance charcteristics of pullets fed diets supplemented with five different proprietary vitamin-mineral premixes in two housing systems from 17 to 21 weeks of age

Performance characteristics of pullets fed diets supplemented with five different PVmP in two HS from point of lay at week 17 to 22 weeks is presented in Table 7. The main effect of HS on final live weight (FLW) and daily feed intake (DFI) were different ($p<0.05$). Pullets in DL had higher ($p<0.05$) daily feed intake (DFI) (88.00g/bird/day) and final live weight (FLW) (1.73kg/bird) compared with 86.86 and 1.68, respectively in BC. The regression of feed intake and age of pullets in BC were positive and strong than in DLas shown by equations 3 and 4 below:

BC: $y = 6.09x + 68.6$ …… (R^2=0.82)…… ……3
DL: $y = 5.124x + 72.498$ …… (R^2=0.57) …… 4

Housing systems did not affect ($p<0.05$) total feed intake (TFI), live weight changes (LWC), feed conversion ratio (FCR) and mortality (M). Pullets in DL recorded 3.08kg/bird (TFI) to gain 11.43g/bird/day (LWC) at feed effeiciencey of 7.70 (FCR) compared with 3.04; 10.29 and 8.44 respectively for those in BC.

The regression of live weight and age in weeks of pullets in the two HS are represented in equations 5 and 6 below. The regression values obtained for growing pullets in both HS were positive, strong and similar.

BC: $y = 0.0746x + 1.2189$ …… ($R^2 = 0.98$)……5
DL: $y = 0.0758x + 1.2469$…… ($R^2 = 0.98$) …… ……. 6

Effects of PVmP on FLW differed ($p>0.05$). Pullets fed diets supplemented with Nutripoult had 1.74 kg/bird FLW similar 1.73 kg/bird for fed diets without PVmP but higher ($p<0.05$) compared with 1.69, 1,69, 1.69 and 1.69 kg/bird for pullets on diets with Hi-Nutrient, Agrited, Daram vita and Micro-mix respectively. The main effect of PVmP supplementation on TFI, DFI, BWC, FCR and mortality were not different ($p>0.05$).

Pullets fed diets supplemetened with Agrited (D4) had highest TFI (4.93 kg/bird), DFI (87.98 g/bird/day) and LWC (11.14 g/bird/day). Pullets on diets supplemented with Nutripoult at week 16 grew from 1.36kg/bird to attain the highest body weight (1.74kg/bird) at week 21. Pullets fed diet supplemented with Hi-Nutrient, Agrited, Daram vita-mix and Micro-mix recorded lower live weights compared to those on diets without PVmP (D1). There were positive and strong regreesion values of live weight and age of pullets fed different PVmP as shown in equations 7, 8, 9, 10, 11 and 12 below:

D1: y =0.074x + 1.2427 …… (R^2 = 0.96)…….. 7
D2: y = 0.0737x + 1.262…… (R^2 = 0.95) …….. 8
D3: y =0.0789x + 1.214 …… (R^2 = 0.98) …….. 9
D4: y = 0.0777x + 1.2047…… (R^2 = 0.99) ……10
D5: y = 0.0749x + 1.2247…… (R^2 = 0.99)…… ……11
D6: y = 0.0714x + 1.2533…… (R^2 = 0.99) …… 12

There was no mortality (%) among pullet on diets supplemented with Hi-Nutrient (D3), while those fed diets without PVmP (D1) recorded 2.08, and those supplemented with Nutripoult (D2), Agrited (4), Daram vita-mix (5) and Micro-mix (6) 2.08, 1.04, 1.04 and 1.04 respectively.

Table 7: Performance characteristics of pullets fed diets supplemented with five different proprietary vitamin-mineral premixes in two housing systems from 17 to 21 weeks of age

	Factors	ILW (kg/bird)	FLW (kg/bird)	TFI (kg/bird)	DFI (g/bird/day)	LWC (g/bird/day)	FCR	M (%)
	BC	1.32	1.68^{b}	3.04	86.86^{b}	10.29	8.44	1.74
HS	DL	1.33	1.73^{a}	3.08	88.00^{a}	11.43	7.70	0.00
	SEM	0.01	0.01	0.01	1.25	0.07	0.04	1.23
	D1	1.34	1.73^{ab}	4.85	87.15	11.14	12.44	2.08
	D2	1.36	1.74^{a}	4.89	87.37	10.86	12.87	2.08
	D3	1.32	1.69^{b}	4.90	87.62	10.57	13.24	0.00
PVmP	D4	1.30	1.69^{b}	4.93	87.98	11.14	12.64	1.04
	D5	1.31	1.69^{b}	4.92	87.81	10.86	12.95	1.04
	D6	1.32	1.69^{b}	4.84	86.31	10.57	13.08	1.04
	SEM	0.01	0.02	0.23	0.33	0.11	0.16	0.32

$^{a-b}$Means with different superscripts within the same column are significantly different ($P<0.05$). HS-Housing systems, PVmP-Proprietary vitamin-mineral premix, BC-Battery cage, D-Deep litter, ILW–Initial live weight, FLW–Final live weight, TFI–Total feed intake, DFI–Daily feed intake, LWC–Live weight change, FCR–Feed conversion ratio, M–Mortality, D1-diet without PVmP, D2, D3, D4, D5 and D6-diets with Nutripoult (K), Hi-Nutrient (L), Agrited (M), Daram vita-mix (N) and Micro-mix (P) respectively, SEM- Standard error of means

The interaction effects of PVmP x HS on performance characteristics of pullets fed diets supplemented with five different PVmP from week 17 to 22 is shown in Table 8. There were significant ($p<0.05$) interaction effects of PVmP x HS on FLW. However, interaction effects of Nutripoult x DL (D2 x DL) on FBW (kg/bird) (1.53) was the highest and similar to Nutripoult x BC (D2 x BC) (1.51); D1 and DL (D1 x DL) (151); Hi-Nutrient x DL (D3 x DL) (1.51); Agritedx DL (D4 x DL) (1.50); Daram vita-mix x DL (D5 x DL) (1.51); and Micro-mixx DL (D6 x DL) (1.51) but higher ($p<0.05$) compared with D1 x BC (1.49), Hi-Nutrient x BC (1.47), Agrited x BC (1,45), Daram vita-mix x BC (1.46) and Micro-mix x BC (1.50). The interaction effect of PVmP x HS on TFI and DFI were not different ($P>0.05$).

Table 8: Interaction effects of proprietary vitamin-mineral premixes and housing systems on performance characteristics of pullets from 17 to 21 weeks of age

	Factors	FLW (kg/bird)	TFI (kg/bird)	DFI (g/bird/day)
	D1 x BC	1.49^{bc}	4.88	87.2
	D2 x BC	1.51^{ab}	4.85	86.61
PVmP x BC	D3 x BC	1.47^{cd}	4.92	87.80
	D4 x BC	1.45^{d}	4.87	86.96
	D5 x BC	1.46^{d}	4.86	86.79
	D6 x BC	1.50^{bc}	4.81	85.86
	D1 x DL	1.51^{ab}	4.86	86.85
	D2 x DL	1.53^{a}	4.94	88.13
PVmP x DL	D3 x DL	1.51^{ab}	4.90	87.44
	D4 x DL	1.50^{ab}	4.98	88.99
	D5 x DL	1.51^{ab}	4.97	88.81
	D6 x DL	1.51^{ab}	4.87	87.02
	SEM	0.01	0.12	2.18

$^{a-d}$Means with different superscripts within the same column are significantly different (P<0.05). PVmP-Proprietary vitamin-mineral premix, BC-Battery cage, DL-Deep litter, FLW–Final live weight, TFI–Total feed intake, DFI–Daily feed intake, D1-diet without PVmP, D2, D3, D4, D5,and D6-diets with Nutripoult, Hi-Nutrient, Agrited, Daram vita-mix and Micro-mix respectively, x-Interaction, SEM-Standard error of means

4.2.2: Hen day egg production of pullets fed diets supplemented with five different proprietary vitamin-mineral premixes in two housing systems from 17 to 21 weeks of age

Hen Day Egg production (HDEP) of pullets fed diets supplemented with five different PVmP in two HS from 17 to 21 weeks of age is shown in Figure 1. The HS affected ($p<0.05$) number of eggs produced (EP) and HDEP of pullets from 17 to 21 weeks of age. Pullets in the two HS started egg laying at about week 18 with birds in BC commencing earlier and produced more eggs than those in DL. At week 21, pullets in BC had 33.19% HDEP higher ($p<0.05$) than 16.62% in DL. In Figure 2, PVmP caused variations ($p<0.05$) in HDEP of pullets from 17 to 21 weeks of age. Pullets on diets supplemented with Micro-mix (D6) maintained highest level of egg production from 17 to 19 week. At week 19, pullets on Hi-Nutrient (D2) increased in HDEP over others fed diets with and without PvmP. The HDEP of pullets fed diets supplemented with Darami vita-mix (D5) increased rapidly more than those on Nutripult (D2) at week 21. However, the HDEP of pullets on diets without PVmP supplementation was abysmal lower compared with those on diets containing PVmP.

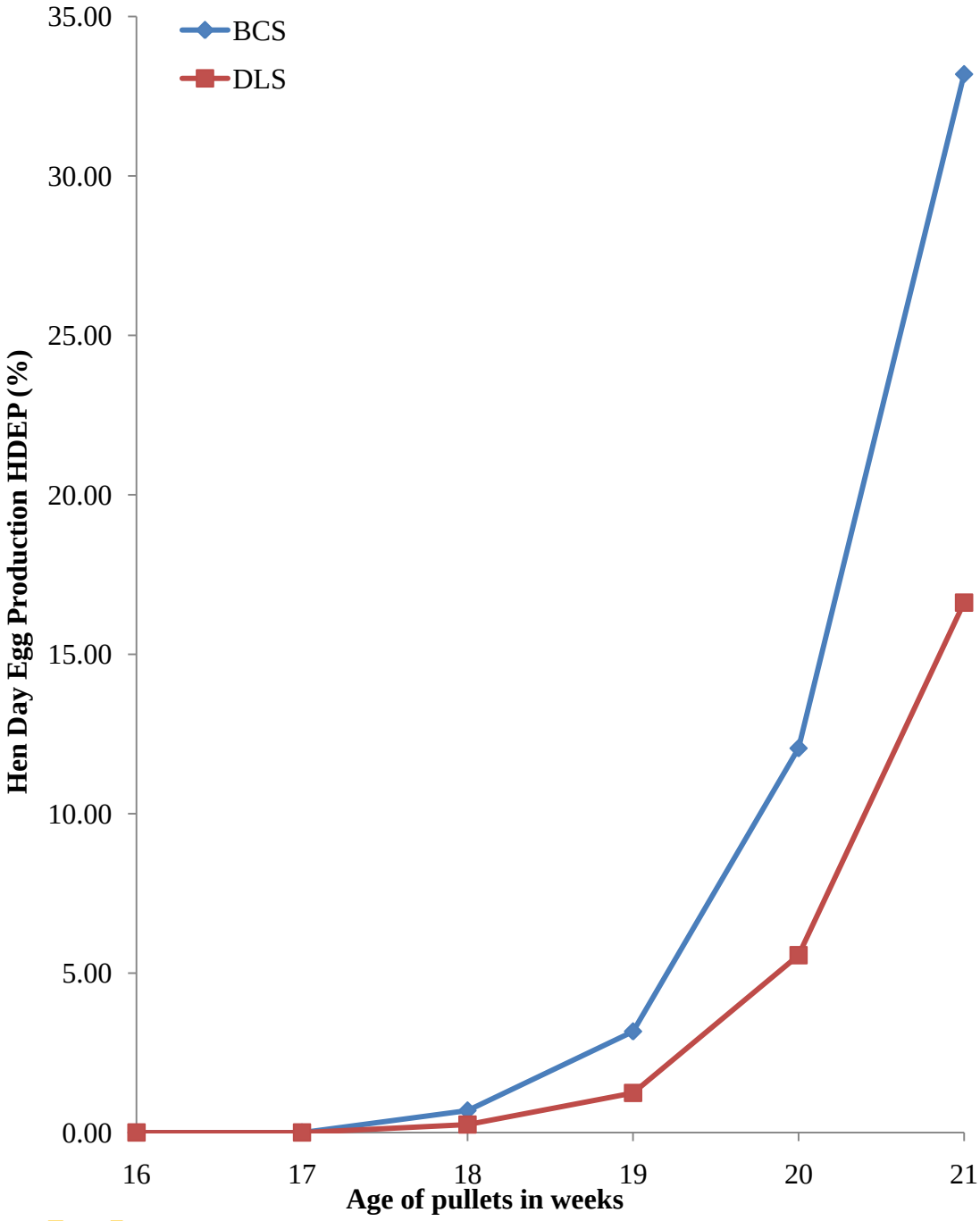

Figure 1: Hen Day Egg Production of pullets in two housing systems from 16 to 21 weeks of age (BCS-Battery cage system, DLS-Deep litter system)

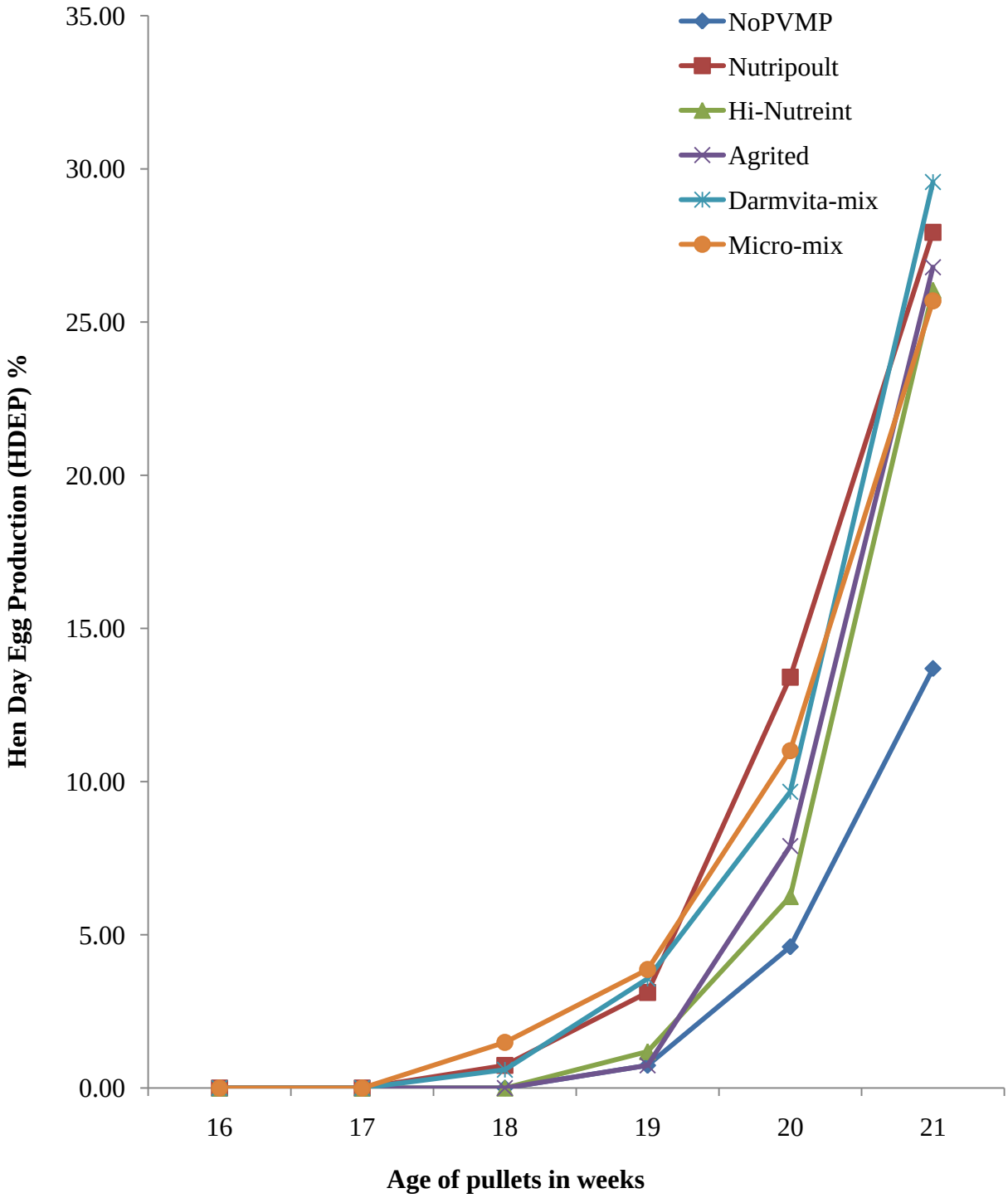

Figure 2: Hen Day Egg Production of pullets fed diets supplemented with five different proprietary vitamin-mineral premixes from 16 to 21 weeks of age

Study Three

4.3.1: Ambient temperature (^{o}C) and relative humidity in the two housing systems

Ambient temperature and relative humidity in the two housing systems in the period of production are presented in Table 9. The ambient temperatures (^{o}C) and relative humidity (%) range recorded in BC and DL were 25.7-32.1 and 22.6-82.2; 25.3-31.3 and 27.8- 87.8 with the corresponding mean values of 28.5±1.6 and 68.2±13.7; 28.3±1.7 and 73.6±13.5 respectively.

.

Table 9: Ambient temperature (°C) and relative humidity (%) of two housing systems

	BC		DL	
Age (weeks)	**T**	**RH**	**T**	**RH**
22	29.3	73.4	29.1	76.0
23	29.3	69.6	29.2	72.8
24	27.7	77.8	27.8	81.2
25	28.9	71.2	29.1	76.6
26	29.5	69.5	29.3	75.5
27	29.1	70.3	29.0	77.2
28	28.1	78.6	28.0	82.5
29	28.5	71.6	28.4	79.3
30	28.8	71.5	29.0	77.9
31	27.8	75.8	27.6	81.4
32	27.6	77.5	27.5	82.6
33	27.7	77.3	27.2	80.2
34	27.5	78.5	27.4	83.4
35	26.9	81.1	26.7	86.3
36	26.4	82.2	26.2	87.8
37	26.2	81.4	26.0	87.6
38	25.9	77.5	25.9	82.6
39	25.7	81.4	26.1	86.0
40-60	.	.	.	.
61	29.8	57.4	27.9	59.0
62	30.9	58.9	30.7	62.3
63	31.2	62.5	31.3	64.8
64	31.1	60.9	31.0	64.1
65	30.0	64.7	30.3	67.2
66	30.8	52.0	31.1	57.3
67	31.3	53.2	31.1	58.1
68	32.1	58.7	32.1	63.0
69	31.1	64.0	30.9	68.0
70	30.4	62.0	30.5	66.4
71	29.7	63.2	29.7	66.7
M±SD	**28.5±1.6**	**68.2±13.7**	**28.3±1.7**	**73.1±13.5**

BC-Batter cage, DL - Deep litter, T-Temperature, RH-Relative humidity, M-Mean, SD - Standard deviation, ·Hidden data

4.3.2: Performance characteristics of layers fed diets supplemented with five different proprietary vitamin-mineral premixes in two housing systems from 22 to 35 weeks of age

The performance characteristics of layers fed diets supplemented with five different PVmP in two HS in from 22 to 35 weeks of age is presented in Table 10. Main effects of HS significantly ($p<0.05$) affected liveweight (LW) and daily feed intake (DFI) of layers. The LW (1.73kg/bird) and DFI (98.54g/bird/day) of layers in DL were higher ($p<0.05$) than 1.54 and 90.09 respectively in BC. There were variations ($p<0.05$) across dietary PVmP supplementation on LW and DFI. The DFI (g/bird/day) of layers fed diets without PVmP (98.16) was higher ($p<0.05$) compared with 86.21, 94.35 and 94.68 for layers diets supplemented with Micro-mix, Daram-vita and Agrited respectively. Layers fed diets without PVmP recorded the highest feed intake (98.16 g/bird/day), while those on diets supplemented with Micro-mix (86.21 g/bird/day) the least. Layers fed diets supplemented with Nutripoult (D2) had the highest LW (1.66 kg/bird) similar to those on Hi-Nutrient (1.65 kg/bird) but higher ($p<0.05$) compared with 1.60, 1.62, 1.64 and 1.64 for those on Daram-vita, Agrited, Micro-mix and without PVmP respectively.

Table 10: Performance characteristics of layers fed diets supplemented with five different proprietary vitamin-mineral premixes in two housing systems 22 to 35 weeks of age

	Factors	BW (kg)	DFI (g/h/d)
	BC	1.54[b]	90.09[b]
HS	DL	1.73[a]	98.54[a]
	SEM	0.01	0.87
	D1	1.64[b]	98.16[a]
	D2	1.66[a]	96.31[ab]
	D3	1.65[ab]	96.20[ab]
PVmP	D4	1.62[c]	94.68[b]
	D5	1.60[d]	94.35[b]
	D6	1.64[b]	86.21[c]
	SEM	0.01	1.51

[a–d]Mean values with different superscripts on the same column are significantly different ($p<0.05$). BW-Body weight, FI-Feed intake, HS-Housing systems, DL-Deep litter, BC-Battery cage, PVMP- Proprietary vitamin-mineral premixes, D1-diet without PVmP, D2, D3, D4, D5,and D6-diets with Nutripoult, Hi-Nutrient, Agrited, Daram vita-mix and Micro-mix respectively, SEM--Standard error of mean

The interaction effects of PVmP x HS on LW and DFI of layers fed diets supplemented with five different PVmP in two HS in from 22 to 35 weeks of age is shown in Table 11. The PVmP x HS interaction effect on DFI and LW were significant ($p<0.05$). Micro-mix x DL interaction effect on DFI (101.11 g/day/bird) was similar to Nutripoult x BC (93.38), Hi-Nutrient x DL (99.45), Agrited x DL (99.44) and Daram-vit x DL (99.30) but higher ($p<0.05$) compared with diet without PVmP x BC (79.40), diet without PVmP x DL (93.06), Micro-mix x BC (87.59), Daram-vita x BC (90.06), Agrited x BC (92.96) and Hi-Nutrient x BC (93.17).

Nutripoult x DL interaction effect on LW (1.75 kg/bird) was similar to Hi-Nutrient x DL (1.73), Daram vita-mix x DL (1.73) and Micro-mix x DL (1.74) but higher ($p<0.05$) compared with diets without PVmP x BC (1.57), Nutripoult x BC (1.57), Hi-Nutrient x BC (1.57), Agrited x BC (1.53), Daram vita-mix x BC (1.47), Micro-mix x BC (1.53), diet without PVmP x DL (1.72) and Agrited x DL (1.70).

Table 11: Interaction effects of proprietary vitamin-mineral premixes and housing systems on performance characteristics of layers from 22 to 35 weeks of age

PVmP x HS	LW (kg)	DFI (g/h/d)
D1 x BC	1.57^{d}	79.40^{e}
D2 x BC	1.57^{d}	93.38^{abc}
D3 x BC	1.57^{d}	93.17^{bcd}
D4 x BC	1.53^{e}	92.96^{cd}
D5 x BC	1.47^{f}	90.06^{d}
D6 x BC	1.53^{e}	87.59^{d}
D1 x DL	1.72^{bc}	93.06^{bcd}
D2 x DL	1.75^{a}	98.93^{ab}
D3 x DL	1.73^{ab}	99.45^{a}
D4 x DL	1.70^{c}	99.44^{a}
D5 x DL	1.73^{abc}	99.30^{a}
D6 x DL	1.74^{ab}	101.11^{a}
SEM	0.01	2.14

$^{a–f}$Mean values with different superscripts on the same column are significantly different (P<0.05). LW-Body weight changes, FI-Feed intake, PVmP-Proprietary vitamin-mineral premix, HS-Housing systems, DL-Deep litter, BC-Battery cage, D1-diet without PVmP, D2, D3, D4, D5,and D6-diets with Nutripoult, Hi-Nutrient, Agrited, Daram vita-mix and Micro-mix respectively, SEM--Standard error of mean, x-Interaction

4.3.3: Egg production characteristics of layers fed diets supplemented with five different proprietary vitamin-mineral premixes in two housing systems from 22 to 35 weeks of age

Egg production characteristics of layers fed diets supplemented with five different PVmP in two HS from 22 to 35 weeks of age is presented in Table 12. The HS significantly ($p<0.05$) influenced number of eggs laid (EP), HDEP, egg mass (EM) and feed conversion ratio per egg mass (FCR/EM). The egg weight was not significantly ($p>0.05$) affected by HS. Layers in DL had higher ($p<0.05$) FCR/EM (2.47), egg laying capacity (HDEP; 71.22%) and egg mass (7.33 g/bird/day) compared with 2.19, 62.58 and 6.48 respectively in BC. Egg production characteristics increased significantly ($p<0.05$) with dietary PVmPs. The FCR/EM of layers fed diets supplemented with Nutripoult (2.46) was similar to 2.42 and 2.37 for those on Hi-Nutrient and Agrited respectively. Layers fed diets supplemental Nutripoult (D2) had higher HDEP (76.65) similar to those on supplemeted with Agrited (76.60) but higher ($p<0.05$) compared with those on diets without PVmP (43.40), Hi-Nutrient (68.45), Daram vita-mix (68.59) and Micro-mix (67.72). The mass of eggs produced by layers fed diets supplemented with Nutripoult (7.79) and Agrited (8.06) were similar and higher ($p<0.05$) compared with those fed diets without PVmP (4.39), Hi-Nutrient (6.95), Daram-vita (7.05) and Micro-mix (7.17)

Table 12: Egg production characteristics of layers fed diets supplemented with five different proprietary vitamin-mineral premixesin two housing systems from 22 to 35 weeks of age

	Factors	EP	HDEP (%)	EW (g)	EM (g/h/d)	FCR/EM
	BC	34.02^{b}	62.58^{b}	40.30	6.48^{b}	2.19^{b}
HS	DL	39.87^{a}	71.22^{a}	39.86	7.33^{a}	2.47^{a}
	SEM	0.46	0.83	0.24	0.10	0.02
	D1	24.14^{c}	43.40^{c}	39.35^{b}	4.39^{c}	2.14^{c}
	D2	41.99^{a}	76.65^{a}	39.49^{b}	7.79^{a}	2.46^{a}
	D3	38.23^{b}	68.45^{b}	39.64^{b}	6.95^{b}	2.42^{a}
PVmP	D4	42.54^{a}	76.60^{a}	40.85^{a}	8.06^{a}	2.37^{ab}
	D5	37.33^{b}	68.59^{b}	40.07^{ab}	7.05^{b}	2.32^{b}
	D6	37.33^{b}	67.72^{b}	41.06^{a}	7.17^{b}	2.29^{b}
	SEM	0.80	1.43	0.41	0.18	0.04

$^{a–d}$Mean values with different superscripts on the same column are significantly different ($p<0.05$). EP- Number of egg produced, HDEP-Hen day egg production, EW-Egg weight, EM-Egg mass, FCR/EM-Feed conversion ratio per egg mass, HS-Housing systems, DL-Deep litter, BC-Battery cage, PVmP-Proprietary vitamin-mineral premix, D1-diet without PVmP, D2, D3, D4, D5 and D6-diets with Nutripoult, Hi-Nutrient, Agrited, Daram vita-mix and Micro-mix respectively, SEM-Standard error of mean

The PVmP x HS interaction effects on egg production characteristics of layers fed diets supplemented with five different PVmP in two HS from 22 to 35 weeks of age is shown in Table 13. There were significant ($p<0.05$) interaction effects of PVmP x HS on egg production characteristics. The interaction effects of diets supplemented with Hi-Nutrient x DL (2.55) on FCR/EM was similar to Nutripoult x DL (2.52), Agrited x DL (2.50), Daram-vita x DL (2.50), Micro-mix x DL (2.42) and Nutripoult x BC (2.41) but higher ($p<0.05$) than diets without PVmP x DL (2.32), Hi-Nutrient x BC (2.30), Agrited x BC (2.23). Similarly, interaction effects of Nutripoult x BC (78.71), Agrited x BC (75.13), Nutripoult x DL (76.59), Agrited x DL (78.08) and Micro-mix x DL (75.97) on HDEP were similar and higher ($p<0.05$) compared with other interaction effects. The interaction effect diets without PVmP x BC (26.61) on HDEP was least. The interaction effects of PVmP and HS on EM and HDEP follow similar trends. The interaction effects of Agrited x DL and diets without PVmP x BC on EM were 8.05 and 2.89, respectively.

Table 13: Interaction effects of proprietary vitamin-mineral premixes and housing systems on egg production characteristics of layers from 22 to 35 weeks of age

HS x PVmP	EP	HDEP (%)	EW (g)	EM (g/h/d)	FCR/EM
D1 x BC	16.25^{f}	29.61^{f}	39.01^{d}	2.89^{f}	1.95^{f}
D2 x BC	41.10^{ab}	76.71^{a}	39.46^{cd}	7.79^{a}	2.41^{ab}
D3 x BC	37.35^{cd}	67.05^{c}	40.10^{bcd}	6.87^{cd}	2.30^{bcd}
D4 x BC	41.35^{ab}	75.13^{ab}	41.81^{a}	8.07^{a}	2.23^{cde}
D5 x BC	32.36^{e}	61.21^{de}	40.81^{abc}	6.39^{de}	2.13^{e}
D6 x BC	35.71^{d}	65.77^{cd}	40.61^{abcd}	6.86^{cd}	2.15^{de}
D1 x DL	32.03^{e}	57.19^{e}	39.69^{cd}	5.89^{e}	2.32^{bc}
D2 x DL	42.89^{a}	76.59^{a}	39.53^{cd}	7.80^{a}	2.52^{a}
D3 x DL	39.11^{bc}	69.84^{bc}	39.18^{cd}	7.04^{bcd}	2.55^{a}
D4 x DL	42.72^{a}	78.08^{a}	39.90^{bcd}	8.05^{a}	2.50^{a}
D5 x DL	42.54^{a}	75.97^{a}	39.33^{cd}	7.70^{ab}	2.50^{a}
D6 x DL	38.94^{bc}	69.68^{bc}	41.50^{ab}	7.48^{abc}	2.42^{ab}
SEM	1.13	2.03	0.59	0.25	0.05

$^{a–f}$Mean values with different superscripts on the same column are significantly different ($p<0.05$).EP-Egg production, HDEP-Hen day egg production, HHEP-Hen house egg production, EWEgg weight, EM–Egg mass, FCR/EM-Feed conversion ratio per egg mass, FCR/DE-Feed conversion ratio per dozen egg, HS-Housing systems, PVmP-Proprietary vitamin-mineral premix, DL-Deep litter, BC-Battery cage, D1-diet without PVmP, D2, D3, D4, D5,and D6-diets with Nutripoult, Hi-Nutrient, Agrited, Daram vita-mix and Micro-mix respectively, SEM-Standard error of mean, x-Interaction

4.3.4: Hen Day Egg Production of layers fed diets supplemented with five different proprietary vitamin-mineral premixes in two housing systems from 16 to 70 weeks of age

The Hen Day Egg Production (HDEP, %) of layers in two HS from 16 to 70 weeks of age is presented in Figure 3. The HDEP of layers in BC was higher and maintained steady increase over and above those in DL. Layers in BC attained peak-lay (HDEP; 65.18) at week 23, while those in DL increased steadily to peak (HDEP: 88.99) at week 30, and thereafter declined. Layers in DL remained at higher HDEP than those in BC for the rest of the production period. The HDEP of layers in BC and DL fluctuated and reduced to 52.14 and 57.78 in BC and DL respectively in late-laying phase. The HDEP of layers fed diets supplemented with five different PVmP from 16 to 70 weeks of age is presented in Figure 4. The HDEP values varied ($p<0.05$) with different PVmP supplementations. The HDEP of layers fed Nutripoult (D2), Hi-Nutrient (D3), Agrited (D4), Daram vita-mix (D5) and Micro-mix (D6) were higher compared with those diets without PVmP (D1).

The HDEP of layers increased at comparative rates from point-of-lay (week 18) so that those fed diet without PVmP (D1) attained peak-lay (HDEP: 59.95) earlier at week 23 and then nose-dived sharply to zero HDEP at week 34. Birds fed Nutripoult (D2) and Daram vita-mix (D4) recorded comparatively higher HDEP; 88.71 and 87.67 at weeks 31 and 29 respectively. Layers fed diets supplemented with Micro-mix (D6), Hi-Nutrient (D3) and Daram vita-mix (D4) attained peak HDEP; 83.38, 78.72 and 77.62 at weeks 30, 29 and 29 respectively.

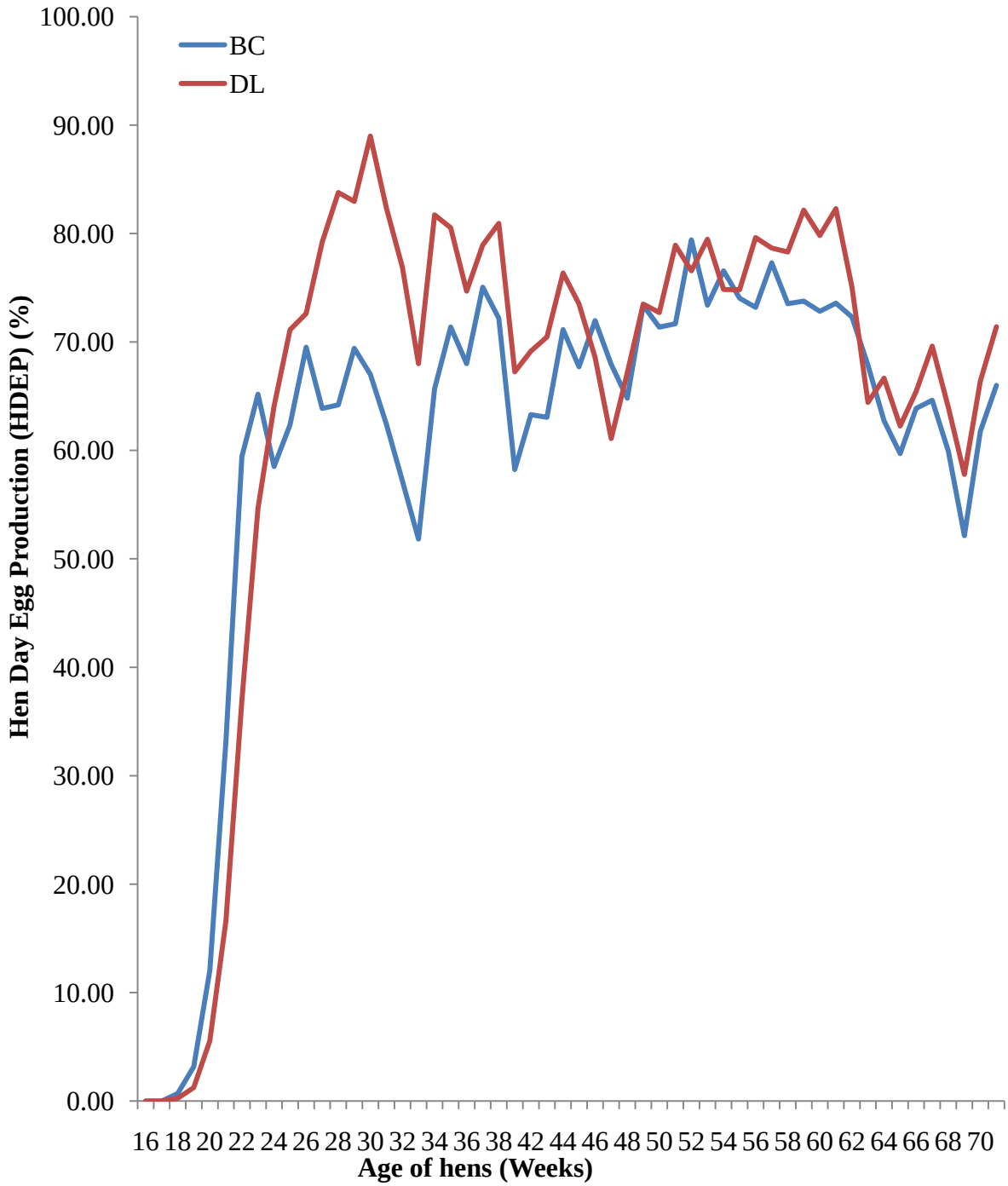

Figure 3: Hen Day Egg Production of laying hens in battery cage and deep litter systems.(DL-Deep litter, BC-Battery cage, HDEP-Hen Day Egg Production)

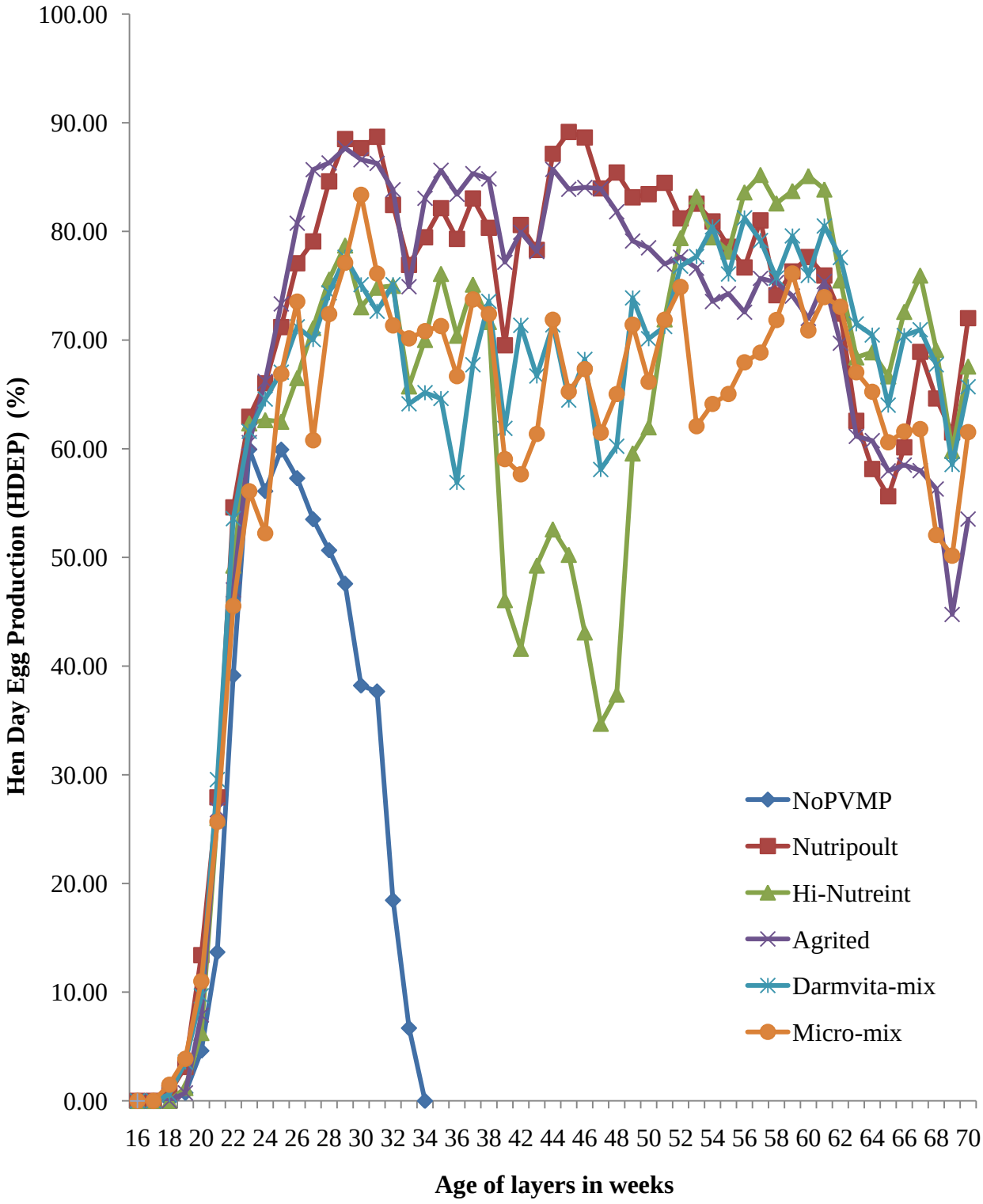

Figure 4: Hen Day Egg Production of laying chickens fed different five different proprietary vitamin-mineral premixes from weeks 16 to 70.

Study Four

4.4.1: External quality indices of eggs from layers fed diets supplemented with five different proprietary vitamin-mineral premixes in two housing systems from 22 to 35 weeks of age

External quality indices of of eggs from layers fed diets supplemeted with five different PVmP in two housing systems from 22 to 35 weeks of age is presented in Table 14. The egg weights (Ew), diameter (ED), shell index (EI) and shell weight (EW) from layers were not affected (P>0.05) by HS. However, Egg length (EL) (37.79) and Eggshell thickness (ET) varied (p<0.05) with HS. The EL from layers in DL (37.79 mm) were higher (p<0.05) than those in BC (37.52). The ET (0.32) from layers in BC was higher (p<0.05) compared with 0.31 in DL. Dietary PVmP significatly (p<0.05) increased Ew, ED, ET and EW. Eggs produced by layers fed diets supplemented with Micro-mix (41.06 g) had highest Ew which was similar to eggs produced by those on Agrited (40.85) and Daram vita-mix (40.07) but higher (p<0.05) than values obtained for eggs produced by those on diets without PVmP(38.35), Nutripoult (39.49) and Hi-Nutrient (39.64).

Eggs from layers fed diets supplemented with Hi-Nutrient (26.03) had highest value of ED which was similar to obtained values for eggs produced by those on Nutripoult (25.94) and Micro-mix (25.89) but higher (p<0.05) than values obtained for eggs produced by those fed diets without PVmP (25.56), Agrited(25.66) and Daram vita-mix (25.65). The highest EI was obtained for eggs produced by layers fed diets without PVmP (1.47), Daram vita-mix (1.47) and Agrited (1.47) which were similar to eggs laid by thos on Nutripoult (1.45) and Micro-mix (1.46) but higher (p<0.05) than 1.44 for those on HDEP.

Table 14: External quality indices of eggs from layers fed with diets supplemented with five different proprietary vitamin-mineral premixes in two housing systems from 22 to 35 weeks of age

	Factors	Ew (g)	EL (mm)	ED (mm)	EI	ET (mm)	EW (g)
	BC	40.30	37.52^{b}	25.76	1.47	0.32^{a}	4.01
HS	DL	39.86	37.79^{a}	25.81	1.46	0.31^{b}	3.93
	SEM	0.24	0.073	0.05	0.51	0.003	0.04
	D1	39.35^{b}	37.64	25.56^{c}	1.47^{a}	0.30^{b}	3.84^{b}
	D2	39.49^{b}	37.69	25.94^{a}	1.45^{ab}	0.32^{a}	3.99^{ab}
	D3	39.64^{b}	37.57	26.03^{a}	1.44^{b}	0.31^{ab}	4.03^{a}
PVmP	D4	40.85^{a}	37.67	25.66^{bc}	1.47^{a}	0.30^{ab}	3.92^{ab}
	D5	40.07^{ab}	37.58	25.65^{c}	1.47^{a}	0.32^{a}	4.02^{a}
	D6	41.06^{a}	37.81	25.89^{ab}	1.46^{ab}	0.32^{a}	4.00^{ab}
	SEM	0.41	0.13	0.09	0.01	0.005	0.06

$^{a-c}$Mean values with different superscript in the same column are significantly different ($p<0.05$).Ew-Egg weight; EL-Egg length; ED-Egg diameter; EI-Egg shape index; ET-Eggshell thickness, EW-Eggshell weight, DL-Deep litter, BC-Battery cage, HS-Housing systems, PVMP- Proprietary vitamin-mineral premixes, D1-diet without proprietary vitamin-mineral premix, D2, D3, D4, D5 and D6 – dietswith Nutripoult, Hi-Nutrient, Agrited, Daram vita-mix and Micro-mix respectively, SEM-Standard error of mean

The ET of eggs produced by layers fed diets supplemented with Nutripoult (0.32 mm), Daram vita-mix (0.32) and Micro-mix (0.32) were similar to eggs produced by those on Hi-Nutrient (0.31) and higher ($p<0.05$) than those on diets without PVmP (0.30). Supplemetntation of Hi-Nutrient (4.03) in diets of layers induced the highest EW which was similar to eggs produced by those on Nutripoult (3.99), Agrited (3.92), Daram vita-mix (4.02) and Micro-mix (4.00) but higher ($p<0.05$) than eggs laid by layers fed diets without PVmP (3.84). The interaction effects of dietary PVmP x HS on external quality characteristics of eggs from layers from 22 to 35 weeks of age is presented in Table 15. The combined effect of dietary PVmP supplementations and HS on external quality characteristics of eggs from layers fed diets supplemented with five different proprietary vitamin-mineral premixes in two housing systems were significant ($p<0.05$). The interaction effects of D4 x BC (41.81) on Ew had the highest and similar to D6 x DL (41.50), D5 x BC (40.81) and D6 x BC (40.61). The interaction effect of D1 x BC (39.01) on Ew was the least. Interaction effect of D1 x DL (38.10) on EL recorded the highest value which was similar to D2 x BC (37.80), D3 x BC (37.61), D4 x BC (37.67), D6 x BC (37.67), D4 x DL (37.68), D5 x DL (37.94) and D6 x DL (37.94). The interaction effect of D1 x BC (37.19) on EL was the least.

The interaction effects of D3 x BC on ED was highest (26.03) and similar to D2 x BC (25.93), D4 x BC (25.87), D6 x BC (25.82), D1 x DL (25.81), D2 x DL (25.94), D3 x DL (26.02), D5 x DL (25.70) and D6 x DL (25.96). The interaction effect of D1 x BC (25.31) was the least. Intraction effects of D1 x DL, D4 x DL and D5 x DL on EI were the same in value (1.48) but similar to D1 x BC (1.47) and D6 x DL (1.46) and higher ($p<0.05$) than D3 x BC (1.45), D5 x BC (1.45), D2 x DL (1.45) and D3 x DL (1.44). Interaction effects of D6 x BC on ET had the highest value (0.33) which was similar to D1 x BC (0.31), D2 x BC (0.32), D3 x BC (0.31), D4 x BC (0.32), D5 x BC (0.32), D3 x DL (0.32), D5 x DL (0.32) and D6 x DL (0.32). The least value of interaction effect (0.29) on ET was obtained for D1 x DL and D4 x DL. The highest interaction effects of D3 x DL (4.18) on EW was similar to D5 x BC (4.17), D6 x BC (4.15), D2 x BC (4.08) and D4 x BC (3.97) and higher ($p<0.05$) than D1 x BC (3.78), D3 x BC (3.88), D1 x DL (3.90), D2 x DL (3.90), D4 x DL (3.88), D5 x DL (3.86) and D6 x DL (3.85).

Table 15: Interaction effects of proprietary vitamin-mineral premixes and housing systems on external quality indices of eggs from layers at week 22 to 35 weeks of age

PVmP x HS	Ew(g)	EL(mm)	ED(mm)	EI	ET(mm)	EW(g)
D1 x BC	39.01^{d}	37.19^{c}	25.31^{d}	1.47^{ab}	0.31^{ab}	3.78^{c}
D2 x BC	39.46^{cd}	37.80^{ab}	25.93^{ab}	1.46^{ab}	0.32^{ab}	4.08^{ab}
D3 x BC	40.10^{bcd}	37.61^{abc}	26.03^{a}	1.45^{a}	0.31^{abc}	3.88^{bc}
D4 x BC	41.81^{a}	37.67^{abc}	25.87^{ab}	1.46^{ab}	0.32^{ab}	3.97^{abc}
D5 x BC	40.81^{abc}	37.22^{c}	25.60^{bcd}	1.45^{b}	0.32^{ab}	4.17^{a}
D6 x BC	40.61^{abcd}	37.67^{abc}	25.82^{ab}	1.46^{ab}	0.33^{a}	4.15^{a}
D1 x DL	39.69^{cd}	38.10^{a}	25.81^{ab}	1.48^{a}	0.29^{c}	3.90^{bc}
D2 x DL	39.53^{cd}	37.58^{bc}	25.94^{a}	1.45^{b}	0.31^{bc}	3.90^{bc}
D3 x DL	39.18^{cd}	37.54^{bc}	26.02^{a}	1.44^{b}	0.32^{ab}	4.18^{a}
D4 x DL	39.90^{bcd}	37.68^{abc}	25.44^{cd}	1.48^{a}	0.29^{c}	3.88^{bc}
D5 x DL	39.33^{cd}	37.94^{ab}	25.70^{abc}	1.48^{a}	0.32^{ab}	3.86^{bc}
D6 x DL	41.50^{ab}	37.94^{ab}	25.96^{a}	1.46^{ab}	0.32^{ab}	3.85^{bc}
SEM	0.59	0.18	0.12	0.00	0.01	0.09

$^{a-d}$ Mean values with different superscripts on the same column are significantly different ($p<0.05$).Ew-Egg weight; EL-Egg length; ED-Egg diameter; EI-Egg shape index; ET-Eggshell thickness; EW-Eggshell weight, DL-Deep litter, BC-Battery cage, HS-Housing systems, PVmP-Proprietary vitamin-mineral premixes, D1-diet without PVmP, D2, D3, D4, D5 and D6-diets with Nutripoult, Hi-Nutrient, Agrited, Daram vita-mix and Micro-mix respectively, SEM-Standard error of means, x-Interaction

4.4.2: Internal quality indices of eggs from layers fed diets supplemented with five different proprietary vitamin-mineral premixes in two housing systems from 22 to 35 weeks of age

Table 16 shows internal quality indices of eggs from layers fed diets supplemented with five different PVmPin two HS from 22 to 35 weeks of age. Albumen height (AH) and Haugh Unit (HU) of eggs were not affected ($p>0.05$) by HS. Egg albumen weight (AW), yolk weight (YW), height (YH), diameter (YD) and index (YI) varied ($p<0.05$) with HS. Layers in BC produced eggs with higher ($p<0.05$) AW (25.49 g), YH (17.54 mm) and YI (0.65) compared with 24.74, 17.31 and 0.63 respectively for eggs from those in DL. Thus, DL induced higher ($p<0.05$) YW (12.42 g) and YD (29.65 mm) in eggs than in BC. Albumen quality of eggs reduced significantly ($p<0.05$) with dietary PVmP supplementtions. Layers fed diets without PVmP (D1) produced eggs with higher ($p<0.05$) AW (25.60 g), AH (5.99 mm) and HU (83.08) compared with eggs fed diets supplemented with PVmPs. The AW of eggs from layers on diets supplemented with Nutripoult (25.48 g), Hi-Nutrient (25.38 g) and Micro-mix (25.11 g) were similar to those fed diets without PVmP (25.60 g) but higher ($p<0.05$) compared with eggs from those on diets supplemented with Daram vita-mix (24.44). The YW, YD and Yolk Colour (YC) increased ($p<0.05$) with dietary PVmPs, while YH and YI decreased ($p<0.05$).

Table 16: Internal quality indices of eggs from layers fed diets supplemented with five different proprietary vitamin-mineral premixes in two housing systems fromduring early laying phase (22 to 35 weeks of age)

Factors	AW (g)	AH (mm)	HU	YW (g)	YH (mm)	YD (mm)	YI	YC
BC	25.49[a]	5.62	80.49	11.71[b]	17.54[a]	28.38[b]	0.65[a]	2.94
DL	24.74[b]	5.53	80.11	12.42[a]	17.31[b]	29.65[a]	0.63[b]	2.82
SEM	0.18	0.04	0.27	0.08	0.05	0.19	0.1	0.07
D1	25.60[a]	5.99[a]	83.08[a]	11.47[b]	17.69[a]	27.87[b]	0.68[a]	2.32[b]
D2	25.48[ab]	5.49[c]	79.62[bc]	12.13[a]	17.41[b]	28.91[a]	0.64[ab]	2.40[b]
D3	25.38[ab]	5.51[bc]	79.75[bc]	12.21[a]	17.42[b]	29.30[a]	0.63[ab]	2.55[b]
D4	24.68[bc]	5.36[c]	79.07[c]	12.21[a]	17.30[b]	29.47[a]	0.62[b]	2.48[b]
D5	24.44[c]	5.43[c]	79.45[c]	12.21[a]	17.36[b]	29.15[a]	0.66[ab]	5.09[a]
D6	25.11[abc]	5.67[b]	80.85[b]	12.25[a]	17.37[b]	29.40[a]	0.62[b]	2.45[b]
SEM	0.32	0.07	0.47	0.14	0.09	0.33	0.02	0.11

[a-c]Mean values with different superscripts on the same cvolumn are significantly different ($p<0.05$).AW-Albumen weight; AH-Albumen height; HU-Haugh Unit, YW-Yolk weight; YH-Yolk height; YD-Yolk diameter; YI-Yolk index; YC Yolk Colour; DL-Deep litter, BC-Battery cage, PVmP-Proprietary vitamin-mineral premix, D1-diet without PVmP, D2, D3, D4, D5 and D6-diets with Nutripoult, Hi-Nutrient, Agrited, Daram vita-mix and Micro-mix respectively, SEM-Standard error of means

The YW of eggs from layers fed diet supplemented with Micro-mix (12.25 g) was similar to eggs from those on diets with Nutripoult (12.13 g), Hi-Nutrient (12.21 g), Agrited (12.21 g) and Daram vita-mix (12.21 g) but higher ($p<0.05$) compared with eggs laid by those fed diets without PVmP (11.47). The YH of eggs from layers fed diets without PVmP (17.69 mm) was higher ($p<0.05$) than those fed PVmP supplementations.

The YD of eggs from layers fed diets supplemented with Agrited (29.47 mm) was similar to those on diets supplemeted with Nutripoult (28.91 mm), Hi-Nutrient (29.20 mm), Daram vita-mix (29.15) and Micro-mix (29.40 mm) but higher ($p<0.05$) than eggs produced by those on diets without PVmP (27.87 mm). The YI of eggs from layers fed diets without PVmP (0.68) and supplemented with Nutripoult(0.64), Hi-Nutrient (0.63) and Daram vita-mix (0.66) were simlar bu higher ($p<0.05$) compared with eggs laid by those on Agrited (0.62) and Micro-mix (0.62). The YC of eggs produced by layers on diets that contained Daram vita-mix (5.09) was higher ($p<0.05$) compared with eggs laid by those fed diets without PVmP (2.32), Nutripoult (2.40), Hi-Nutrient (2.55), Agrited (2.48) and Micro-mix (2.45). Table 17 indicates significant interaction effects ($p<0.05$) of dietary PVmP and HS on all parameters of internal quality of eggs from layers fed diets supplemented with five different PVmP in two HS from 22 to 35 weeks of age.

Table 17: Interaction effects of proprietary vitamin-mineral premixes and housing systems on internal quality indices of eggs from layers at week 22 to 35 week of age

PVmP x HS	AW (g)	AH (mm)	HU	YW (g)	YH (mm)	YD (mm)	YI	YC
D1 x BC	25.80^{abc}	6.24^{a}	84.81^{a}	10.83^{d}	17.97^{a}	26.45^{e}	0.73^{a}	2.23^{b}
D2 x BC	26.32^{a}	5.49^{cd}	79.24^{c}	11.77^{c}	17.47^{bc}	28.58^{cd}	0.65^{b}	2.35^{b}
D3 x BC	26.12^{ab}	5.61^{bc}	80.36^{bc}	11.79^{c}	17.56^{b}	28.87^{bcd}	0.64^{b}	2.44^{b}
D4 x BC	24.94^{bcd}	5.38^{cd}	79.13^{c}	12.06^{bc}	17.28^{bc}	29.68^{abc}	0.62^{b}	2.49^{b}
D5 x BC	24.47^{d}	5.39^{cd}	79.12^{c}	11.89^{c}	17.54^{bc}	28.32^{d}	0.65^{b}	4.88^{a}
D6 x BC	25.29^{abcd}	5.60^{bc}	80.26^{bc}	11.91^{c}	17.45^{bc}	28.38^{d}	0.64^{b}	2.55^{b}
D1 x DL	25.41^{abcd}	5.75^{b}	81.35^{b}	12.10^{abc}	17.42^{bc}	29.28^{abcd}	0.63^{b}	2.42^{b}
D2 x DL	24.64^{cd}	5.49^{cd}	80.00^{bc}	12.48^{ab}	17.35^{bc}	29.23^{abcd}	0.62^{b}	2.46^{b}
D3 x DL	24.65^{cd}	5.42^{cd}	79.15^{c}	12.63^{a}	17.28^{bc}	29.73^{abc}	0.61^{b}	2.65^{b}
D4 x DL	24.42^{d}	5.33^{d}	79.01^{c}	12.19^{abc}	17.32^{bc}	29.27^{abcd}	0.62^{b}	2.47^{b}
D5 x DL	24.41^{d}	5.47^{cd}	79.78^{bc}	12.53^{ab}	17.19^{c}	29.98^{ab}	0.67^{ab}	5.29^{a}
D6 x DL	24.94^{bcd}	5.75^{b}	81.35^{b}	12.58^{ab}	17.29^{bc}	30.43^{a}	0.60^{b}	2.35^{b}
SEM	0.45	0.09	0.66	0.20	0.13	0.46	0.03	0.16

$^{a-c}$Mean values with different superscripts on the same column are significantly different ($p<0.05$). AW-Albumen weight; AH-Albumen height; HU- Haugh Unit, YW-Yolk weight; YH-Yolk height; YD-Yolk diameter; YI-Yolk index; YC - Yolk Colour; DL-Deep litter, BC-Battery cage, PVmP-Proprietary vitamin-mineral premix,T1-diet without PVmP, D2, D3, D4, D5 and D6-diets with Nutripoult, Hi-Nutrient, Agrited, Daram vita-mix and Micro-mix respectively, SEM-Standard error of means, x-Interaction

4.4.3: Effect of duration of storage on external quality indices of eggs from layers fed diets supplemented with five different proprietary vitamin-mineral premixes in two housing systems from 36 to 52 weeks of age

Effect of duration of storage on external quality indices of eggs from layers fed diets supplemented with five different PVmP in two housing systems from 36 to 52 weeks of age are presented in Table 18. The EW, ET and Egg weight Loss (EwL) varied significantly ($p<0.05$) with HS, while Ew and EI were not affected. The EW (5.89 g) and ET (0.35 mm) of eggs of layers in BC were higher ($p<0.05$) compared with 5.58 and 0.33 respectively for eggs produced in DL. The EwL of layers in DL (1.70 %) was higher ($p<0.05$) compared with 1.60% for eggs produced in BC. The different dietary PVmP did not affect ($p>0.05$) Ew, ET and EI but varied ($p<0.05$) with EW and EwL. The EW of eggs produced by layers fed Nutripoult (5.89 g) was similar to those fed fed diets supplemented with Daram vita-mix (5.73) and Micro-mix (5.84) but were higher ($p<0.05$) than those fed diets supplemented with Hi-Nutrient (5.59) and Agrited (5.61).

The highest EwL value was recorded for eggs laid by layers fed Hi-Nutrient (1.80%) which was similar to those fed diets supplemented with Nutripoult (1.70) and Agrited (1.70) but higher ($p<0.05$) than those on Daram vita-mix (1.50) and Micro-mix (1.50). The EW, ET and EwL varied ($p<0.05$) with days of egg storage. The Ew and EI were not affected ($p>0.05$) by days of egg storage. The EW and EwL increased ($p<0.05$) with days of storage, while ET decreased ($p<0.05$). The interaction effectd of PVmP and HS (PVmP x HS), HS and DoS (HS x DoS), PVmP and DoS (PVmP x DoS) and PVmP, HS and DoS (PVMP x HS x DES) on external quality indices eggs layers fed diets supplemented with five different PVmP in two HS from 36 to 52 weeks of age were not significant ($p>0.05$). Also, interaction effects of PVmP and DoS (PVmP x DoS) and PVmP, HS and DoS (PVmP x HS x DoS) on ET were significant ($p<0.01$).

Table 18: Effect of duration of storage on external quality indices of eggs from layers fed diets supplemented with five different proprietary vitamin-mineral premixes in two housing systems from 36 to 52 weeks of age

	Factors	Ew (g)	EW (g)	ET (mm)	EI	EwL (%)
	BC	60.65	5.89^{a}	0.35^{a}	1.34	1.60^{b}
HS	DL	59.74	5.58^{b}	0.33^{b}	1.37	1.70^{a}
	SEM	0.56	0.58	0.00	0.14	0.00
	D2	60.96	5.89^{a}	0.34	1.29	1.70^{a}
	D3	60.09	5.59^{b}	0.33	1.37	1.80^{a}
PVmP	D4	59.81	5.61^{b}	0.33	1.35	1.70^{a}
	D5	59.04	5.73^{ab}	0.34	1.39	1.50^{b}
	D6	61.08	5.84^{ab}	0.34	1.37	1.50^{b}
	SEM	0.89	0.92	0.00	0.02	0.10
	0	60.63	5.54^{c}	0.35^{a}	1.32	0.00^{e}
	7	59.23	5.60^{bc}	0.32^{b}	1.36	0.90^{d}
DoS	14	60.33	5.81^{ab}	0.33^{b}	1.34	1.70^{c}
	21	60.47	5.76^{abc}	0.35^{a}	1.38	2.50^{b}
	28	60.31	5.95^{a}	0.35^{a}	1.38	3.20^{a}
	SEM	0.89	0.09	0.00	0.02	0.10
	PVmPxHS	0.0550^{NS}	0.4137^{NS}	0.4448^{NS}	0.5325^{NS}	0.6123^{NS}
	HSxDoS	0.6110^{NS}	0.0403^{NS}	0.1227^{NS}	0.0642^{NS}	0.1685^{NS}
	PVmPxDoS	0.6560^{NS}	0.0769^{NS}	0.0001**	0.4698^{NS}	0.0844^{NS}
	PVmPxHSxDoS	0.8830^{NS}	0.7889^{NS}	0.0007**	0.3572^{NS}	0.7168^{NS}

$^{a–e}$ Mean values with different superscripts on the same column are significantly different ($p<0.05$). Ew-Egg weight, EW-Eggshell weight, ET-Eggshell thickness, EI-Egg shape index, EwL-Egg weight loss, HS-Housing systems, PVmP- Proprietary vitamin-mineral premixes, DoS-Days of egg storage, D2, D3, D4, D5 and D6-diets with supplemental PVmPN, H, A, D and M respectively, SEM -Standard error of mean, x-Interaction, **- (p,0.01), NS-Not significant

4.4.4: Effect of duration of storage on internal quality indices of eggs from layers fed diets supplemented with five different proprietary vitamin-mineral premixes in two housing systems from 36 to 52 weeks of age

Effect of duration of storage on internal quality indices of eggs from layers fed diets supplemented with five different dietary PVmP in two housing systems from 36 to 52 weeks of age are presented in Table 19. Albumen pH (ApH) and AW of eggs were not affected ($p>0.05$) by HS, while AH and HU varied ($p<0.05$). Eggs produced by layers in BC had higher ($p<0.05$) AH (3.69 mm) and HU (48.68) than 3.50 and 44.78, respectively in DL. Yolk quality indices of eggs were not affected ($p>0.05$) by HS. Albumen and yolk quality indices of eggs did not vary ($p>0.05$) with dietary PVmP supplementation aside AH and HU. The highest AH value was obtained from eggs produced by layers fed diet supplemented with Micro-mix (3.72 mm) and simllar to eggs from those fed diets with Nutripoult (3.69), Hi-Nutrient (3.57) and Daram vita-mix (3.67) but higher ($p<0.05$) than those supplemented with Agrited (3.33).

The HU of eggs produced by layers fed diets supplemented with Daram vita-mix (48.64) was similar to those fed Nutripoult (46.13) and Micro-mix (48.03) and higher ($p<0.05$) than those on Hi-Nutrient (46.08) and Agrited (44.75). The ApH, AH, HU, YH and YI varied ($p<0.05$) with DoS. However, AW, YW, YR and YAR were not affected ($p>0.05$) in DoS. As ApH of eggs increased ($p<0.05$), AH, HU, YH and YI decreased ($p<0.05$) in DoS. The interaction effects of PVmP and HS (PVmP x HS); HS and DoS (HS x DoS); PVmP and DoS (PVmP x DoS); and PVmP, HS and DoS (PVmP x HS x DoS) on internal quality indices of eggs from layers fed diets supplemented with five different PVmP in two HS were not different ($p>0.05$). The interaction effect of PVmP and DoS (PVmP x DoS) affected ($p<0.05$) AH and HU

Table 19: Effect of duration of storage on internal quality indices of eggs from layer fed diets supplemented with five different proprietary vitamin-mineral premixes in two housing systems from 36 to 52 weeks of age

		Albumen quality				Yolk quality				
Effects	Factors	ApH	AW (g)	AH (mm)	HU	YW (g)	YH (mm)	YI	YR	YAR
	BC	9.21	40.63	3.69^{a}	48.68^{a}	14.13	9.30	22.9	23.32	0.36
HS	DL	9.23	40.26	3.50^{b}	44.78^{b}	13.91	9.67	22.9	23.31	0.36
	SEM	0.01	0.72	0.07	1.03	0.65	0.44	1.1	1.06	0.02
	D2	9.22	42.23	3.69^{a}	46.13^{ab}	12.85	9.61	22.3	21.03	0.37
	D3	9.22	40.04	3.57^{ab}	46.08^{b}	14.46	9.78	22.9	24.13	0.38
PVmP	D4	9.23	40.65	3.33^{b}	44.75^{b}	13.55	8.72	21.4	22.64	0.35
	D5	9.23	38.71	3.67^{a}	48.64^{a}	14.61	9.51	23.6	24.89	0.36
	D6	9.21	40.58	3.72^{a}	48.03^{a}	14.65	9.83	24.2	23.89	0.37
	SEM	0.01	1.14	0.10	1.64	1.03	0.69	1.70	1.68	0.03
	0	8.77^{d}	40.21	6.99^{a}	83.08^{a}	14.88	15.52^{a}	41.90^{a}	24.61	0.37
	7	9.24^{c}	39.12	3.92^{b}	56.52^{b}	14.5	11.09^{b}	26.80^{b}	24.39	0.38
DoS	14	9.33^{b}	41.55	3.22^{c}	48.03^{c}	12.96	8.37^{c}	19.50^{c}	21.31	0.34
	21	9.39^{a}	41.64	2.22^{d}	30.63^{d}	13.08	7.03^{cd}	14.80^{cd}	21.89	0.35
	28	9.39^{a}	39.69	1.62^{e}	15.38^{e}	14.68	5.41^{d}	11.40^{d}	24.39	0.39
	SEM	0.01	1.14	0.10	1.64	1.03	0.69	1.70	1.68	0.03
	PVmPxHS	0.7430^{NS}	0.2830^{NS}	0.9110^{NS}	0.8220^{NS}	0.7390^{NS}	0.7220^{NS}	0.8240^{NS}	0.4940^{NS}	0.4540^{NS}
	HSxDoS	0.2200^{NS}	0.2480^{NS}	0.2570^{NS}	0.4060^{NS}	0.8630^{NS}	0.5950^{NS}	0.7380^{NS}	0.7810^{NS}	0.7720^{NS}
	PVmPxDoS	0.4690^{NS}	0.8400^{NS}	0.0360*	0.2740*	0.9440^{NS}	0.9860^{NS}	0.9840^{NS}	0.9330^{NS}	0.9430^{NS}
	PVmPxHSxDoS	0.6870^{NS}	0.6390^{NS}	0.7740^{NS}	0.2590^{NS}	0.9250^{NS}	0.6610^{NS}	0.6930^{NS}	0.8530^{NS}	0.8220^{NS}

$^{a–d}$Mean values with different superscripts on the same column are significantly different ($p<0.05$). ApH-Albumen pH, AW-Albumen weight, AH-Albumen height , HU-Haugh unit, YW-Yolk weight, YH-Yolk height, YI-Yolk index, YR-Yolk ratio, YAR-Yolk-Albumen ratio, HS-Housing systems, PVmP- Proprietary vitamin-mineral premixes, DoS-Days of egg storage, D2, D3, D4, D5 and D6-diets with Nutripoult, Hi-Nutrient, Agrited, Daram vita-mix and Micro-mix respectively, SEM -Standard error of mean, x-Interaction,*-(p,0.05), NS-Not significant

4.4.5: Effect of duration of storage on external quality indices of eggs from layers fed diets supplemented with five different proprietary vitamin-mineral premixes in two housing systems from 53 to 70 weeks of age

Effect of duration of storage on external quality characteristics of eggs from layers fed diets supplemented with five different PVmP in two HS from 53 to 70 weeks of age is presented in Table 20. All indices of external quality characteristics of eggs were not influenced ($p>0.05$) by HS aside egg diameter (ED). Eggs produced by layers in BC were wider ($p<0.05$) than eggs from DL. However, the dietary PVmP did not affect ($p>0.05$) all indices of external quality of eggs. Egg length (EL), diameter (ED) and shell index (EI) were not affected ($p>0.05$) by DoS, while egg weight (Ew), shell weight (EW), shell thickness (ET) and weight loss (EwL) varied ($p<0.05$). The ET and EwL increased ($p<0.05$) with DoS, while EW and EW decreased ($p<0.05$).

Table 20: Effect of duration of storage on external quality indices of eggs from layers fed diets supplemented with five different proprietary vitamin-mineral premixes in two housing systems from 53 to 70 weeks of age

Effects	Factors	Ew (g)	EL (mm)	ED (mm)	EW (g)	ET(mm)	EI (%)	EwL (%)
	BC	59.44	56.00	40.70[a]	5.81	0.59	72.27	5.44
HS	DL	58.96	55.90	41.00[b]	5.71	0.59	71.61	5.46
	SEM	0.65	1.90	0.20	0.08	0.04	0.56	0.30
	D2	60.03	56.40	40.50	5.72	0.57	72.00	5.75
	D3	59.30	55.90	40.20	5.79	0.60	72.22	5.10
PVmP	D4	59.32	55.90	40.80	5.75	0.07	71.42	5.88
	D5	58.68	55.90	40.10	5.80	0.07	71.86	5.28
	D6)	58.66	55.80	40.10	5.73	0.07	72.21	5.22
	SEM	1.02	0.50	0.32	0.12	0.38	0.87	0.55
	0	63.70[a]	56.20	40.80	6.10[a]	0.14[c]	72.68	-
	7	60.35[b]	56.60	40.40	5.71[b]	0.14[c]	70.09	2.16[d]
DoS	14	58.92[cb]	55.70	40.30	5.52[b]	0.93[a]	72.61	4.73[c]
	21	56.14[d]	55.60	39.90	5.62[b]	0.83[a]	71.90	6.73[b]
	28	56.88[cd]	55.80	40.30	5.85[ab]	0.59[b]	72.43	8.18[a]
	SEM	0.88	0.50	0.30	0.12	0.02	0.85	0.26

[a–e]Mean values with different superscripts on the same column are significantly different ($p<0.05$). Ew-Egg weight, EL-Egg length, EB-Egg width, EW-Eggshell weight, ET-Eggshell thickness, EI-Egg shape index, EwL-Egg weight loss, HS-Housing systems, PVmP- Proprietary vitamin-mineral premixes, DoS-Days of egg storage, D2, D3, D4, D5 and D6-diets with Nutripoult, Hi-Nutrient, Agrited, Daram vita-mix and Micro-mix respectively, SEM -Standard error of mean.

4.4.6: Internal quality indices of eggs from layers as affected by duration of storage, proprietary vitamin-mineral premixes and two housing systems from 53 to 70 weeks of age

Internal quality indices of eggs from layers as affected by duration of storage, proprietary vitamin-mineral premixes and two housing systems from 53 to 70 weeks of age is presented in Table 21. Albumen weight was not affected ($p>0.05$) by HS. Albumen height and Haugh Unit (HU) varied ($p<0.05$) with HS. Egg-yolk quality characteristics were not affected ($p>0.05$) by HS. Albumen and yolk quality indices varied ($p<0.05$) with PVmP supplementations. Layers fed diet supplemented with Nutripoult (D2) produced eggs that had similar AW with eggs from those on diets supplemented with Agrited (D4) and Daram vita-mix (D5) which were higher ($p<0.05$) than eggs from those produced by layers on Hi-Nutrient (D3) and Micro-mix (D6). Albumen height (AH) and HU of eggs produced by layers fed Daram vita-mix (D5) was higher ($p<0.05$) and similar to eggs laid by those on diets supplemented with Nutripoult (D2) and Hi-Nutrient (D3) but higher ($p<0.05$) than eggs from those on Agrited (D4) and Micro-mix (D6).

Eggs produced by layers on diets supplemented with Hi-Nutrient (D3) were widest and similar to those colleceted from layers on diets supplemented with Nutripoult (D2), Daram vita-mix (D5) and Micro-mix (D6). Yolk index of eggs from layers fed diet supplemented with Daram vita-mix (D5) were similar to eggs produced by those fed D2, D4 and D6 and higher ($p<0.05$) than eggs produced by those fed diets supplemented with Hi-Nutrient (D3). Yolk weight (YW) and width (YD) increased ($p<0.05$) with decrease ($p<0.05$) in AW, AH, HU, YH and YI in DoS. Albumen and yolk quality indices decreased ($p<0.05$) in DoS.

Table 21: Internal quality indices of eggs as affected by duration of storage, proprietary vitamin-mineral premixes and two housing systems from 53 to 70 weeks of age

	Factors	AW (g)	AH (mm)	HU	YW (g)	YH (mm)	YD (mm)	YC	YI (%)
	BC	35.96	52.20^{a}	77.98^{a}	17.93	8.30	47.80	1.00	19.29
HS	DL	35.55	48.60^{b}	73.12^{b}	17.88	8.70	47.60	1.00	20.27
	SEM	0.60	2.50	6.26	0.25	3.1	1.00	0.00	1.61
	D2	37.44^{a}	5.08^{ab}	80.63^{ab}	17.61	0.86	4.64^{c}	1.00	20.41^{a}
	D3	35.20^{b}	5.07^{ab}	79.33^{ab}	18.42	0.81	4.91^{a}	1.00	18.49^{b}
PVmP	D4	35.41^{ab}	4.86^{c}	77.77^{c}	18.30	0.86	4.85^{ab}	1.00	19.66^{ab}
	D5	35.69^{ab}	5.16^{a}	81.63^{a}	17.25	0.87	4.69^{c}	1.00	20.53^{a}
	D6	35.07^{b}	4.75^{b}	78.90^{b}	17.91	0.86	4.75^{cb}	1.00	20.02^{ab}
	SEM	0.94	0.39	2.00	0.38	0.09	0.16	0.00	2.57
	0	40.86^{a}	8.17^{a}	97.39^{a}	17.05^{b}	1.48^{a}	3.70^{e}	1.00	40.11^{a}
	7	36.90^{b}	4.68^{b}	75.48^{b}	17.71^{b}	1.09^{b}	4.36^{d}	1.00	25.16^{b}
DoS	14	34.51^{c}	4.60^{b}	74.87^{b}	18.94^{a}	0.65^{c}	4.95^{c}	1.00	13.32^{c}
	21	32.29^{d}	3.58^{c}	65.98^{c}	18.04^{ab}	0.43^{d}	5.44^{b}	1.00	7.92^{d}
	28	32.80^{cd}	3.54^{c}	65.86^{c}	17.90^{ab}	0.39^{d}	5.78^{a}	1.00	6.78^{d}
	SEM	0.70	0.16	1.13	0.36	0.03	0.06	0.00	0.62

$^{a-c}$ Mean values with different superscripts on the same column are significantly different ($p<0.05$). AW-Albumen weight; AH-Albumen height; HU- Haugh Unit, YW-Yolk weight; YH-Yolk height; YD-Yolk diameter; YI-Yolk index; YC Yolk Colour; DL-Deep litter, BC-Battery cage, DoS-Days of egg storage, SEM- Standard error of means, D2, D3, D4, D5 and D6-diets with Nutripoult, Hi-Nutrient, Agrited, Daram vita-mix and Micro-mix respectively, F-Factors

4.4.7: Relationship among external quality indices of eggs as affected by duration of storage

Variations in external and internal quality indices of eggs with days of storage were observed. These variations were shown by regression of EW on DoS from 36 to 52 and 53 to 70 weeks of age in equations 13 and 14 respectively below:

Mid-laying phase; $y = -0.0173x^2 + 0.3297x + 5.026$ $(R^2 = 0.85)$ ……13

Late-laying phase; $y = 0.0022x^2 - 0.0709x + 6.0966$ $(R^2 = 0.99)$ ……14

Shell weight (EW) of stored eggs in mid-laying phase increased up to day 7 of storage and then nose dived. However, at late-laying (53 to 70 weeks of age), the EW remained fairly constant as shown in Figure 5 below:

Equations 15 and 16 are regreesions of ET on DoS from 36 to 52 and 53 to 70 weeks of age as shown below:

Mid-laying phase; $y = 0.0001x^2 - 0.0024x + 0.344$ $(R^2 = 0.55)$ …… ..15

Late-laying phase; $y = -0.002x^2 + 0.0786x + 0.0123$ $(R^2 = 0.69)$ …… 16

The ET remained fairly constant in mid-laying phase (36 to 52 weeks of age) and later increased rapidly but decreased after day 21of storage as shown in Figure 6.

The regressions of EwL on DoS from 36 to 52 and 53 to 70 weeks of age are represented by equations 17 and 18 respectively below:

Mid-laying phase; $y = -0.0006x^2 + 0.1306x + 0.0029$ $(R^2 = 0.99)$ …….. 17

Late-laying phase; $y = -0.0029x^2 + 0.3802x - 0.1103$ $(R^2 = 0.99)$ …… ..18

Egg weight loss increased linearly in with DoS but more rapid in eggs collected for storage between 53 to 70 comapred with eggs colleceted for storage between 36 to 52 weeks of age.

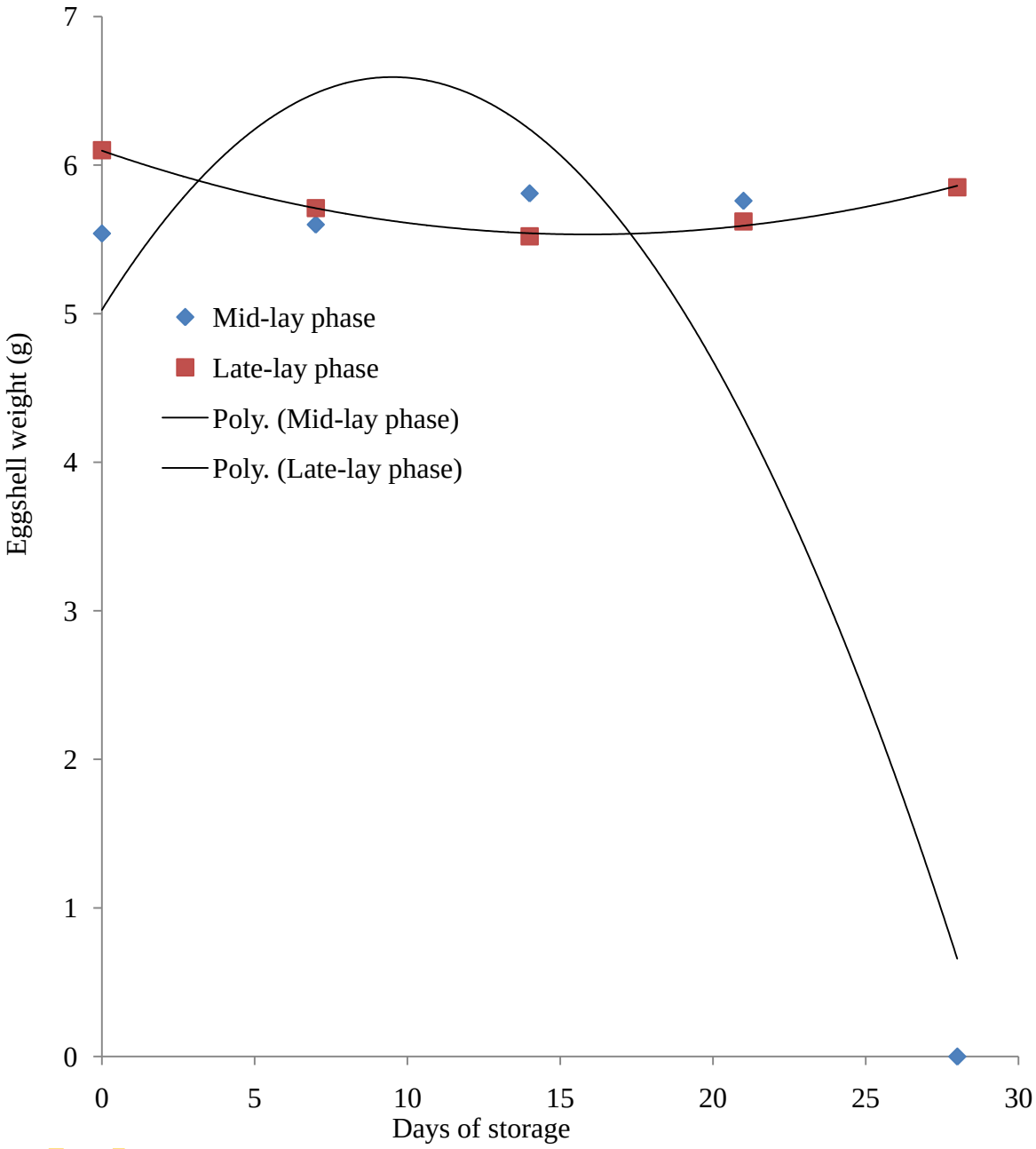

Figure 5: Regression of eggshell weight on days of storage from 36 to 52 and 53 to 70 weeks of age

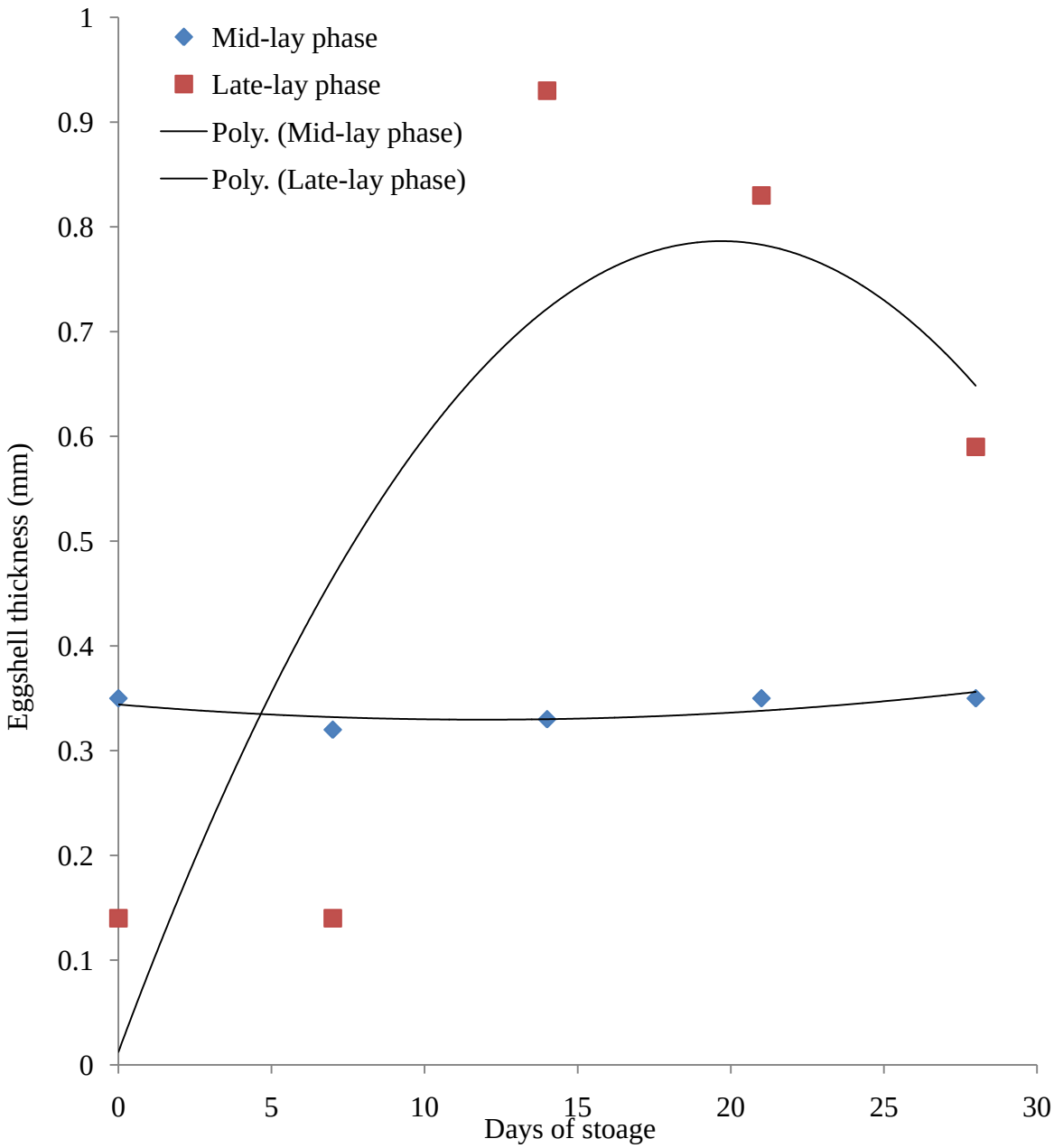

Figure 6: Relationships between eggshell thickness on days of storage from 36 to 52 and 53 to 70 weeks of age

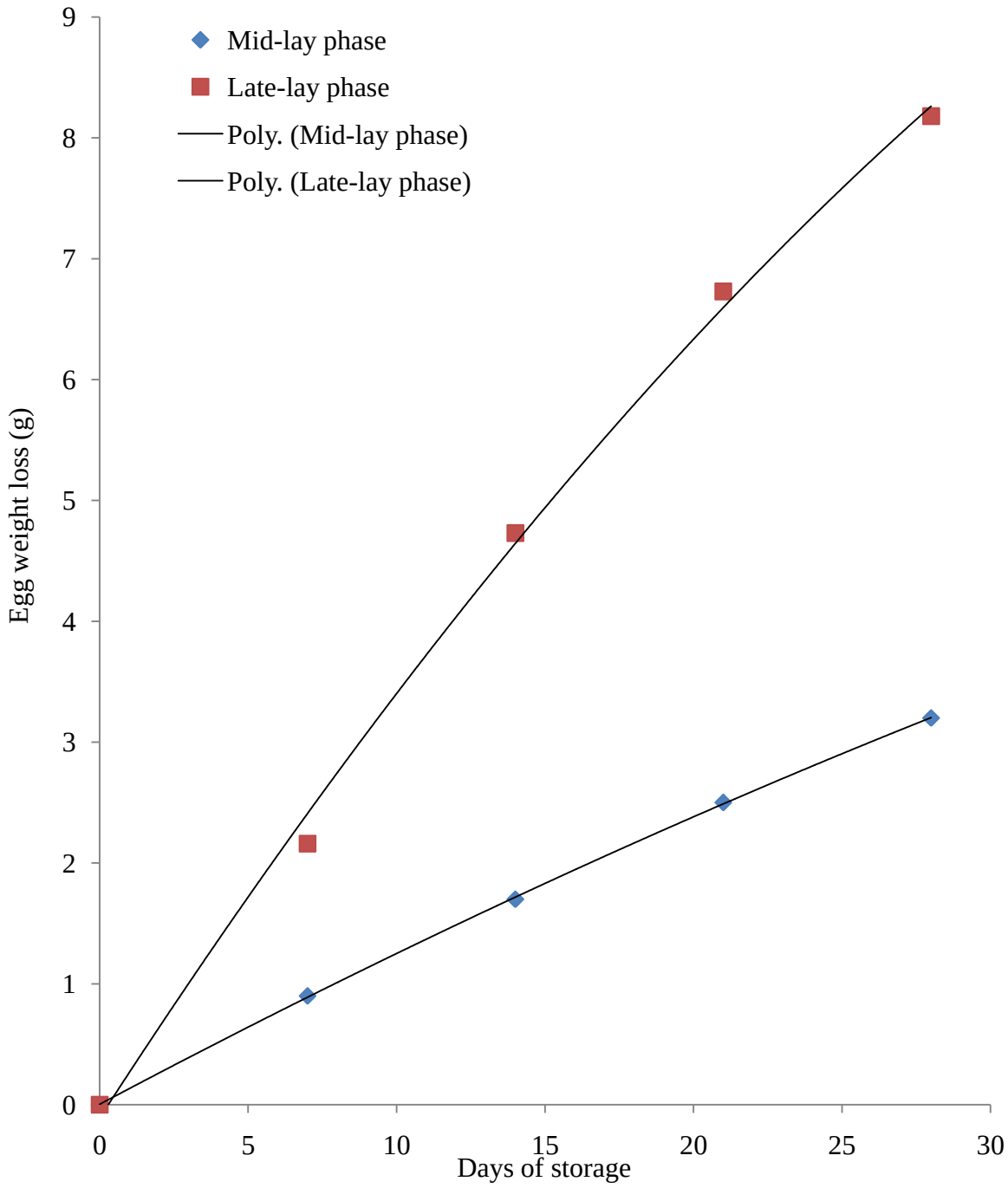

Figure 7: Relationships between egg weight losses on days of storage from 36 to 52 and 53 to 70 weeks of age

4.4.8: Relationship among internal quality indices of eggs from layers as affected by duration of storage

The regressions of albumen quality (HU) on DoS from 36 to 52 and 53 to 70 weeks of age are presented in equations 19 and 20 respectively below:

Mid-layig phase; $y = 0.02x^2 - 2.8637x + 80.945$ ($R^2 = 0.98$) ……. …19

Late-laying phase;$y = 0.0515x^2 - 2.4774x + 95.471$ ($R^2 = 0.93$) …….20

Albumen quality of stored eggs decreased in DoS from 36 to 52 and 53 to 70 weeks of age. Albumen quality of eggs produced from 36 to 52 weeks deterioriated faster than from 36 to 52 weeks from 36 to 52 weeks as graphically shown in Figure 8.

Regression equations 21 and 22 described the changes in yolk quality of eggs in days of storage from 36 to 52 and 53 to 70 weeks of age respectively and plotted graphs in Figure 9 below:

Mid-laying phase; $y = 0.0379x^2 - 2.1041x + 41.194$ ($R^2 = 0.99$) …….21

Late-laying phase;$y = 0.0497x^2 - 2.5888x + 40.304$ ($R^2 = 0.99$) …… 22

Yolk quality of stored eggs deterioriated faster from 36 to 52 weeks compared with eggs from 53 to 70 weeks. Also, albumen and yolk qualities of stored eggs were compared in regression equations 23 and 24 respectively, and plotted graphs in Figure 10 below:

Albumen (HU) quality; $y = 0.02x^2 - 2.8637x + 80.945$ ($R^2 = 0.98$) ……. 23

Yolkquality;$y = 0.0379x^2 - 2.1041x + 41.194$ ($R^2 = 0.99$) ……. 24

The regression grahs of albumen and yolk quatilies in Figure 10 shows similar rates of quality deterioration. However, egg quality deterioriation relatively procceded faster in albumen than in egg-yolk.

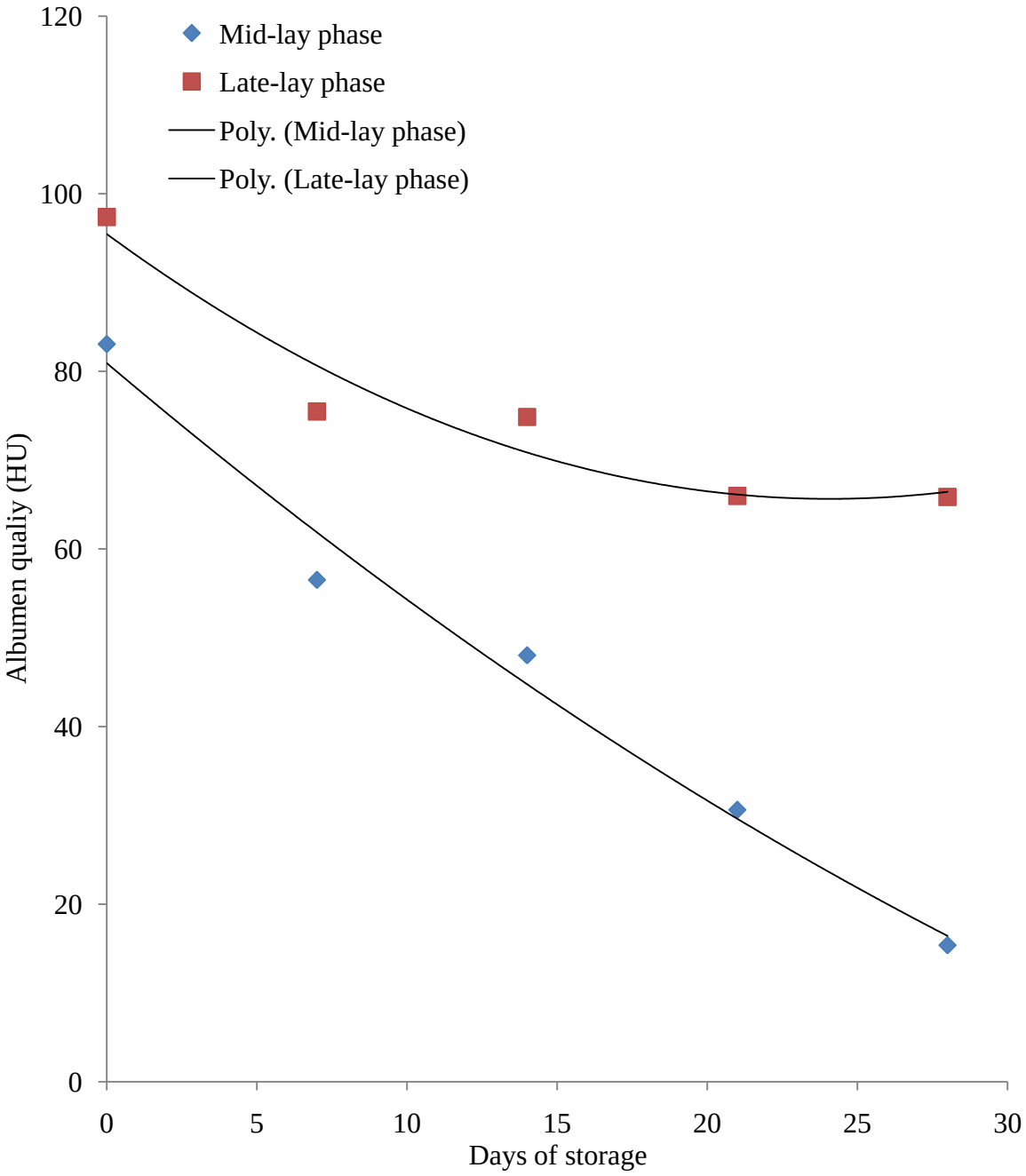

Figure 8: Regression of albumen quality (Haugh Unit) on days of storage of eggs from 36 to 52 and 53 to 70 weeks of age

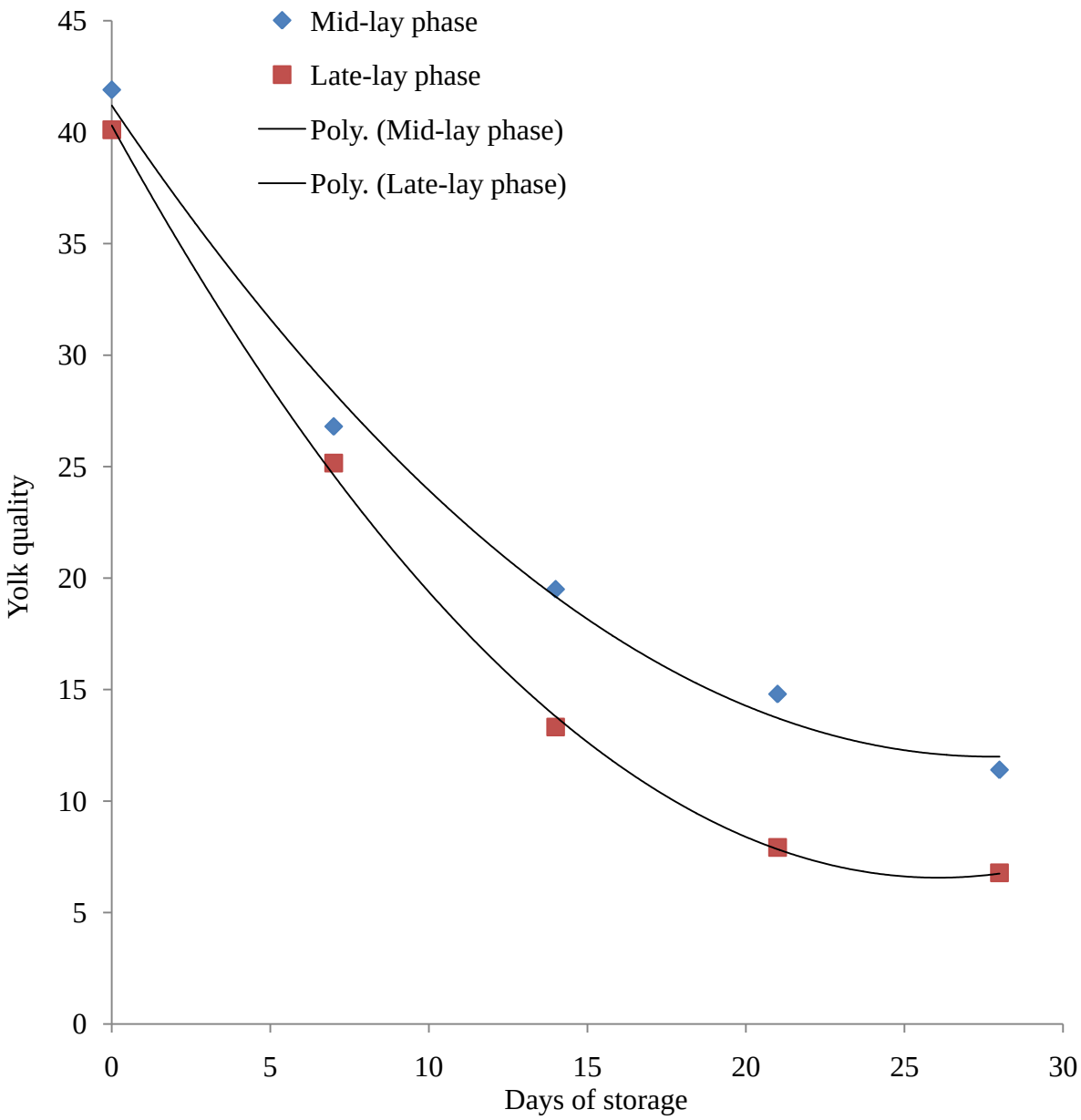

Figure 9: Regression of egg-yolk quality on days of storage of eggs at the mid- and late-laying phases

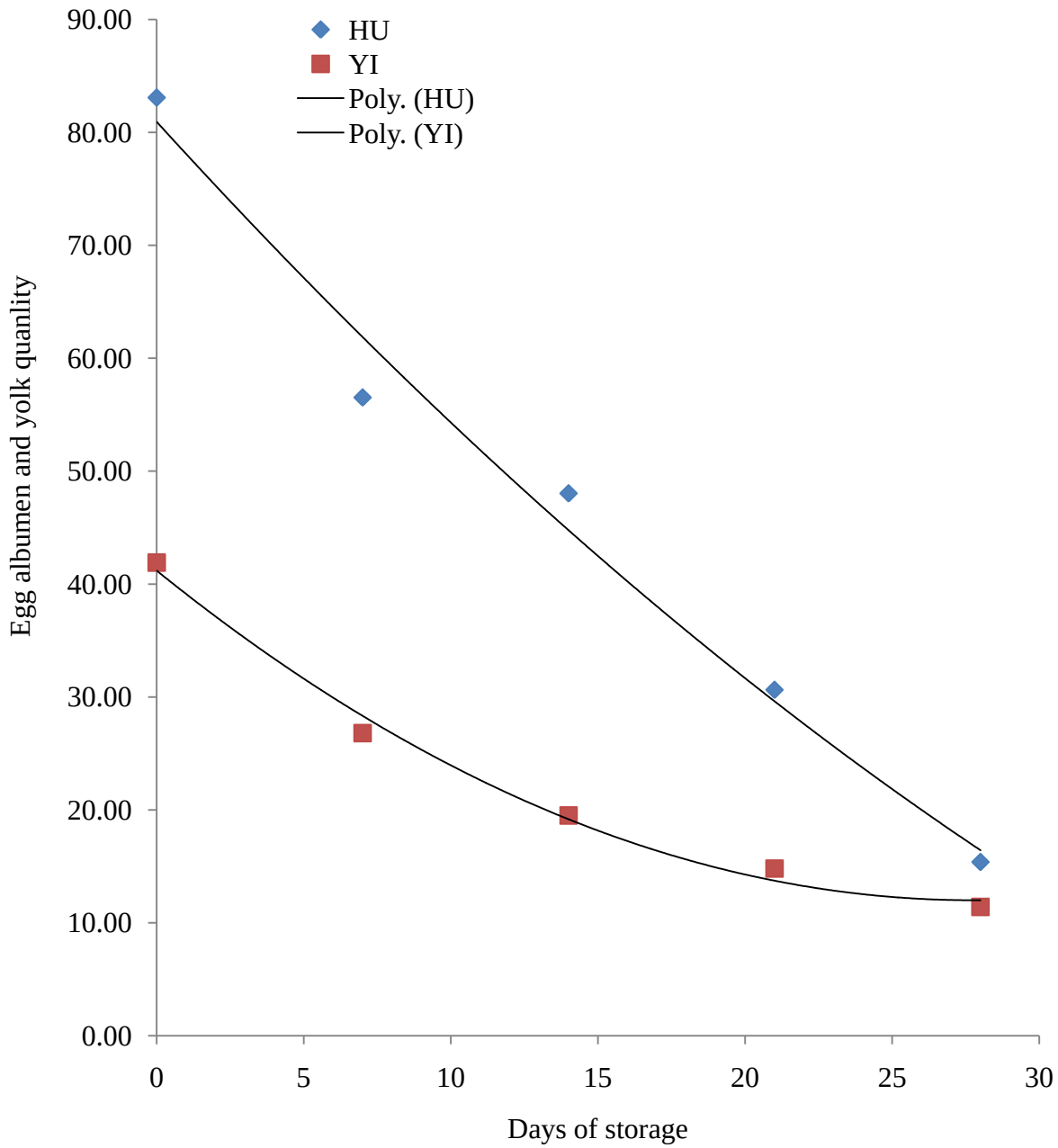

Figure 10: Regression of albumen and yolk quality on days of storage of eggs

Study Five

4.5.1: Chemical compositions of eggs from layers fed diets supplemented with five different proprietary vitamin-mineral premixes in two housing systems at week 22 to 35 weeks of age

The chemical composition of eggs from layers fed diets supplemented with five different PVmP in two HS at weeks 22 to 35 weeks of age is presented in Table 22. Composition of eggs varied ($p<0.05$) with HS. Layers in BC produced eggs that were higher ($p<0.05$) in moisture, (69.96%), crude and true proteins (15.36 and 13.20%), ash (1.02%), ether extract (13.20%), gross energy (1.40 KJ/g), calcium (43.11 mg/100g) and phosphorous (185.54 mg/100g) but lower dry matter (30.10%) contents compared with eggs from DL. Also, composition of eggs were significantly ($p<0.05$) affected by dietary PVmP supplementations.

The moisture content of eggs from layers fed diet supplemented with Hi-Nutrient (69.83%) was highest; while eggs from those fed diets supplemented with Agrited (69.24%) was lowest ($p<0.05$). The misture contentof eggs by layers on Nutripoult (D2), Daram vita-mix (D5) and Micro-mix (D6) were similar to eggs produced by those on diets without PVmP (D1). Layers on diets supplemented with Hi-Nutrient (D3) and Agrited (D4) produced eggs with 30.17 and 30.76% lower ($p<0.05$) and higher ($p<0.05$) in dry matter content, respectively. The dry matter content of eggs by layers on Nutripoult (D2), Hi-Nutrient (D3), Agrited (D4) and Micro-mix (D6) were similar to eggs produced by those on diets without PVmP (D1). Eggs produced by chickens fed supplementalDaram vita-mix (D5) recorded 14.88 and 12.72% highest crude and true proteins respectively while those on Nutripoult (D2) and Micro-mix (D6) were lowest.

Crude protein of eggs produced by layers on Daram vita-mix (D5) was similar to those fed diets supplemented with Micro-mix (D6). Layers on Nutripoult (D2) laid eggs that contained crude protein similar to those on Agrited (D4). However, the true protein of eggs by layers on Nutripoult (D2), Hi-Nutrient (D3) and Agrited (D4) were similar with those on diet without PVmP (D1) ($p>0.05$). The ash content of eggs by layers on Micro-mix 1.10% was highest, while eggs produced by those on Nutripoult 0.88% were the least. The ash content of eggs by layers on Micro-mix (D6) was similar to those on

Daram vita-mix (D5). Eggs produced by layers fed diets supplemented with Hi-Nutrient (D3), Agrited (D4) and Daram vita-mix (D5) were similar in ash content with those fed diet without PVmP (D1). Layers on Micro-mix (1.41 KJ/g) produced eggs with higher gross energy similar to those on Agrited (D4), Daram vita-mix (D5) and those fed diet without PVmP (D1) but higher ($p<0.05$) than eggs from those on Nutripoult (D2) and Hi-Nutrient (D3). Layers fed Micro-mix (D6) produced eggs with highest calcium (42.87 mg/100g) and phosphorus (186.28 mg/100g).

Calcium content of eggs by chickens fed diets supplemented with Micro-mix (42.87 mg/100g) was similar to those on Daram vita-mix (D5) but significantly higher ($p<0.05$) than those on Nutripoult (D2), Hi-Nutrient (D3) and Agrited (D4) and those fed diets without PVmP (D1). The calcium content of egg produced by layers on Hi-Nutrient (40.40 mg/100g) was the lowest. The phosophorous content of eggs produced by layers fed diets supplemented with Hi-Nutrient (180.33mg/100g), Agrited (181.85 mg/100g) and those without PVmP (182.21 mg/100g) were similar but higher ($p<0.05$) than those on Nutripoult (179.33 mg/100g).

Table 22: Chemical compositions of eggs from layers fed diets supplemented with five different proprietary vitamin-mineral premixes in two housing systems from 22 to 35 weeks of age

Effects	Factors	MC (%)	DM (%)	CP (%)	TP (%)	Ash (%)	EE (%)	GE (KJ/g)	Ca (mg/100g)	P (mg/100g)
	BC	69.96^{a}	30.10^{b}	15.36^{a}	13.20^{a}	1.02^{a}	13.20^{a}	1.40^{a}	43.11^{a}	185.54^{a}
HS	DL	69.21^{b}	30.79^{a}	13.81^{b}	11.79^{b}	0.94^{b}	11.17^{b}	1.38^{b}	40.50^{b}	178.87^{b}
	SEM	0.14	0.14	0.08	0.07	0.03	0.05	0.02	0.18	0.78
	D1	69.55^{bc}	30.45^{bc}	14.58^{c}	12.50^{bc}	0.98^{bc}	12.19^{bc}	1.39^{ab}	41.81^{b}	182.21^{cd}
	D2	69.63^{b}	30.37^{bc}	14.17^{d}	12.41^{cd}	0.88^{d}	12.02^{d}	1.38^{b}	41.15^{bc}	179.33^{e}
	D3	69.83^{a}	30.17^{c}	14.73^{b}	12.61^{b}	0.94^{c}	12.17^{bc}	1.38^{b}	40.40^{c}	180.33^{d}
PVmP	D4	69.24^{d}	30.76^{a}	14.32^{cd}	12.41^{cd}	0.98^{bc}	12.41^{a}	1.40^{a}	41.97^{b}	181.85^{cd}
	D5	69.62^{b}	30.38^{bc}	14.88^{a}	12.72^{a}	1.02^{ab}	12.08^{d}	1.39^{ab}	42.65^{a}	183.24^{b}
	D6	69.42^{c}	30.58^{b}	14.83^{a}	12.35^{d}	1.10^{a}	12.27^{b}	1.41^{a}	42.87^{a}	186.28^{a}
	SEM	0.17	0.33	0.61	0.60	0.07	0.35	0.07	0.15	1.02

$^{a-d}$ Means with different superscripts on the same column are significantly different ($p<0.05$).MC-Moisture content, CP-Crude protein, TP-True protein, EE-Ether extract, A-Ash, NFE-Nitrogen free extract, GE-Gross energy, HS-Housing systems, PVMP-Proprietary vitamin-mineral premixes, D1-diet without PVmP, D2, D3, D4, D5 and D6-diets withNutripoult, Hi-Nutrient, Agrited, Daram vita-mix and Micro-mix respectively, SEM-Standard error of means

The interaction effects of PVmP and HS on chemical composition of eggs from chickens fed diets supplemented with five different PVmP in two HS from 22 to 35 weeks of age is presented in Table 23. There were significant interactions ($p<0.05$) of PVmP and HS on chemical compositions of eggs in the early-laying phase.

Table 23: Interaction effects of proprietary vitamin-mineral premixes and housing systems on chemical composition of eggs from 22 to 35 weeks of age

Factors	MC (%)	DM (%)	CP (%)	TP (%)	Ash (%)	EE (%)	GE (KJ/g)	Ca (mg/100)	P (mg/100)
D1xBC	69.96^{a}	30.11^{c}	15.36^{a}	13.20^{a}	1.02^{ab}	13.20^{a}	1.40^{ab}	43.11^{a}	185.54^{b}
D2xBC	69.98^{a}	30.02^{c}	14.85^{a}	13.23^{a}	0.92^{d}	13.13^{a}	1.39^{bc}	42.60^{b}	184.13^{bc}
D3xBC	70.15^{a}	29.85^{c}	15.45^{a}	13.17^{a}	0.96^{cd}	13.13^{a}	1.38^{c}	40.97^{c}	181.33^{c}
D4xBC	69.96^{a}	30.40^{b}	15.27^{a}	13.50^{a}	1.06^{ab}	13.29^{a}	1.40^{ab}	43.53^{ab}	186.90^{ab}
D5xBC	69.87^{a}	30.13^{c}	15.64^{a}	13.39^{a}	1.02^{bc}	13.15^{a}	1.41^{a}	43.96^{a}	185.60^{b}
D6xBC	69.86^{a}	30.14^{c}	15.59^{a}	12.72^{a}	1.14^{a}	13.32^{a}	1.41^{a}	44.50^{a}	189.73^{a}
D1xDL	69.21^{ab}	30.79^{ab}	13.81^{ab}	11.79^{ab}	0.94^{bc}	11.17^{b}	1.38^{b}	40.50^{ab}	178.87^{b}
D2xDL	69.29^{ab}	30.71^{ab}	13.48^{b}	11.58^{b}	0.83^{c}	10.90^{c}	1.36^{b}	39.70^{c}	174.53^{c}
D3xDL	69.51^{a}	30.49^{b}	14.00^{a}	12.04^{a}	0.92^{bc}	11.20^{b}	1.37^{b}	39.83^{bc}	179.33^{b}
D4xDL	68.91^{b}	31.09^{a}	13.37^{b}	11.32^{c}	0.90^{c}	11.53^{a}	1.40^{a}	40.40^{b}	176.80^{c}
D5xDL	69.37^{ab}	30.63^{ab}	14.12^{a}	12.05^{a}	1.01^{ab}	11.00^{c}	1.36^{b}	41.33^{a}	180.87^{ab}
D6xDL	68.97^{ab}	31.03^{ab}	14.07^{a}	11.97^{a}	1.05^{a}	11.21^{b}	1.41^{a}	41.23^{a}	182.83^{a}
SEM	0.17	0.33	0.61	0.60	0.07	0.35	0.07	0.15	1.02

$^{a-f}$Means with different superscripts on the same column are significantly different ($p<0.05$). MC-Moisture content, TP-True protein, EE-Ether extract, A-Ash, NFE-Nitrogen free extract, GE-Gross energy, E-effects, F-Factors, HS-Housing systems, PVmP- Proprietary vitamin-mineral premixes, PVMP-Proprietary vitamin-mineral premixes, D1-diet without PVmP, D2, D3, D4, D5 and D6-diets with Nutripoult, Hi-Nutrient, Agrited, Daram vita-mix and Micro-mix respectively,SEM-Standard error of means, x-Interactions

4.5.2: Chemical compositions of eggs as affected by five different proprietary vitamin-mineral premixes, two housing systems and duration of storage from 36 to 52 weeks of age

The chemical compositions of eggs as affected by five different proprietary vitamin-mineral premixes, two housing systems and duration of storage as well as interaction effects of PVmP and HS in DoS from 36 to 52 weeks of age are presented in Table 24. Ether extracts, ash and nitrogen free extract differed significantly ($p<0.05$) with HS, while moisture, dry matter and crude protein were not significantly ($p>0.05$) affected. Significantly ($p<0.05$) higher ether extract and ash values were obtained from eggs produced by layers in DL (7.64 and 1.30%, respectively) than eggs from BC (7.59 and 1.28%). On the other hand, layers in BC (1.15%) produced eggs that were significantly higher ($p<0.05$) in nitrogen free extract than eggs from DL (1.08%).

The moisture, dry matter, crude protein and ether extract contents of eggs significantly ($p<0.05$) varied with different dietary PVmP, while ash and nitrogen free extract content were not significantly affected ($p>0.05$). The moisture content of eggs from layers on diets supplemented with Agrited (D4) was higher ($p<0.05$) and similar to those on Micro-mix-diets (D5) but significantly ($p<0.05$) higher compared with eggs from layers on Nutripoult (D2), Hi-Nutrient (D3) and Micro-mix (D6). Eggs produced by layers fed diets supplemented with Nutripoult (D2) had the highest dry matter content. Layers fed diets supplemented with Agrited (D4) recorded the least dry matter content was similar to eggs produced by those on Daram vita-mix (D5). The dry matter content of eggs produced by layers on diet supplemented with Hi-Nutrient, (D3) and Daram vita-mix (D5) were similar and lower ($p<0.05$) than eggs from those on Micro-mix (D6).

Eggs laid bychickens fed diets supplemented with Nutripoult recorded highest level (11.63%) of crude protein. Diet supplemented with Agrited (11.44%) induced the least level crude protein in eggs. The crude protein of eggs from layers fed diets supplemented with Hi-Nutrient (D3), Daram vita-mix (D5) and Micro-mix (D6) were similar but lower ($p<0.05$) compared with eggs from those on Nutripoult (D2). Eggs from layers on diets supplemented with Nutripoult (D2) was the highest in ether extract and similar to eggs produced by those on Agrited (D4) and higher ($p<0.05$) than eggs

from those on Hi-Nutrient (D3), Daram vita-mix (D5) and Micro-mix (D6). Eggs produced by layers fed diets supplemented with Micro-mix (D6) were lowest in ether extract. Moisture content and ether extract content of eggs signifcantly decreased ($p<0.05$) with DoS, while dry matter, crude protein and ash increased ($p<0.05$). The interaction effects of HS x PVmP were highly significant ($p<0.01$) for all indices of chemical compositions, while PVmP x DoS interaction was significant ($p<0.05$) on ether extract and nitrogen free extract. There were no significant ($p>0.05$) interaction effects of HS x DoS and PVmP x HS x DoS on chemical composition parameters.

Table 24: Chemical compositions of eggs as affected by five different proprietary vitamin-mineral premixes, two housing systems and duration of storage from 36 to 52 weeks of age

Effects	Factors	MC (%)	DM (%)	CP (%)	EE (%)	A (%)	NFE (%)
HS	BC	78.42	21.58	11.56	7.59^{b}	1.28^{b}	1.15^{a}
	DL	78.43	21.57	11.54	7.64^{a}	1.30^{a}	1.08^{b}
	SEM	0.01	0.01	0.01	0.01	0.01	0.02
PVmP	D2	78.32^{d}	21.68^{a}	11.63^{a}	7.67^{a}	1.28	1.09
	D3	78.45^{b}	21.55^{c}	11.55^{b}	7.57^{c}	1.29	1.14
	D4	78.50^{a}	21.50^{d}	11.44^{c}	7.65^{ab}	1.28	1.12
	D5	78.48^{ab}	21.52^{cd}	11.54^{b}	7.62^{b}	1.30	1.04
	D6)	78.37^{c}	21.63^{b}	11.59^{b}	7.56^{c}	1.29	1.18
	SEM	0.02	0.02	0.021	0.02	0.01	0.04
DoS	0	78.51^{a}	21.49^{c}	11.45^{b}	7.61^{a}	1.24^{c}	1.15
	7	78.49^{a}	21.51^{c}	11.54^{a}	7.60^{ab}	1.23^{c}	1.12
	14	78.41^{b}	21.62^{a}	11.60^{a}	7.61^{ab}	1.29^{b}	1.13
	21	78.36^{c}	21.65^{a}	11.55^{a}	7.62^{a}	1.35^{a}	1.10
	28	78.35^{c}	21.59^{b}	11.59^{a}	7.59^{b}	1.34^{a}	1.06
	SEM	0.02	0.02	0.02	0.02	0.01	0.04
	HSxPVmP	< 0.0001**	< 0.0001**	<0.0001**	< 0.0001**	< 0.0001**	< 0.0001**
	HSxDoS	0.1860^{NS}	0.1860^{NS}	0.1983^{NS}	0.9652^{NS}	0.8000^{NS}	0.9323^{NS}
	PVmPxDoS	0.2090^{NS}	0.2095^{NS}	0.9237^{NS}	0.0071*	0.7047^{NS}	0.0116*
	HSxPVmPxDoS	0.4210^{NS}	0.4219^{NS}	0.8890^{NS}	0.6826^{NS}	0.2026^{NS}	0.6230^{NS}

$^{a–d}$Means with different superscripts on the same column are significantly different ($p<0.05$). HS-Housing systems, PVmP-Proprietary vitamin-mineral premix, DoS-Days of storage, MC-Moisture content, DM-Dry matter, CP-Crude protein, EE-Ether extract, A-Ash, NFE-Nitrogen free extract, D2, D3, D4, D5 and D6-diets Nutripoult, Hi-Nutrient, Agrited, Daram vita-mix and Micro-mix, respectively, x-Interaction,*-(p0.05), **-(p0.01), SEM-Standard error of mean

4.5.3: Chemical compositions of eggs as affected by five different proprietary vitamin-mineral premixes, two housing systems and duration of storage from 53 to 70 weeks of age

The effects of duration of storage on chemical compositions of eggs from layers fed diets supplemented with five different proprietary vitamin-mineral premixes in two housing systems from 53 to 70 weeks is presented in Table 25. The moisture (MC) and dry matter (DM) content of eggs did not vary ($p>0.05$) with HS, while crude protein (CP), ether extract (EE), ash and nitrogen free extract (NFE) affected ($p<0.05$). Eggs produced by layers in BC were significantly higher ($p<0.05$) in CP than eggs from DL. On the other hand, layers in DL produced eggs higher ($p<0.05$) in EE, ash and NFE than eggs from BC. The MC, CP, EE, ash and NFE of eggs varied ($p<0.05$) with dietary PVmP supplementations. The DM of eggs was not affected($p>0.05$) by dietary PVmP. Eggs laid by layers fed diets supplemented with Nutripoult (D2) had MC similar to eggs produced by those on Hi-Nutrient (D3) and Micro-mix (D6) but higher ($p<0.05$) than eggs form those on Agrited (D4) and Daram vita-mix (D5).

Agrited (D4) induced the highest level of CP in eggs, while eggs from layers fed diets supplemeneted with Nutripoult (D2) had the least. The CP of eggs from layers on Nutripoult (D2) was similar to those on Hi-Nutrient (D3). Crude protein of eggs of layers on Daram vita-mix (D5) ranked second and similar to eggs laid by those on Hi-Nutrient (D3). Eggs from layers on Agrited (D4) was highest in EE and similar to those on Micro-mix (D6) but higher ($p<0.05$) compared with eggs produced by those fed diets supplelenemted with Nutripoult (D2), Hi-Nutrient (D3) and Daram vita-mix (D5). Layers on diets supplemented with Nutripoult (T2) were lowest in EE. Eggs laid by layers on diets supplemented with Micro-mix (D6) was higher ($p<0.05$) in ash content and similar to eggs fromt those on Hi-Nutrient (D3) and Daram vita-mix (D5) but higher ($p<0.05$) than eggs form those on Nutripoult (D2) and Agrited (D4). Nutripoult supplementation (D2) induced the highest NFE which was similar to eggs from layers on Hi-Nutrient (D3) and Daram vita-mix (D5) but higher ($p<0.05$) than eggs laid by those on Agrited (D4) and Micro-mix (D6). Chemical compositions were affected ($p<0.05$) with moisture content reducing ($p<0.05$), while DM, CP, EE, ash and NFE increasing ($p<0.05$).

Table 25: Chemical compositions of eggs as affected by five different proprietary vitamin-mineral premixes, two housing systems and duration of storage from 53 to 70 weeks of age

Effects	Factors	MC (%)	DM (%)	CP (%)	EE (%)	Ash (%)	NFE (%)
	BC	74.44	25.56	7.97^{a}	10.38^{b}	1.02^{b}	6.20^{b}
HS	DL	74.35	25.65	7.09^{b}	10.61^{a}	1.10^{a}	6.85^{a}
	SEM	0.17	0.05	0.11	0.12	0.03	0.16
	D2	74.77^{a}	25.23	7.11^{c}	9.94^{c}	1.02^{bc}	7.17^{a}
	D3	74.47^{ab}	25.53	7.38^{bc}	10.43^{b}	1.10^{ab}	6.61^{ab}
PVmP	D4	73.95^{c}	26.05	8.27^{a}	10.95^{a}	0.96^{c}	5.87^{c}
	D5	74.36^{b}	25.64	7.64^{b}	10.32^{b}	1.08^{ab}	6.60^{ab}
	D6	74.43^{ab}	25.57	7.24^{c}	10.84^{a}	1.12^{a}	6.37^{bc}
	SEM	0.26	0.13	0.17	0.17	0.04	0.25
	0	76.03^{a}	23.97^{e}	6.64^{e}	10.14^{c}	0.93^{c}	6.25^{b}
	7	75.47^{b}	24.53^{d}	7.10^{d}	10.30^{bc}	1.02^{c}	6.11^{b}
DoS	14	74.63^{c}	25.37^{c}	7.49^{c}	10.21^{c}	0.98^{c}	6.68^{ab}
	21	73.31^{d}	26.69^{b}	7.92^{b}	10.59^{b}	1.13^{b}	7.05^{a}
	28	72.53^{e}	27.47^{a}	8.48^{a}	11.22^{a}	1.24^{a}	6.52^{ab}
	SEM	0.16	0.65	0.16	0.17	0.04	0.25

$^{a–d}$Means with different superscripts on the same column are significantly different ($p<0.05$). HS-Housing systems, PVmP- Proprietary vitamin-mineral premix, DoS-Days of egg storage, MC-Moisture content, DM-Dry matter, CP-Crude protein, EE-Ether extract, A-Ash, NFE-Nitrogen free extract, D2, D3, D4, D5 and D6-diets Nutripoult, Hi-Nutrient, Agrited, Daram vita-mix and Micro-mix respectively, SEM -Standard error of mean

4.5.3: Relationship parameters or chemical compositions of eggs as affected by duration of storage from 53 to 70 weeks of age

The regression of crude protein on days of storage of eggs at the early- and late-laying phases are shown in equations 25 and 26, respectively and plotted graphs in Figure 11 below:

Early-laying phase; $y = -0.0003x^2 + 0.0127x + 11.458$ … ($R^2 = 0.82$) ……. 25
Late-laying phase;$y = 0.0003x^2 + 0.0545x + 6.6603$… .....($R^2 = 0.99$) ……. 26

Crude protein of eggs produced and stored at the early-laying phase was higher than those at late-laying phase. The crude protein of eggs in days of storage at the early-laying remain fairly contant ($R^2 = 0.82$) but increased at the late-laying phase ($R^2 = 0.99$) as shown in Figure 11.

Equations 27 and 28 shows regression of egg fat on days of storage at the early- and late-laying phases, respectively.

Late-laying phase; $y = 0.0021x^2 - 0.0226x + 10.203$ ($R^2 = 0.95$) ……27
Early-laying phase; $y = -6E\text{-}05x^2 + 0.0013x + 7.6043$ ($R^2 = 0.30$) …….. 28

Fat in stored eggs at the late-laying phase was higher than in early-laying phase. At eraly-laying phase, fat content of eggs ($R^2 =0.30$) remained fairly contant but increased ($R^2 =0.95$) at the late-laying phase as shown in Figure 12 below:

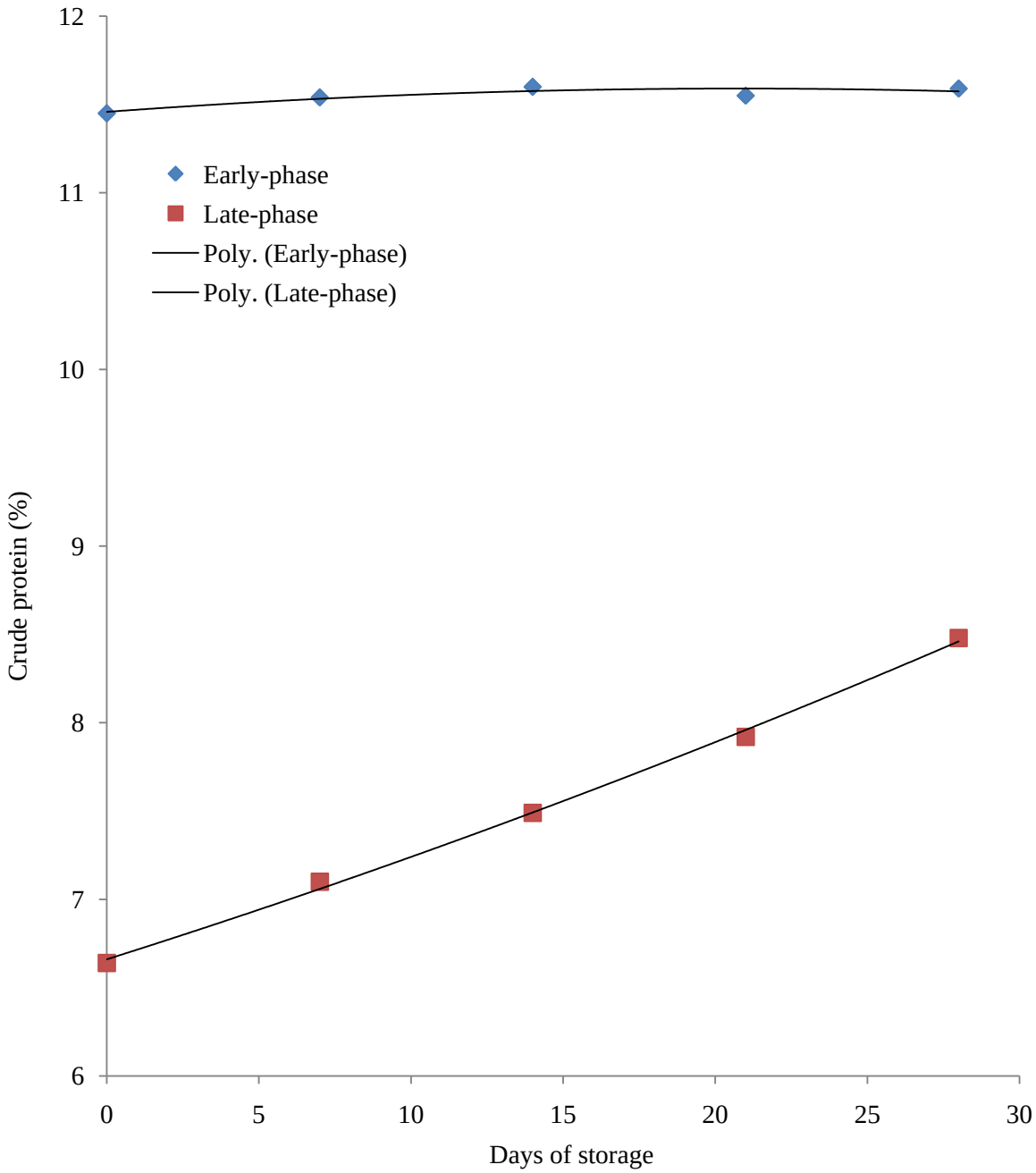

Figure 11: Regression of eggscrude protein on days of storageat the early- and late-laying phases

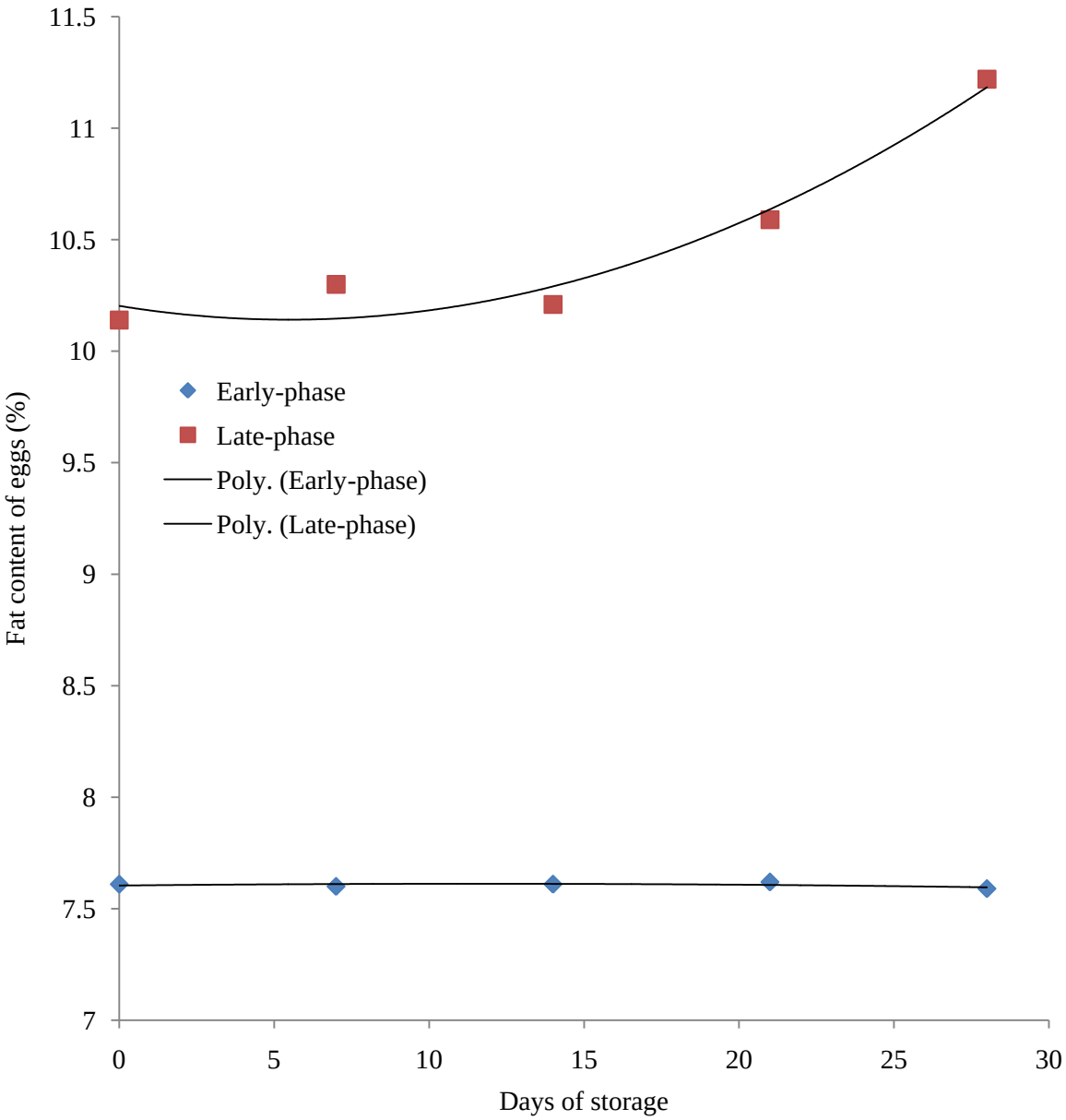

Figure12: Regression of egg fat on days of storageat the early- and late-laying phases

Study Six

4.6.1: Cholesterol profile of whole-egg from layers fed diets supplemented with five different proprietary vitamin-mineral premixes in two housig systems from 36 to 52 weeks of age

The cholesterol profile of whole-egg from layers fed diets supplemented with five different PVmP in two HS at mid-laying phase is presented in Table 26. Cholesterol profile of whole-egg was not affected ($p>0.05$) by HS but varied ($p<0.05$) with different dietary PVmP supplementation. The cholesterol (TC) and low density lipoprotein (LDL) follow a similar trend. Layers fed diets supplemented with Nutripoult (D2) laid eggs with highest whole-egg TC (567.67 mg/dL) and LDL (397.33 mg/dL), while those on Agrited (345.67 and 182.50 respectively) were least. The TC and LDL of whole-egg of laye3rs fed diets supplemented wth Hi-Nutrient (465.17 and 277.67), Daram vita-mix (434.55 and 247.33) and Micro-mix (428.33 and 245.00) were similar but lower ($p<0.05$) compared with eggs laid by those on Nutripoult (D2) and higher ($p<0.05$) than in eggs produced by those fed diets supplemented with Agrited (D4).

Table 26: Cholesterol profiles of whole-egg of eggs from layers fed diets supplemented with five proprietary vitamin-mineral premixes in two different housing systems from 36 to 52 weeks of age

Effects	Factors	TC (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
	BC	441.80	264.33	122.80	265.60	52.93
HS	DL	454.81	265.60	126.93	274.33	53.53
	SEM	11.47	1.78	2.40	9.51	0.53
	D2	567.67^{a}	262.83^{b}	117.83^{b}	397.33^{a}	52.17^{b}
	D3	465.17^{b}	263.00^{b}	135.33^{a}	277.67^{b}	52.67^{b}
PVmP	D4	345.67^{c}	262.67^{b}	110.67^{b}	182.50^{c}	52.50^{b}
	D5	434.33^{b}	271.83^{a}	131.33^{a}	247.33^{b}	54.50^{a}
	D6	428.33^{b}	264.50^{ab}	129.17^{ab}	245.00^{b}	54.33^{a}
	SEM	18.14	2.82	3.80	15.03	0.84

$^{a–c}$Means within the same column with different superscripts differ significantly ($p<0.05$).TC-Total cholesterol, TG-Triglyceride, HDL-High Density Lipoprotein, LDL-Low Density Lipoprotein, VLDL-Very Low Density Lipoprotein, DL-Deep litter, BC-Battery cage, HS-Housing systems, PVmP-Proprietary vitamin-mineral premixes, D2, D3, D4, D5 and D6-diets with Nutripoult, Hi-Nutrient, Agrited, Daram vita-mix and Micro-mix, respectively, SEM-Standard Error of Mean.

The triglycerides (TG) and very low density lipoprotein (VLDL) in whole-eggs of layers fed diets supplemented with Daram vita-mix (D5) were similar to those from on Micro-mix (D6) but higher ($p<0.05$) compared with those on Nutripoult(D2), Hi-Nutrient (D3) and Daram vita-mix (D4). Layers on Nutripoult (D2) and Hi-Nutrient (D3) laid eggs that contained lower ($p<0.05$) VLDL and TG. Whole-eggs of layers on Hi-Nutrient (D3) was higher ($p<0.05$) in high density lipoprotein (HDL) though similar to eggs from those on Daram vita-mix (D5) and Micro-mix (D6) but higher ($p<0.05$) than eggs from those on Nutripoult (D2) and Agrited (D4). The whole-egg HDL of layers on Nutripoult (D2), Agrited (D4) and Micro-mix (D6) were similar too. The interaction effects of PVmP and HS on cholesterol profile of whole-egg of layers from 36 to 52 weeks of age is shown in Table 27. The interaction effect of PVmP and HS on cholesterol profile of whole-egg was significant ($p<0.05$).

Table 27: Interaction effects of proprietary vitamin-mineral premixes and housing systems on cholesterol profile of whole-egg of layers from 36 to 52 weeks of age

Effects	Factors	TC (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
	D2 x BC	607.33^{a}	266.00^{bcd}	132.00^{a}	422.00^{a}	53.00^{abc}
	D3 x BC	459.00^{bc}	264.00^{bcd}	137.67^{a}	269.33^{b}	53.00^{abc}
	D4 x BC	273.67^{d}	254.67^{d}	88.67^{b}	134.00^{c}	51.00^{c}
	D5 x BC	459.00^{bc}	264.00^{bcd}	133.00^{a}	269.67^{b}	53.00^{abc}
HS x PVmP	D6 x BC	410.00^{c}	273.00^{ab}	122.67^{a}	233.00^{b}	54.67^{ab}
	D2 x BC	520.00^{b}	259.67^{cd}	103.67^{b}	392.67^{a}	51.33b^{c}
	D3 x BC	471.33^{bc}	262.00b^{cd}	133.00^{a}	286.00^{b}	52.33^{bc}
	D4 x BC	417.67^{c}	270.67^{abc}	132.67^{a}	231.00^{b}	54.00^{abc}
	D5 x BC	410.67^{c}	279.67^{a}	129.67^{a}	225.00^{b}	56.00^{a}
	D6 x BC	446.67^{c}	256,00^{d}	135.67^{a}	257.00^{b}	54.00^{abc}
	SEM	25.66	3.99	5.37	21.26	1.18

$^{a\ -d}$ Means with different superscripts within the same column differ significantly ($p<0.05$). TC-Total cholesterol, TG-Triglyceride, HDL-High Density Lipoprotein, LDL-Low Density Lipoprotein, VLDL-Very Low Density Lipoprotein, DL-Deep litter, BC-Battery cage, HS-Housing systems, PVmP-Proprietary vitamin-mineral premixes, D2, D3, D4, D5 and D6-diets with Nutripoult, Hi-Nutrient, Agrited, Daram vita-mix and Micro-mix, respectively, x-Interactions SEM-Standard Error of Mean

4.6.2: Cholesterol profile of egg-yolk from layers fed diets supplemented with five different proprietary vitamin-mineral premixes in two housing systems from 36 to 52 weeks of age

The of cholesterol profile of egg-yolk from layers fed diets supplemented with five different PVmP in two HS from 36 to 52 weeks of age is shown in Table 28. The HS did not affect ($p>0.05$) total cholesterol (TC) and high density lipoprotein (HDL) of egg-yolk, while trigylecrides (TG), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) varied ($p<0.05$). Eggs from layers in BC were higher ($p<0.05$) in yolk TG and VLDL but lower in LDL compared with those from DL. The cholesterol profile of egg-yolk were affected ($p<0.05$) by dietary PVmP. Eggs produced by layers fed diets supplemented with Micro-mix (D6) was higher in yolk TC, TG and VLDL and similar to eggs laid by those on Nutripoult (D2), Hi-Nutrient (D3) and Agrited (D4) but higher ($p<0.05$) than eggs from those on Daram vita-mix (D5).

Table 28: Cholesterol profile of egg-yolk from layers fed diets supplemented with five differnt proprietary vitamin-mineral premixes in two housing systems from 36 to 52 weeks of age

Effects	Factors	TC (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
HS	BC	14.73	50.80^{a}	4.01	0.60^{b}	9.47^{a}
	DL	14.80	41.01^{b}	3.67	2.13^{a}	8.40^{b}
	SEM	0.41	1.28	0.16	0.16	0.28
PVmP	D2	15.33^{ab}	44.50^{b}	5.33^{a}	1.17^{b}	8.83^{bc}
	D3	15.80^{ab}	48.67^{b}	3.00^{b}	2.83^{a}	9.67^{b}
	D4	14.00^{ab}	42.83^{b}	3.33^{b}	1.33^{b}	6.83^{c}
	D5	12.33^{b}	28.00^{c}	4.83^{a}	1.00^{b}	6.50^{c}
	D6	16.67^{a}	65.67^{a}	2.83^{b}	0.50^{b}	12.83^{a}
	SEM	0.26	0.81	0.10	0.10	0.17

$^{a-c}$ Means with different superscripts on the same column are significantly different ($p<0.05$).TC- Total cholesterol, TG-Triglyceride, HDL-High Density Lipoprotein, LDL-Low Density Lipoprotein, VLDL-Very Low Density Lipoprotein, HS-Housing systems, PVmP-Proprietary vitamin-mineral premixes, D2, D3, D4, D5 and D6-diets with Nutripoult, Hi-Nutrient, Agrited, Daram vita-mix and Micro-mix respectively, SEM-Standard error of means

The egg-yolk of layers fed diets supplemented with Daram vita-mix (D5) was lower in TC, TG and VLDL. The HDL of egg-yolk produced by layers on Nutripoult (D2) was similar to those on Daram vita-mix (D5) and higher ($p<0.05$) than eggs from those on Hi-Nutrient (D3), Agrited (D4) and Micro-mix (D6). Egg-yolk of layers fed diets supplemented with Micro-mix (D6) was the lowest in HDL. The egg-yolk of layers on Hi-Nutrient (D3) and Micro-mix (D6) had highest and lowest LDL respectively. The LDL of egg-yolk oflayers feed diets supplemented with Nutripoult (D2), Agrited (D4), Daram vita-mix (D5) and Micro-mix (D6) were similar. The interaction effects of dietary PVmP and HS on cholesterol profile of egg-yolk of layers from 36 to 52 weeks of age are presented in Table 29. There were interaction effects ($p<0.05$) of PVmP and HS on parameters of cholesterol profile of egg-yolk of layers.

Table 29: Interaction effects of proprietary vitamin-mineral premixes and housing systems on cholesterol profile of egg-yolk of layers from 36 to 52 weeks of age

Effects	Factors	TC (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
PVmP x HS	D2 x BC	17.67^{ab}	51.67^{bcd}	6.67^{a}	0.67^{b}	10.33^{bc}
	D3 x BC	12.00^{c}	39.67^{cd}	3.33^{bc}	0.67^{b}	8.00^{bcd}
	D4 x BC	12.00^{c}	48.33^{bcd}	2.67^{c}	0.67^{b}	6.00^{d}
	D5 x BC	13.67^{abc}	36.00^{de}	4.67^{abc}	1.00^{b}	8.00^{bcd}
	D6 x BC	19.67^{a}	78.33^{a}	3.00^{bc}	0.67^{b}	15.00^{a}
	D2 x DL	13.00^{bc}	37.33^{cd}	4.00^{bc}	1.67^{b}	7.33^{cd}
	D3 x DL	19.00^{ab}	57.67^{b}	2.67^{c}	2.00^{a}	11.33^{ab}
	D4 x DL	16.00^{abc}	37.33^{cd}	4.00^{bc}	1.00^{b}	7.67^{bcd}
	D5 x DL	11.00^{c}	20.00^{e}	5.00^{ab}	1.67^{b}	5.00^{d}
	D6 x DL	14.67^{abc}	53.00^{bc}	2.67^{c}	1.33^{b}	10.67^{bc}
	SEM	0.18	0.57	0.07	0.07	0.13

$^{a–d}$Means different superscripts within the same column with differ significantly ($p<0.05$). TC-Total cholesterol, TG-Triglyceride, HDL-High Density Lipoprotein, LDL-Low Density Lipoprotein, VLDL-Very Low Density Lipoprotein, DL-Deep litter, BC-Battery cage, HS-Housing systems, PVmP-Proprietary vitamin-mineral premixes, D2, D3, D4, D5 and D6-diets Nutripoult, Hi-Nutrient, Agrited, Daram vita-mix and Micro-mix, respectively,SEM-Standard Error of Mean, x-interaction

Study Seven

4.7.1: Lipid oxidation in egg-yolk of layers fed diets supplemented with five different proprietary vitamin-mineral premixes under two different housing systems in days of storage from 36 to 52 weeks of age

Lipid oxidation in egg-yolk of layers fed diets supplemented with five different PVmP under two different HS in DoS from 36 to 52 weeks of age is presented in Table 30. The HS caused variations ($p<0.05$) in lipid oxidation of egg-yolk. Lipid oxidation (mg/kg) in egg-yolk of layers in DL (0.034) was higher ($p<0.05$) compared with 0.028 in BC. Dietary PVmP influenced ($p<0.05$) lipid oxidation in egg-yolk of layers at the mid-laying phase. Lipid oxidation of egg-yolk of layers fed diet supplemented with Agrited (D4) (0.033) had the highest value; while those on Micro-mix (D6) was least (0.027). Egg-yolk lipid oxidation increased ($p<0.05$) in DoS from 0.021 in freshly laid eggs to 0.036 at day 28 of storage

Table 30: Lipid oxidation of egg-yolk of layers fed diets supplemented with five different proprietary vitamin-mineral premixes as affected by housing systems and duration of storage from 36 to 52 weeks of age

Factors	HS	PVmP	DoS
BC	0.028[b]		
DL	0.034[a]		
SEM	0.00021		
D2		0.028[d]	
D3		0.031[c]	
D4		0.033[a]	
D5		0.032[b]	
D6		0.027[e]	
SEM		0.000021	
0			0.021[e]
7			0.026[d]
14			0.033[c]
21			0.035[b]
28			0.036[a]
SEM			0.000021

[a-c] Means with different superscripts on the same column are significantly different ($p<0.05$). HS-Housing systems, PVmP- Proprietary vitamin-mineral premixes, DoS-Duration of egg storage, BC-Battery cage, DL-Deep litter, PVmP-Proprietary vitamin-mineral premixes, D2, D3, D4, D5 and D6-diets with Nutripoult, Hi-Nutrient, Agrited, Daram vita-mix and Micro-mix respectively, SEM-Standard error of means

4.7.2: Lipid oxidation of egg albumen and whole-egg of chickens fed diets supplemented with five proprietary vitamin-minerals premixes as affected by two housing systems and duration of storage from 53 to 70 weeks of age

The Lipid Oxidation (LO) of egg albumen and whole-egg of chickens fed diets supplemented with five different PVmP as affected by two housing systems and duration of storage from 53 to 70 weeks of age is presented in Table 31. The LO in albumen and whole-egg varied ($p<0.05$) with HS. Eggs produced by layers in DL had higher ($p<0.05$) 0.06 LO in albumen and 0.16 in whole-egg compared with 0.04 and 0.15 respectively in BC. Dietary PVmP supplementations impacted ($p<0.05$) on LO of albumen and whole-egg. Albumen of eggs from layers on diets supplemented with Micro-mix (D6) had the highest value of LO (0.056), while albumen of eggs from those fed diet supplemented with Nutripoult (D2) had the least (0.048). The highest LO in whole-egg was recorded in eggs from layers on diets with Hi-Nutrient (D3) (0.156) and Agrited (D4) (0.156), while LO of whole-egg produced by those on diets with Micro-mix (D6) was least (0.151). The LO of whole-egg produced by layers on diets with Nutripoult (D2) and Daram vita-mix (D5) were similar. The LO in albumen and whole-eggs increased ($p<0.05$) in DoS.

Table 31: Lipid oxidation of egg albumen and whole-egg of chickens fed diets supplemented with five proprietary vitamin-minerals premixes as affected by two housing systems and duration of storage from 53 to 70 weeks of age

	Egg albumen			Whole-egg		
Factors	HS	PVmP	DoS	HS	PVmP	DoS
BC	0.04[b]			0.15[b]		
DL	0.06[a]			0.16[a]		
SEM	0.00			0.00		
D2		0.048[e]			0.155[b]	
D3		0.051[c]			0.156[a]	
D4		0.049[d]			0.156[a]	
D5		0.054[b]			0.153[b]	
D6		0.056[a]			0.151[c]	
SEM		0.000			0.000	
0			0.008[e]			0.010[e]
7			0.019[d]			0.125[d]
14			0.051[c]			0.162[c]
21			0.078[b]			0.216[b]
28			0.102[a]			0.256[a]
SEM			0.000			0.000

[a-c]Means with different superscripts on the same column are significantly different ($p<0.05$). HS-Housing systems, PVmP- Proprietary vitamin-mineral premixes, DoS-Days of egg storage, BC-Battery cage, DL-Deep litter, D2, D3, D4, D5 and D6-diets with Nutripoult, Hi-Nutrient, Agrited, Daram vita-mix and Micro-mix respectively, SEM-Standard error of means

4.7.3: Regression of lipid oxidation of egg albumen, yolk and whole-egg with duration of storage at the late-laying phase

The regression of lipid oxidation on egg albumen, yolk and whole-egg of chickens fed diets supplemented with five different PVmP in HS in DoS at the late-laying phase are shown in equations 29, 30 and 31 and plotted graphs in Figure 13 below:

Albumen; $y = 3E\text{-}05x^2 + 0.0027x + 0.0052$ …… ($R^2 = 0.99$) ……..29

Yolk; $y = -2E\text{-}05x^2 + 0.0011x + 0.0205$ …… ($R^2 = 0.98$) …… ..30

Whole-egg; $y = -0.0002x^2 + 0.0138x + 0.0182$……..($R^2 = 0.98$) ……..31

Lipid oxidation was highest in whole-egg and least in yolkbut increased more rapidly in whole-egg and albumen than in egg-yolk as shown in Figure 13 below:

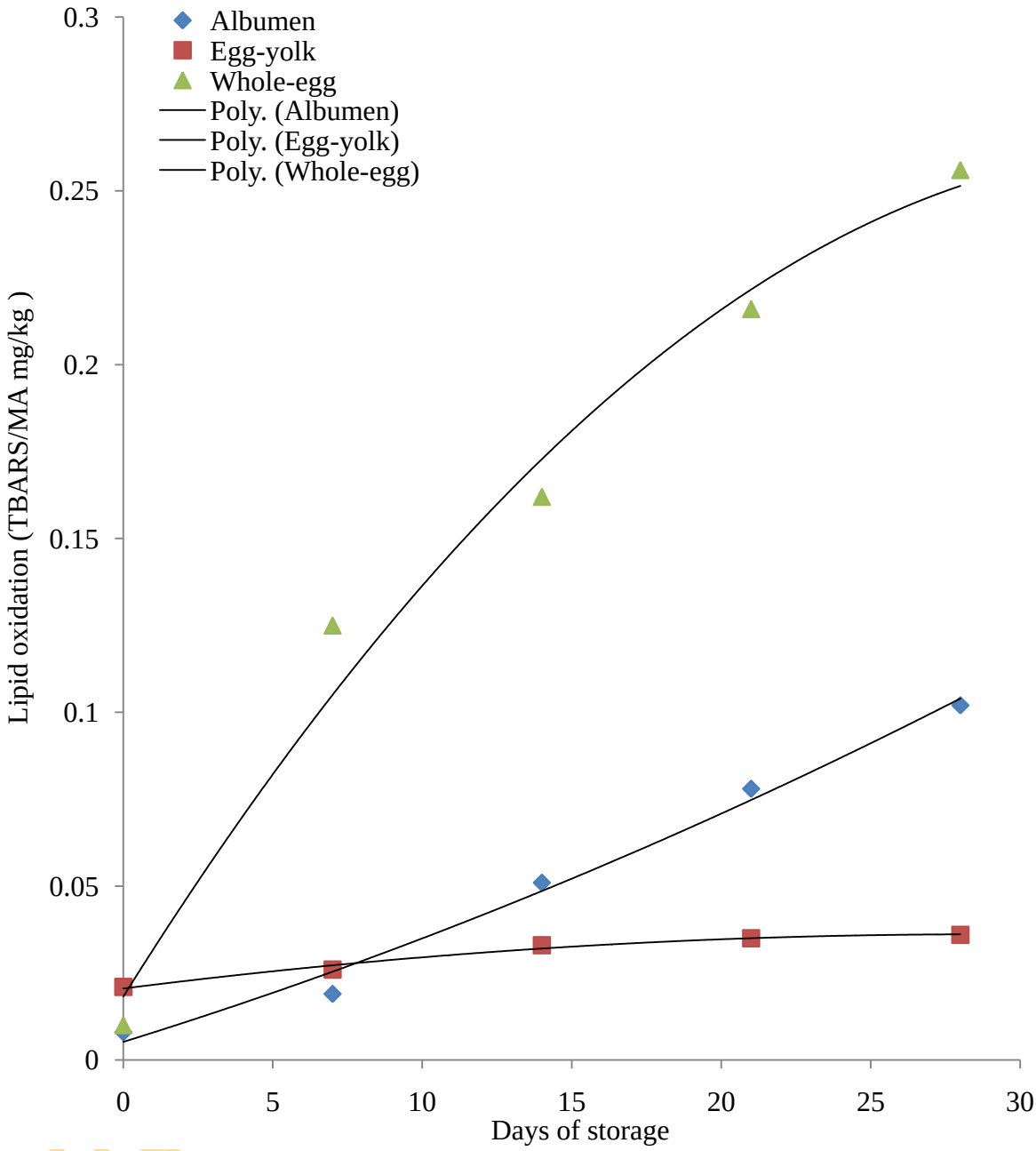

Figure 13: Regressionof lipid oxidation on albumen, yolk and whole-eggin days of storage

CHAPTER FIVE

5.0 DISCUSSION

Study One

Effects of two housing systems on performance characteristics of growing pullets from 13 to 16 weeks of age

Performance characteristics of growing pullets were indicated that daily feed intake (g/bird) and feed cost per live weight gain varied ($p<0.05$) with HS. Feed consumed (g/bird/day) in this period were 100.00±0.06 and 80.00±0.08 for pullets in BC and DL, respectively. Growing pullets in BC consumed feed more ($p<0.05$) than those in DL. Although, earlier study (Al-Rawi and Abu-Ashour, 1983) claimed that birds in DL consumed more ($p<0.05$) feed than those in BC, the finding in this study agrees with recent report (Bannga-Mboko *et al*., 2010) that feed consumption in BC (199.2 g/bird/day) was higher ($p<0.05$) than 155.7 in DL.

Natural habits of feeding on litter and feacal materials provided marginal nutrients to pullets in DL (Asaduzzaman *et al*., 2005; DEFRA, 2011). Pullets in BC were not deined such environment. Thus, feeding on litter and feacal materials and feed wasting by pullets in DL could possibly compensate for reduced feed intake despite similar feed efficiency in both HS. The comparative advantages of limited space per birds, higher ($p<0.05$) feed intake and feed cost per live weight and reduced feed wastage of birds in BC than in DL as reported (Pistikova *et al*., 2006; Voslářova *et al*., 2006; Bannga-Mboko *et al*., 2010) could be responsible for thse differences. The increase in feed intake by pullets in both HS was necessary because individual pullet adjust to physiological development and readiness for egg production. This was evident as pullets in both HS commenced egg production at approximately 122 days with those in BC staring earlier than those in DL. It was possible that the higher ($p<0.05$) feed intake of pullets in BC was utilised for egg production, hence production of heavier of first egg (32.00g) than 31.83g in DL.

There was no significant difference in feed conversion ratio of pullets in the two HS. This was contrary to the finding by Bannga-Mboko *et al*. (2010) that feed efficiency in BC (2.7) was better ($p<0.05$) than in DL (3.42). The live weight and weight gain of pullets were not influenced by HS. However, pullets in the two HS increased in weight in the course of study. This was evident by strong and positive relationship between live weight and age of pullets as indicated by regression values BC ($R^2 = 0.96$) and DL ($R^2 = 0.97$).

Relationships between liveweight and age of pullets in both HS were linear which explains positive growth in age. Pullets in DL increased in weight slightly more than those in BC contrary to earlier findings by Pistikova *et al*. (2006), Voslářova *et al*. (2006) and Banga-Mboko *et al*. (2010) that birds in BC achieve better feed efficiency and growth rate than in DL. There was difference ($p<.0.05$) in feed cost per live weight gain of pullet. The higher ($p<0.05$) feed cost per live weight in BC than DL recorded in this study disagrees with the report (Appleby, 2001) that birds in BC lower feed cost. There were no records of mortality in BC and DL which possibly implied that the two HS were safe for managing growing pullets. However, Voslářova *et al*. (2006) reported lower mortality and better performance in BC ($p<0.05$) than DL and recommended that DL meet animal welfare policy requirement.

Study Two

Effects of five different proprietary vitamin-mineral premixes and two housing systems on performance and egg production characteristics of pullets from 16 to 21 weeks of age

Feed consumption and utilisation are important factors that contribute to final live weight. The HS did not affect total feed consumption but influenced ($p<0.05$) daily feed intake of pullets. The relationship between DFI and age of pullets in BC and DL were positive and significant ($p<0.05$). Pullets in BC consumed more feed than those in DL. At early stage of egg producton, feed consumption was observed to increase with increase rate of egg production. The results were contrary to earlier findings (Hargreave, 1982; Al-Rawi and Abu-Ashour, 1983) but confirmed by other reports (Pistikova *et al*., 2006; Voslářova *et al*., 2006; Bannga-Mboko *et al*., 2010) that birds in BC utilised the advantages of spatial density to reduced feed wastage and grow better ($p<0.05$) than those in DL. The daily feed consumption of pullets in BC and DL increased ($p<0.05$) linearly ($R^2=0.82$ and $R^2=0.57$ respectively) with age which could be due to higher nutrients demand for maintenance requirement, metabolisable energy, body weight, growth, on-set of egg production; due to increase in size of ovary, oviducts, combs and nutrient storage for egg-yolk precursors in liver, particularly calcium phosphate in medullary bones, increase in size and number of eggs, chickens' activity and ambient temperature of the housing system (Singh and Panda, 1988).

Pullets utilised feed in BC and DL to meet requirements for maintenance, growth, development of eggs forming organs and early egg production. Thus, the increased daily feed intake of pullets in DL could mean demand for more nutrients to meet requtrement for maintenance, growth and exercise activity arising from higher floor space allowance per pullet. However, pullets fed on litter and faecal materials to obtain exogenous nutrients such as vitamins and minerals to partly meet these requirements. The efficiency of feed utilization indicated that pullets were not meat-type and effect of HS on feed convsersion ratio was not significant ($p>0.05$). Thus, variations in vitamin and trace minerals content of dietary PVmP supplemented did not cause significant difference ($p>0.05$) in total daily feed consumptions and feed conversion ratio. It could therefore be deduced that the amount and quality of vitamins and trace minerals in test

ingredients (supplemental PVmP) were adequate to met requirement for growth and initiation of egg production. In addition, some vitamins such as thiamine (B_1), riboflavin B_2), pyridoxine (B_6) and niacin which stimulate appetite were probably synthesized by gut microbes in adequate amount to meet requirement for growth and preparation for egg production despite competition with dietary source or sparing effect and nutrients synergetic relationship. The findings in this study agree with explanation provided by Oduguwa (1991) that chickens are better stabilised when riboflavin (B_2), pyridoxine (B_6) and niacin in combination with thiamine are adequately supplied in diets. The differences in vitamins and trace minerals in PVmP did not cause adverse effect on feed consumption and utilisation by pullets. The interaction of supplemental PVmP and HS did not affect ($p>0.05$) total and daily feed consumptions of pullets.

At week 21, HS affected ($p<0.05$) final live weight of pullets. The final live weight of pullets in DL was higher ($p<0.05$) compared with those in BC. Pullets in the two HS grew in the period of study. Pullets in DL increased in daily live weight more than those in BC. This was indicated by similar and strong positive linear relationship ($R^2= 0.98$) between live weight and age of pullets in both HS. The higher ($p<0.05$) final live weight of pullets in DL could be due to higher ($p<0.05$) feed consumption among other factors. This shows that feed was properly utilised for growth and in preparation for egg production. Pullets in DL had access to richer environment that provided extra nutrients through feeding on litter and feacal materials as well as more floor space requirement. The possibility of pullets in DL obtaining extra natural feed materials rich in protein, amino acids, vitamins and minerals could not be ruled out. This probably explains the reason for higher ($p<0.05$) final live weight attained by pullets in DL compared with those in BC. Supplementation of diets with PVmP did not affect daily live weight while final live weight of pullets varied ($p<0.05$).

The main effect of PVmP on final live weight was significant ($p<0.05$). Pullets fed diets supplemented with PVmP grew differently in the period of study. Higher ($p<0.05$) feed intake of pullets on diets supplemented with Nutripoult could be due to balanced vitamin and trace mineral profile. The reduction in final live weight of pullets fed diets supplemented with Hi-Nutrient, Agrited, Daram-vita and Micro-mix could be due to relatively imbalanced, lower and/or excess of some vitamins and minerals. Low or

excess of thiamine, biotin, cyanocobalamin (B_{12}), folic acid and zinc have been reported by Ogunmodede (1974; 1978; 1982; 1991) to impair utilisation of carbohydrate, fat and proteins. The final live weight of pullets fed diets supplemented with Hi-Nutrient, Agrited, Daram vita-mix and Micro-mix were similar to those on diets without PVmP. Imbalanced dietary vitamins and minerals are often implicated in complicity arising from nutrient toxicity and/or antagonism. The combine effect of PVmP and HS affected ($p<0.05$) live weight of pullets.

The HS influenced ($p<0.05$) number of eggs produced (EP) and hen day egg production (HDEP) of pullets. Pullets in BC consumed lower feed but produced more eggs compared with those in DL. At week 16 to 21 weeks of egg-lay, pullets in BC increased rapidly in egg production more than in DL. This shows that feed consumption of pullets in BC and DL was more properly channelled to egg production than maintenance and growth. These results were in agreement with reports by Al-Rawi and Abu-Ashour (1983), Anderson and Adams (1994), Abrahamsson *et al*. (1996), Pistikova *et al*. (2006), Voslářova *et al*. (2006) and Bannga-Mboko *et al*. (2010) indicated that layers in BC had comparative advantages of spatial density; controlled micro-climate; less contact with feacal materials as a source of disease infection, better health condition and reduced feed wastage to produced more egg than in DL. The findings in this study corroborates reports by Voslářova *et al*. (2006) and Bannga-Mboko *et al*. (2010) that layers in BC produced higher number of eggs and improved number of egg (+55%), laying capacity (+25.5%) and feed efficiency (2.7 versus 3.42) than in DL.

The HDEP of pullets was affected ($p<0.05$) by PVmP supplementation. The difference in composition of vitamin and trace minerals in diets supplemented with PVmP possibly explains the variation in HDEP. Pullets fed diets supplemented with different PVmP had higher HDEP. At about week 17, pullets fed diets with and without PVmP supplementation were in lay irrespective of the differences in vitamin and trace minerals in the diets. Pullets fed diets supplemented with PVmP had higher ($p<0.05$) HDEP than those on diet without PVmP. The difference in HDEP was due to supply of additional vitamins and trace minerals suuplied by the tested PVmP in the diets. Also, higher HDEP of pullets fed Daram vita mix could be due to relatively more balanced vitamins

and trace minerals profile compared with lower HDEP values of those on diets supplemented with Nutripoult, Hi-Nutrient, Agrited, and Micro-mix. Thus, diets supplemented with PVmP and interaction effects with HS improved ($p<0.05$) HDEP.

Study Three

Effects of five different proprietary vitamin-mineral premixes and two housing systems on performance and hen day egg production of laying chickens (22 to 70 weeks of age)

The ambient temperatures (^{o}C) and relative humidity (%) in BC (28.5±1.6and 68.2±13.7) and DL (28.3±1.7 and 73.1±13.5) were higer than thermoneutral zones (18-22 ^{o}C) documented for poultry (Charles, 2002; USDC-ESSA, 1970 as modified by Tao and Xin, 2003). Higher ambient temperature and relative humidity have implication on nutrients requirement for growth and egg production. Nutrient requirement for maintenance and productive functions varies with changes in ambient conditions. The daily variations in ambient temperature and relative humidity had noticeable effect on rates of daily feed consumption and egg production. The rates of daily feed consumption were affected ($p<0.05$) by HS. Earlier reports (Ajakaiye *et al*., 2011) indicated that reduction in feed and dry matter intake of chickens under high ambient conditions caused decline of egg production.

The high ambient tempartures and relative humidity probably affected production to cause reduction in hen day egg production. Reports of earlier study (Hughes *et al*., 1985) showed that high ambient temperature imposed heat stress on layers and cause reduction hen day egg production and quality. This observation agree with reports by Sahin and Kucuk (2001), Balmvave (2004), Robert (2004), Ciftci *et al*. (2005) Karaman *et al*. (2007), Daghir (2009) and Ajakaiye *et al*. (2011) that layers managed in housing system maintained at ambient temperature outside thermoneutral zones (18-22 ^{o}C) declined in egg production drastically because of the presence of feather covering and lack of sweat gland which made heat dissipation very difficult. Also, high ambient temperature higher than thermoneutral zones (18-22 ^{o}C) create heat stress on ovarian activities thereby causing differential ovarian blood flow pattern to leading to reduction in production of eggs. Hence, reported study of Oguntunji and Alabi (2010) indicated that fluctuation in egg production pattern was due to combined effect of high alternating day and night ambient temperatures and humidity which stimulate higher level of corticosteroids from hypothalamus. Therefore, the high level concentration

corticosteroids could be responsible for the negative influence onoviposition and cause decline in egg production.

At early- (22 to 35 weeks), mid- (36 to 52) and late- (53 to 70) laying phases, layers in DL had higher ($p<0.05$) daily feed intake than those in BC but there were differences ($p<0.05$) in daily feed consumption among layers fed diets supplemented with PVmP. The interaction of PVmP and HS affected ($p<0.05$) rate of daily feed consumption and efficiency of feed utilisation. This observation was contrary to reports of Bannga-Mboko *et al*. (2010) that feed consumption and feed efficiency of birds in BC was higher ($p<0.05$) than those in DL. The feed consumption per egg mass of layers in BC was lower ($p<0.05$) compared with those in DL which implied better feed efficiency for egg production rather than growth. Layers in DL consumed feed on daily basis to meet nutrients requirement for more active movement and exercise because of allowed higher floor space. The higher daily feed consumption by layers in DL could probably be due to feed wastage. This finding coborrates authors reports of Pistikova *et al*. (2006), Voslářova *et al*. (2006) and Bannga-Mboko *et al*. (2010) that birds in BC utilised advantages of spatial density to reduced feed wastage and perform better than those in DL.

The label compositions of five different PVmPs contained varying amount of vitamins and trace minerals. Micro-mix had relatively higher levels vitamins and minerals profiles compared with other PVmPs. Adequate dietary levels of vitamins and minerals enhance appetite but when in excess could cause toxicity or nutrient antagonism. Daily feed consumption of chickens fed diets supplemented with Nutripoult, Hi-Nutrient, Agrited and Daram vita-mix were higher due to enhanced appetite by vitamins and minerals compared with those on diets without PVmP. This finding perhaps indicates importance of PVmP supplementation in enhancing and stimulation of birds' appetite to increase feed consumption and egg production. However, layers on diets supplemented with Micro mix recorded lower daily feed intake probably due to reduced appetite as a result of imbalance vitamins and mineral profile.

The live weight (LW) of layers varied ($p<0.05$) with HS, dietary PVmP supplementation and its interactions with HS. Layers in DL was higher ($p<0.05$) in live weight than those in BC. Efficiency of feed utilisation was determined in term of feed conversion ratio per egg mass. Feed conversion ratio per egg mass of layers fed diets without PVmP was better than those fed dietary PVmP supplementation. The implication of thses findings is that HS and PVmP supplememtation in the diets caused differences weight, efficiency of feed utilization for unit mass of egg produced. Layers fed diets with PVmP supplementation possibly utilized vitamins such as thiamine (B_1), riboflavin B_2), pyridoxine (B_6) and niacin which stimulate appetite leading to higher feed intake, egg weigt and mass and feed conversion ratio per egg mass. Variations in daily feed consumption and efficiency of utilisation of egg production could be due to differences of the different HS and PVmP compositions.

The importance of PVmP supplementation in diets of layers was underscored by the results obtained in this study. Number of eggs produced, egg weight, egg mass and hen day egg production varied ($p<0.05$) with HS, PVmP supplementation and PVmP interactions with HS. Layers in DL was higher ($p<0.05$) in number of egg produced, egg mass and hen day egg production than in BC. This finding is contrary to reports of Bannga-Mboko *et al*. (2010) that BC improved ($p<0.05$) number of egg, hen day egg production, egg mass and egg weight than DL. Layers in DL probably obtained extra nutreints by feeding on litter and faecal materials for maintenance, growth and egg production than those in BC.

Hen day egg production of layers varied ($p<0.05$) with HS. The hen day egg production of layers in DL was higher ($p<0.05$) than in BC. Variations in egg production in the two HS could be due to higher ($p<0.05$) feed intake and richer nutrient environment birds feed on litter and faecal materials as well as the comfort of egg-laying on soft litter materials in nesting boxes in DL. At late-laying phase (53 to 70 weeks), egg production declined as birds approach the end of first laying cycle. The rate of decline of egg production in BC was quite higher ($p<0.05$) than in DL. This observation was in agreement with earlier reports of Anderson and Adams (1994) that layers in BC always produce lesser number of eggs and heavier eggs at end of egg production cycle than in

DL. The hen day egg production of layers in BC was relatively higher than in DL at week 22 and increased steadily in DL from week 22 more than those in BC at week 23.

The hen day egg production of layers in DL remained higher until week 33. The different PVmP compositions influenced ($p<0.05$) hen day egg production. Higher ($p<0.05$) live weight, number of egg produced and hen day egg production of layers on diets supplemented with Nutripoult (D2) probably indicates adequacy of vitamin and trace minerals supply in diets. The layers on diets without supplemental PVmP (D1) probably utilised feed for growth than egg production compared with those on diets with PVmP supplementation. Layers on diets without PVmP (D1) decresed in hen day egg production from week 23, and later dive-nosed to zero value at week 34, while those on diets supplemnetd with PVmP increased steadily in egg production. This finding is consistent with earlier reports of Singh and Panada (1988) that any marked deficiency of one or more of vitamins and trace minerals caused reduction or cessation of growth and/or egg production in layers.

Research reports of Sahin and Kucuk (2001) and Ciftci *et al*. (2005) indicated that differences in dietary vitamins and minerals and the degree in which micro-nutrients mitigate heat stress were responsible for variations in egg production. Thus, in agreement with the findings of Mori *et al.* (2003), Çiftçi *et al*, (2005) and Seven (2008), vitamin and mineral profile of PVmPs probably maintained synergetic relationship in thermoregulatory control of physiological processes in layers under heat stress to impacted differences in hen day egg production. The differences in antioxidants profile of PVmPs could participate in supply of egg precursors in plasmato reduce ACTH concentration and decrease blood carbon dioxide (CO_2) to cause variations in hen day egg production (Kevin, 1982; Koelkebeck, 1999; Ny *et al*., 1999)

Study Four

Effects of five different proprietary vitamin-mineral premixes, two housing systems and duration of storage on external and internal quality indices of eggs

The egg length (EL) of eggs from layers in DL was higher than those from BC which supported earlier reports of Silversides and Scott, (2001), Wang *et al*. (2009) and Ojedapo (2013) that egg weight (Ew), EL, diameter (EB), shell weight (EW) and shell thickness (ET), yolk weight (YW) and colour (YC), albumen weight (AW) and heght (AH) were better in eggs produced in DL than in BC. Eggshell was thicker for eggs from BC than in DL. This finding is contrary to the report of Ojedapo (2013). External [EW, EB and eggshell index (EI)] and internal (albumen and yolk) quality indices of eggs varied ($p<0.05$) with different dietary PVmP. The YW, YB and YC increased ($p<0.05$) with PVmP, while AW, HU and YH and YI decreased ($p<0.05$) in accordance with reported studies of Silversides (1994), Monria *et al*. (2003) and Silversides and Budgell (2004).

Egg quality indices of layers were affected by quality and composition of feed according to reported studies (Van den Brand *et al*., 2004; Jones, 2006; Pavlovski *et al*., 2012). Layers on diets without PVmP (D1) probably shut down all biochemical processes necessary for egg formation and other body metabolism at week 34 due to insufficient supply or lack of vitamin and minerals. The compositions of vitamin and trace mineral profile in PVmP were different which explained the variations in egg quality indices. Whitehead (1996) reported variations in number of egg produced and egg quality of layers when given varied vitamin D3. This could be due to synergistic relationships of vitamin D3 with other nurients. Also, the difference in vitamin E and its synergetic relationship with other nutrients could probably increase ($p<0.05$) EP, EW and ET when layers were under heat stress (Çiftçi *et al.*, 2005). The EI and ET of eggs were not different from the values obtained for eggs produced by those on diets without PVmP as reported also by Qi and Sim (1998).

The variation in amount of microminerals in PVmP possibly accounted for the differences in quality indices of eggs since vitamins and trace minerals serves as co-enzymes in shell formation and associated membranes as reported by Mas and Arola

(1985) and Miles (2001). Trace minerals, particularly zinc, copper, iron, selenium and manganese in PVmP are key components of shell matrix and shell integrity. Reported study by Zamani *et al*. (2005) indicated that poor quality indices of eggs were due to variation in dietary supply of micronutrients. The deficiency of dietary copper affect biochemical and mechanical properties of eggshell membrane resulting in deformation of egg shape, while dietary selenium supplementation up to 0.8mg/kg caused no negative impact on eggshell quality indices as reported by reported Chowdury (1990).

Housing conditions in the two HS affected egg production and quality indices. The EW, ET and AH of eggs of layers in BC were higher ($p<0.05$) than those from DL which was contrary to reported studies by Silversides and Scott (2001), Wang *et al*. (2009) and Ojedapo (2013). Variations in productive performance of layers on diets supplemented with PVmPs could be due to difference in micronutrient profile of different PVmPs. The findings of Roland (2000) and Zamani *et al*. (2005) indicated that deficiency or excess of micronutrients in diets of layers impair efficiency of egg production leading to production of poor egg quality. However, earlier reported study by Kershavarz and Nakajima (1993) showed that excess dietary micronutrients such as calcium, phosphorous, zinc and managanese above their requirements did not improve shell quality. In similar reported studies by Taylor (1965), Boorman *et al.* (1989), Kershavarz and Austic (1990), Nys (1995), and Pavlovski *et al*. (2012) imbalanced dietary micronutrients such as phosphorous was reported to cause heat stress which inhibited calcium mobilization with attendant poor bones development and eggshell breakage. Also, the reports of Mas and Arola (1985) and Miles (2001) showed that dietary zinc, copper, iron and manganese play crucial role as co-enzymes in metabolic reactions, shell matrix and membranes formation and eggshell integrity.

There were no effects of HS on EW and ET contrary to reported studies of Jin and Craig (1994), Pavlovski *et al*. (2001) and Hidalgo *et al*. (2008). However, present study indicates difference ($p<0.05$) in egg diameter of layers in the two HS. This was consistent with reports of studies by Mohan *et al*. (1991), Anderson and Adams (1994) and Abrahamsson *et al*. (1996). In this study, external quality indices of eggs from layers in BC were better contrary to the findings of Hughes *et al*. (1985), Pavlovski *et*

al. (2001), Hidalgo *et al*. (2008) and Ojedapo (2013). Reported studies by Morris (1985) and Keshavarz and Nakajima (1995) indicated that differences in external quality indices of eggs from layers in HS were due to production practices and physiological stress. The high ambient temperature and relative humidity and competition among layers for dust bathing was reported by Hughes *et al*. (1985) and Short (2001) to increase stress in DL. Also, reported studies by Okoli *et al.* (2006) and Oguntunji and Alabi (2010) showed that higher ambient temperatures outside thermonuetral zone reduced voluntary feed intake and availability of micronutrients for shell deposition and adversely affect oviposition and oviposition interval leading to reduction in egg production and weak eggshell.

The findings in late-laying (53 to 70 weeks) was contrary to reports of Silversides and Scott (2001) and Wang *et al*. (2009) that internal quality indices of eggs from layers in DL were better than eggs from BC. Thus, variations and utilisation of micronutrients in PVmPs critically affected internal quality indices of eggs. Reported studies by Williams (1992), Franchini *et al*. (2002), Kirunda *et al*. (2001), Puthpongsiriporn *et al*. (2001) and Ajakaiye *et al.* (2011) showed that albumen quality (Haugh Unit) was not greatly influenced by variation in dietary nutrients. The duration of egg storage (DoS) affected ($p<0.05$) external and internal quality characteristics of eggs. The Ew, EW, ET and egg weight loss (EwL) change with DoS in agreement with findings of Jin *et al*. (2011). The Ew decreased with DoS due of loss of egg moisture through shell pores in agreement with the reports by Brake *et al*. (1997). The decrease in Ew with DoS agreed with reported studies by ACIAR (1998) and Samli *et al*. (2005) that eggs reduced drasticaly in weight within 10 days of storage at 29^{o}C.

The EI, EL, EB and ET were not affected by DoS in agreement with reported studies of Hamilton (1982), Tilki and Inal (2004) and Alade *et al*. (2009). In the reported studies of Dudusola (2009) and Alsobayel and Albadry (2011), decrease in Ew with DoS was due to metabolic process leading to loss of moisture, carbon dioxide, ammonia, nitrogen and hydrogen sulphide gases. The decrease in AH and HU with DoS occurred more quickly at higher ambient temperaturein as reported by Li-Chan and Nakai (1989) and Dudusola (2009). At ambient temperatures and relative humidity lower than 70%,

Natalie (2009) indicated that stored eggs reduced in HU by 10–15 after few days of storage but increased to 30 HU at 35 days of storage, while AH of eggs stored for 10 days decreased from 9.16-4.75 mm (Scott and Silversides, 2000; Ihsan, 2012). Albumin index of eggs were influenced ($p<0.05$) by DoS. The increase in albumen pH during egg storage could be due to changes in ovomucin (thick albumen) (Kato *et al.*, 1994; Toussant and Latshaw, 1999). Reported study of Okeudo *et al.* (2003) indicated that loss of carbon dioxide (CO_2) through shell pores made albumen more alkaline, transparent and increasingly watery. At higher temperatures of egg storage, loss of carbon dioxide (CO_2) could be faster with increased deterioration of albumin quality (Natalie, 2009). The changes in YW, YD and YH with DoS agreed with reported studies by Fromm and Matrone (1962), Okoli and Udedibe (2003) and Jones (2006) that protein structures of thick albumen and vitelline membrane degenerates faster while water from albumen moves into yolk resulting in enlarged and decreased yolk viscosity so that yolk become flattened and breakdown with increase in internal temperature of eggs.

These changes could account for reduction in YH and YI and increased YW and YB. The YI indicates spherical nature of egg-yolk which decreases progressively when vitelline membranes become weakened and cause liquefaction of egg-yolk due to osmotic diffusion of water from albumen. The YI decreased with increased moisture content in agreement with reported study by Hidalgo *et al.* (1996). There were observed variations in external and internal quality indices of eggs with days of storage. These variations are explained by the regression of EW on DoS at the mid- ($R^2 = 0.85$) and late- ($R^2 = 0.99$) laying phases. The rates of quality deterioration of albumen and egg yolk were similar but proceeded relatively faster in egg yolk than albumen.

Study Five

Effect of supplementing laying chicken feed with five different proprietary vitamin-mineral premixes, two housing systems and duration of storage on chemical compositions of eggs

Eggs produced by layers in BC contained more energy, proteins, fat, ash, calcium and total phosphorous than eggs from DL.These results corroborates reported study of Matt *et al*. (2009) that eggs produced by birds in BC were richer in nutrients than eggs from DL. However, reports of Menill *et al*. (2007) and Matt *et al*. (2009) indicated that organic eggs contained more proteins and carbohydrate than eggs produced in BC. Also, reported study of Menill *et al*. (2007) showed that orgnic eggs were higher in dry matter compared with from those BC. The reason for lower chemical components of eggs produced by layers in DL at early- (22 to 35), mid- (36 to 52) and late- (53 to 70 weeks) laying phases could be due to nutrients partitioning between maitainenace for active movement and exercise.

The chemical composition of eggs was affected by PVmP, which could be due to the varying efficacy of the PVmP. Vitamins and trace minerals are required for different biological process, particularly as co-enzymes in metabolism of carbohydrate, fat and protein, production of eggs shell, albumen and yolk-forming materials in liver and ovaries (Etches, 1996). Crude protein in eggs produced by layers at early laying phase and stored was higher ($p<0.05$) than at late laying phase. The crude protein of eggs during storage at early laying phase remained fairly conatant ($R^2 = 0.82$) but increased more at late laying phase ($R^2 = 0.99$). Fat in stored eggs at late laying phase was higher ($p<0.05$) compared with early laying phase. At early laying phase, fat content in stored eggs was lower ($R^2 = 0.30$) than at the late laying phase ($R^2 = 0.95$).

Study Six

Effects of five different proprietary vitamin-mineral premixes and two housing systems on cholesterol profile of chicken eggs

The cholesterol profile of whole-egg and egg yolk of eggs produced by layers fed different PVmP revealed that HS did not affect triglyceride (TG), total cholesterol (TC), High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL) and Very Low Density Lipoprotein (VLDL) of whole-egg. However, TG and LDL of egg-yolk varied ($p<0.05$) with HS. Eggs produced by layers in BC were higher ($p<0.05$) in TG but lower in LDL than eggs from DL. The TG, TC, HDL, LDL and VLDL of both whole-eggs and egg-yolk varied ($p<0.05$) with different dietary PVmP supplementations. The interaction effects of PVmP supplementation and HS influenced ($p<0.05$) TG and cholesterol profile of whole-egg and egg-yolk. These findings agreed with reported studies of Lopez-Bote *et al*. (1998), Rizzi *et al.* (2006), Rossi (2007), Stefano *et al.* (2008), Józefa *et al.* (2011) and Kamil *et al.* (2012). Eggs produced by layers in DL contained two-thirds amount of cholesterol of those in BC. Also, the finding in this study agreed with report of Zemkovia *et al.* (2007) that HS influenced yolk cholesterol but contrary to the findings by Rizzi *et al.* (2006), Rossi (2007) and Kamil *et al*. (2012).

Lower egg-yolk LDL was observed in eggs produced by layers from BC compared with those in DL. This finding agrees with the reports by Zemkovia *et al.* (2007) and Minelli *et al.* (2007). The TG of egg yolk of layers in BC was higher than those from DL. This finding was contrary to the reports of Cherian *et al.* (2009) that there was no clear effect of HS on lipid composition of egg-yolk. The difference ($p<0.05$) in TG, TC and cholesterol profile of whole-egg and egg-yolk was a reflection of differences in amount of vitamins and trace mineral content of dietary PVmP supplementation. Vitamins and minerals serves primarily as an antioxidant in stabilizing lipid component in poultry by reducing lipid peroxidation leadfing to increase in egg production and quality (Gutteridge, 1995; Vicenzi, 1996; Meluzzi *et al.*, 2000; Leeson and Summers, 2001; Surai, 2003, Mabe *et al*., 2003; Franco and Sakamoto, 2005; Fernandez e*t al*,. 2011).

Vitamins like thiamine, riboflavin pyridoxine, folic acid and niacin stimulate appetite and increase consumption of feed. The higher feed consumption of layers in DL and those fed PVmP supplementation possibly explains the differences in cholesterol profile of eggs. This finding agrees with reports by Vargas and Naber (1984) that egg-yolk cholesterol correlates positively with dietary energy balance because excess dietary energy consumption beyond maintenance and production requirements increased body weight and cholesterol synthesis such that excess cholesterol is transferred and stored in egg-yolk. Conversly, Quirino *et al*, (2009) explained that dietary energy had no effect on egg-yolk cholesterol and fatty acid profile. On the other hand, Hassan *et al*. (2013) indicated that saturated fatty acid decreased with increase in unsaturated fatty acid of egg-yolk with increase dietary metabolisable energy and decreased crude protein. The differential feed consumption implied variation in dietary energy with consequential correlation on egg-yolk cholesterol content.

Study Seven

Effect of five different proprietary vitamin-mineral premixes, two housing systems and duration of storage on lipid oxidation of eggs

At mid-laying phase, egg-yolk TBARS varied ($p<0.05$) with HS and PVmP and DoS interaction effects. The egg-yolk TBARS of layers in BC was higher ($p<0.05$) than those from DL. The egg-yolk TBARS increased ($p<0.05$) linearly ($R^2 = 0.98$) with DoS. Also, at late- (53 to 70 weeks) laying phase, TBARS in albumen and whole-eggs varied ($p<0.05$) with HS and PVmP supplementation. The TBARS increased ($p<0.05$) linearly with DoS in both the albumen ($R^2 = 0.99$) and in whole-eggs ($R^2 = 0.98$) at the late-laying phase. The TBARS increased ($p<0.05$) linearly with DoS at the late-laying phase. The differences in TBARS in egg-yolk, albumen and whole-eggs could be due to different HS and levels of potency of the vitamin and trace mineralin the different supplemental PVmP.

Higher TBARS in whole-egg implied greater degree of lipid oxidation content in egg-yolk. This finding agree with reported studies by Hamilton (1982), Tilki and Inal (2004), Alade *et al*. (2009) and Tebesi *et al.* (2012) that egg quality was affected by storage time. Also, Bou *et al*. (2006) observed that longer periods of supplementation of α-tocopherol decreased lipid hydro-peroxides and lowered TBARS in stored eggs. In this study, dietary supplementation of PVmP caused variations ($p<0.05$) in egg-yolk TBARS. ThUS, higher TBARS in whole-egg implied greater degree of lipid oxidation in egg-yolk. Reported studies of McDowell (1989), Halliwell and Gutteridge (1989), Morrissey *et al*. (1997), Botsoglou *et al*. (2005), Grau *et al*. (2001), Galobart *et al*. (2002) and Bou *et al*. (2006) revealed that dietary vitamin E in syerngistic relation with vitamins C and selenium function as chain-breaking antioxidants in lipid oxidation phases of cellular membrane or low density lipoproteins to reduce ($p<0.05$) TBARS. Lipid oxidation was higher in whole-egg than egg yolk but increased rapidly in whole-egg and albumen than egg yolk.

CHAPTER SIX

6.0: SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1: Summary

Seven studies were carried out to investigate effects of five proprietary vitamin-minreal premixes (PVmP) and two housing systems (HS) on performance, egg production and egg quality indices of laying chickens. Bovan Nera (n=576) pullets at week 13 were divided equally into 288 per HS and used for the study. The two HS were conventional 3-tier Battery Cage (BC) and Deep Litter (DL) systems. The five different PVmPs (growers and layers premixes): Nutripoult, Hi-Nutrient, Agrited, Daram vita-mix and Micro-mix and designated K, L, M, N and P respectively were common brands of premixes used for formulating poultry diets in different tolls of feed milling in Ibadan. The compositions of vitamins and trace minerals in five different PVmP as indicated on respective the labels and two HS were not the same and constituted souces of variation. The findings of the study revealed that;

- Ambient temperature (^{o}C) and relative (%) ranged from 26.5 ± 0.1 to 31.9 ±1.1 and 40.6 ±1.0 to 90.5±8.7 respectively and were above thermoneutrality for laying chickens.
- Layers attained peak-lay at different periods during production irrespective of HS and PVmP type.
- The hen day egg production (HDEP) (%) in BC (64.1 ± 26.4) and DL (82.0 ± 13.8) at peak-lay reduced to 52.1 ± 11.4 and 57.8 ± 14.1 respectively in late-lay (52 to 70 weeks).
- The HDEP of layers fed diets without PVmP at peak-lay declined from 56.1±9.6 to zero at week 34.
- At week 34, HDEP of layers fed diets supplemented with Nutripoult (76.65) and Agrited (76.60) were higher ($p<0.05$) than 68.45, 68.59 and 67.72 on diet with Hi-Nutrient, Daram vita-mix and Micro-mix respectively.
- At week 36, crude protein (%) of eggs from layers on diets supplemented with Nutripoult (11.6 ± 0.17), Hi-Nutrient (11.55 ± 0.23), Daram vita-mix (11.55 ± 0.23) and Micro-mix (11.6 ± 0.23) were higher than those on diets with Agrited (11.4±0.17).

- Low density lipoprotein (mg/dL) and Lipid oxidation (µmol/g) in eggs from layers on DL (2.13 ± 1.63 and 0.04 ± 0.01 respectively) were higher ($p<0.05$) than 0.74 ± 0.15 and 0.028 ± 0.01 respectively in BC.
- At zero duration of storage, Lipid oxidation (µmol/g) of egg from layers on Nutripoult (0.028 ± 0.009), Hi-Nutrient (0.031 ± 0.008), Agrited (0.033 ± 0.008), Daram vita-mix (0.032 ± 0.008) and Micro-mix (0.027 ± 0.010) were different and increased ($p<0.05$) linearly with duration of egg storage.
- The eggshell weight and thickness of eggs from BC (5.89 ± 0.60 and 0.35 ± 0.03) were higher ($p<0.05$) than 5.58 ± 0.48 and 0.34 ± 0.03 respectively in DL.
- Eggs from BC (48.7 ± 24.6) had higher haugh unit than DL (44.8 ± 25.2).
- The haugh unit of egg from layers on Daram vita-mix (48.6 ± 25.2) and Micro-mix (48.0 ± 25.0) were higher ($p<0.05$) than Nutripoult (46.1 ± 26.8), Hi-Nutrient (46.1 ± 23.8) and Agrited (44.8 ± 25.1), and haugh unit decreased ($p<0.05$) with duration of egg storage ($R^2 = 0.98$).

6.2: Conclusion

Empirical findings from this study revealed that laying chickens managed on deep litter produced more eggs than battery cage system from weeks 22 to 70. Diet without PVmP supplementation made laying chickens to attain early peak of egg production at week 25 which subsequently declined to zero at week 34. Diets with PVmP supplementations sustained increased egg production to peak at different weeks. Housing systems and the type of dietary PVmP both affected composition and egg quality characteristics in duration of egg storage. Nutripoult and Micro-mix Micro-mix would be preferred in both HS as they both tend to ensure good albumen height, Haugh unit, yolk height, yolk index, higher shell thickness and lowered weight loss. Quality of eggs was observed to decrease when stored at room temperature. The lipid indices and duration of storage of eggs from both HS were affected by the different dietary PVmP. Also, the interactions of the dietary PVmP and HS as well as duration of egg storage profoundly affected the lipid composition of eggs. Eggs qualities deteriorated below desirable grade before day 7 of storage at room temperature. Micro-mix reduced egg lipid oxidation, while interaction effects of Hi-Nutrient and Daram vita-mix with both housing systems enhanced bird laying capability.

6.3: Recommendations

- Further studies on quality and potentcy of chemical profile of proprietary vitamin-mineral premixes must be undertaken regularly to ensure standards
- Strict compliance of industry standards by different proprietors of vitamin-mineral premixes should be enforced by regulatory agencies and professional bodies such as National Agency for Food and Drug Administration and Control (NAFDAC), Standard Organisation of Nigeria (SON), Nigeria Institute of Animal Science (NIAS) and Poultry Assocaiation of Nigeria (PAN), Animal Science Association of Nigeria (ASAN), Nigerian Society for Animal Production (NSAP).
- Public education and awearness programme should be mounted to provide information on nutritional benefits of egg.
- Alternative methods should be considered for storing excess eggs produced to enhance retention of freshness.

REFERENCES

Abanikannda, O. T. F., Olutogun, O., Leigh, A. O. and Ajayi, L. A. (2007). Statistical modeling of egg weight and egg dimensions in commercial layers. .*International Journal of Poultry Science*, 6 (1):59-63

Abrahamsson, P. and Tauson, R. (1995).Aviary systems and conventional cages for laying hens. *Acta Agricultural Scandenviana Animal Science*, 45:191–203

Abrahamsson, P., Tauson, R. and Elwinger, K. (1996). Effects on production, health and egg quality of varying prop ortions of wheat and barley in diets for two hybrids of laying hens kept in different housing systems. *Acta Agricultural Scandenviana Animal Science*, 46:173–182

Adeogun, I. O. and Amole, F. O. (2004). Some Quality Parameters of Exotic Chicken eggs Under Different Storage Conditions. *Bulletin for Animal Health and Production in Africa (Kenya),* 52 (1):43 – 47

Ahmadi, F. and Rahimi, F. (2011). Factors affecting quality and quantity of egg production in laying hens: A Review.*World Applied Science*, 12(3): 72-384

Ajakaiye, J. J., Perez-Bello, A. and Mollineda-Trujillo, A. (2011). Impact of heat stress on egg quality in layer hens supplemented with l-ascorbic acid and dl-heat stress on egg quality in layer hens supplemented with l-ascorbic acid and dl-tocopherol acetate tocopherol acetate. *Veterinary Arhive*, 81, 119-132

Alade, F. A., Uzeh, R. E. and Bankole, M. (2009).Quality of eggs under varying storage periods, condition and seasons in semi-arid regions of Nigeria.*International Journal of Basic Applied and Innovative Research*, 8(2):21-24

Alahyari-Shahrab, M., Moravej, H., Shivzad, M. and Gerami, A. (2011). Study of possible reduction or withdrawal of vitamin premix during finisher period in floor and battery cage broiler raising system. .*African Journal of Biotechnology*, 33:6337

Aliarabi, H., Ahmadi, A., Ashori, N. and Hosseini, S. A. (2007). Effect of different storage condition and hens age on egg quality parameters. 19th *Australia Poultry Science Symposium,* Sydney, New South Wales, 12-14th February: 106-109

Alleman, F. and Leclercq, B. (1997). Effect of dietary protein and environmental temperature on growth performance and waste composition of male broiler chickens. *British Poultry Science*, 38: 607- 610

Al-Nasser, A., Holleman, K., Al-Khailefa, H., Al-Saffar, A., Behbehani, S., Al-Haddad, A. and Al-Matrouq, F. (1998). Effect of vitamin and trace mineral premixes hen

substituted for concentrates in broiler and layer diets in Kuwait. *Poultry Science*, 77 (Suppl. 1): 86 (Abstr,)

Al-Rawi, B. A. and Abu-Ashour, A. M. (1983).Performance of laying hens under different housing and environmental condition. *World's Review of Animal Production*, 19:54-60

Alsobayel, A.A. and Albadry, M. A. (2011). Effect of storage period and strain of layers on internal and external quality characteristics of eggs marketed in Riyadh area. *Journal Saudi Agricultural Science*, 10:41-45

Altunta, E. and Sekeroglu, A (2007). Effect of egg shape index on mechanical properties of chicken eggs. *Journal Food Engeering*, 85: 606-612.

Anderson, K. E. and Adams, A. W. (1994). Effect of cage versus floor rearing environments and cage floor mesh sizeon bone strength, fearfulness, and production of singlecomb white leghorn hens. *Poultry Science*, *73*, 1233–1240.

Anderson, K. E., Tharrington, J. B., Curtis, P. A. and Jones, F. T. (2004). Characteristics of egg shape from historic strains of single conb White Leghorn chickens and the relationship of egg shape to shell strength. *International Journal of Poultry Science*, 3:17-19

Anisuzzaman, M. (1993). Influence of different types of litter on the performance of broiler chicks, M. Sc. Thesis, Dept. of Poultry Science, BAU

AOAC (2000). Official Methods of Analysis, 15th edition, Association of Official Analytical Chemists, Washington, DC

Appleby, M. C. (2001). Chickens: Layer Housing, Encyclopaedia of Animal Science. Doi: 10 1081/E-EAS-120019534.

Arima, Y., Mather, F. B. and Ahmad, M. M. (1976). Response of egg production and shell quality, to increases in environmental temperature in. two age groups of hens. *Poultry Science Journal*, 55:818-820

Asaduzzaman, M., M. S. Jahan, M. R. Mondol, M. A. Islam, and A. K. Sarkar (2005). Efficacy of different commercial vitamin-mineral premixes on productive performance of caged laying pullets. *International Journal of Poultry Science*, 4(8):589-595

Australian Centre for International Agricultural Research (ACIAR) (1998). Measurement and maintenance of duck and hen egg quality in Vietnam. Research Note: *Research* RN23 12/99

Awoniyi, T. A. M. (2003). The effect of housing on layer-chicken productivity in the 3-tier cage. *International Journal of Poultry Science,* 2:438-441

Aygun, A. and Yetisir, R. (2010). The relationship among egg quality characteristic of different hybrid layers to forced moulting programme with and without feed withdrawal. *Journal of Animal Vetenitary Advance,* 9:710-715

Ayo, J. O. O., Owoyele, O. O and Dzenda, T. (2007). Effects of ascorbic acid on diurnal variation on rectal temperature of Bovan Nera pullets during the harmattan season. *International Journal of Poultry Science,* 6: 612-616.

Bains, B. S. (1999). A Guide to the Application of Vitamins in Commercial Poultry Feed. Rath Design Communications, Australia

Bakken, G. S., Vanderbilt, V. C. Buttemer, W. A.and Dawson. W. R. (1978). Avian eggs: thermoregulatory value of very high near infra-red reflectance. *Poultry Science,* 200:321-323

Banga-Mboko, H., Mabas, J. S. and Adzona, P. P. (2010). Effect of Housing System (Battery Cages Versus Floor Pen) on Performance of Laying Hens under Tropical Conditions in Congo Brazzaville. *Journal of Poultry Science,* 3(1): 1-4

Bar, A., Vax, E. and Striem, S. (1999). Relationship among age, eggshell thickness and vitamin D metabolism and its expression in laying hens. *Comparative Biochemistry and Physiology,* 123: 147-154

Barber, E. F. and Cousin, R. J. (1998). Internaleukin-1-stimulated induction of cerulopiasmin synthesis in normal and copper deficient rats. *Journal of Nutrition,* 118:375-381.

Belnave, D., R. J., Gill, X. Li and Bryden,W. L. (2000). Response of Isa brown laying hens to pre-layer diet containing additional calcium and to dietary protein and lysine concentration during inorganic phosphorous in laying hens. *British Poultry Science,* 51:779-784

Bennett, C. D. (1992). The influence of shell thickness on hatchability in commercial broiler breeder flocks. *Journal of Applied Poultry Research,* 1:61-65

Benson, B. N., Calvert, C. C., Roura, E. and Klasing, K. C. (1993). Dietary energy sources and density modulate of immunologic stress in chicks. *Journal of Nutrition,* 123:1714-1723.

Berry, W. D. and Brake, J. (1991). Research note: Induced moult eggshell quality and calbindin-D28K content of eggshell gland duodenum of egg hens. *Poultry Science,* 70:655-657.

Beyer, R. S. (2005). Factors affecting egg quality Kansas State University. http://www.oznet.ksu.edu/library/lvstk2/ep127.pdf

Bhale S., No H., Prinyawiwaiku, K. and Far, W. (2003). Chitosan coating improves shelf life of eggs. *Journal of Food Science,* 68:2378-2383

Biladeau, A. M. and Keener, K. M. (2009). The effects of edible coatings on chicken egg quality under refrigerated storage. *Poultry Science*, 88:1266-1274.

Blokhuis, H. J. (1989). The effect of a sudden change in floor type on pecking behaviour in chicks. *Applied Animal Behaviour Science*, 22 (1): 65-73

Bolu, S. A. (2013). Vitamins in Poultry Nutrition. T-Babs Printers ISBN: 978-8113-02-8

Boorman K. N., Volynchook, J. G. and Belyavin, C. G. (1989). Egg Shell Formation and Quality, In: Recent Developments in Poultry Nutrition. Eds: Cole, D. J. A. and Haresign, W., Butterworths, Kent, England

Botsoglou, N. A., Florou-Paneri, P., Nikolakakis, I., Giannenas, I., Dotas, V., Botsoglou, E. N., Bou, R., Codony, R., Baucells, M. D. and Guardiola, F. (2005). Effect of heated sunflower oil and dietary supplements on the composition, oxidative stability, and sensory quality of dark chicken meat. *Journal of Agricuture and Food Chemistry*, 53:7792–7801

Bou, R., Grimpa, S., Baucells, M. D., Codony, R. and Guardiola, F. (2006). Dose and duration of dark chicken meat through frozen storage: Influence of dietary fat and alpha-tocopherol and ascorbic acid supplementation. *Poultry Science Journal,* 80:1630–1642

Brake, J., Walsh, T. J., Benton, C. E., Petitte, J. N. Jr., Meijerhof, R. and G. Penalva. (1997). Egg handling and storage. *Poultry Science*, 76:144-151.

Brandão, P. A. (2005). Ácidos graxos e colesterol na alimentação humana. *Agropecuária Técnica,* (26)1:5-14.

Brillard, J. P. (2004). Natural mating in broiler breeders: present and future concerns. *World's Poultry Science Journal,* 60 (4): 439-445

Brown K. I. and Nestor K. E. (1973). Some physiological response of turkey selected for high and low adrenal response to cold stress. *Poultry Science,* 52:1948

Burley, R. W. and Vadehra, D. V. (1989). The Avian Egg Chemistry and Biology, John Wiley and Sons, New York, NY

Burtov, Yu, Z., Goldin, Yu. S. and Krrivopishin, I. P. (1990). Incubation of eggs: Handbook, Agropromizdat, Moscow, Russia

Butcher, G. D. and Miles, R. D. (2003). Concepts of Eggshell Quality, University of Florida http://edis.ifas.ufl.edu/pdffiles/VM/VM01300.pdf

Butcher, G. D., and Miles, R. D. (1995). Factors causing poor pigmentation of brown-shelled eggs, Cooperative Extension Service Fact Sheet VM94 Institute of Food and Agricultural Science, Univrsity of Florida, Gainesville, FL.

Butterwith, S. C. and Griffin, H. D. (1989). The effect of macrophage-derived cytokines on lipid metabolism in chickens (*Gallus domesticus*) hepatocytes and adipocytes. *Comparative Biochemistry Physiology*, 97B:722-724

Campo J. L, Garcia, H. and Gil M. (1998). Internal inclusions in brown eggs: relationships with fearfulness and stress. *Poultry Science,* 77:1743-1747

Carrillo-Domínguez, S., Avila, G. E., Vásquez, P. C., Fuente, B., Calvo, C., C. and Carranco, J. M. (2012). Eggs–No Yolking Matter. *Nutrition Action Health Letter*.

Casagrande Proietti, P., Passamonti, F., and Asdrubali, G. (2001). La gallina ovaiola allevata a terra e in gabbia. *Riv. Avicolt,* 3(5-6):12-15

Catala, A. (2006). An overview of lipid peroxidation with emphasis in outer segments of carbonyl in eggs. *The Lipid Chronicles* 4(1):35-37.

Cerny, K., Kordylas, P., Pospisil, F., Vanbens, O. S. and Zajic, B. (1971). Nutritive value of the winged bean (*Psophocarpus tetragonolobus Desv*). *British Journal of Nutrition*, 26: 293-299.

Chance, B., Sies, H. and Boveris, A. (1979). Hydroperoxide metabolism in mammalian organs. *British Poult Journal* 5(1):12-15

Charles, D. R. (2002). Responses to the thermal environment. In: poultry environment problems, A guide to solutions (Charles, D.A. and Walker, A.W. Eds.), Nottingham University Press, Nottingham, United Kingdom. 1-16

Cherian, G. (2007). Conjugated linoleic acid and fish oil in laying hen diets: effects on egg fatty acids, thiobarbituric acid reactive substances, and tocopherols during storage. *Poultry Science,* 86(5):953-958

Cherian, G. Campbell, A. and Parker, T. (2009). Egg quality and lipid composition of eggs from hens fed *Camelina saliva. Journal of Applied Poultry Research,* 18: 143-150

Cherian, G., Holsonbake, M., and Goeger, P. (2002). Fatty acid composition and egg components of speciality eggs. *Poultry Science Journal, 81*:30-33.

Cherian, G., Wolfe, F. and Sim, J. (1996). Dietary oils with added tocopherols: Effects on egg or tissue tocopherols, fatty acids, and oxidative stability. *Poultry Science Journal,* 75: 423-431.

Chernick, S. S., Lepkovsky, S. and Chaikoff, I. L. (1948). A dietary factor regulating the enzyme content of the pancreas; changes induced in size and proteolytic activity of the chicks' pancreas by the ingestion of raw soyabean meal. *American Journal of Physiology,* 155:33-41

Chowdhury, S. R., Chowdhury, S. D. and Smith, T. K. (1990). Effects of dietary garlic on cholesterol metabolism in laying hens. *Poultry Science,* 81:1856-1862.

Chukwuka, O. K., Okoli, I. C., Okeudo, N. J., Udedibie, A. B. I., Ogbuewu, I. P., Aladi, N. O., Iheshiulor, O. O. M. and Omded, A. A. (2011). Egg Quality Defects in Poultry Management and Food Safety. .*Asian Journal of Agriciltural Research*, 5, 1-16.

Ciftci, M., Nihat, O. and Ertas, T. Guler (2005). Effects of vitamins E and C dietary supplementation on egg production and egg quality of laying hens exposed to a chronic heat stress. *Revue Médical Véterinary*, 156 (Suppl. II), 107-111

Coetzee, C. (2002). The effect of elevated Calcium Levels in drinking water on shell integrity. Spesfeed News, Summer 2002. http://www.spesfeed.co.za/Summer2002.htm

Cook, M. E. (1991). Nutrition and immune response of the domestic fowl. *Critical Reviewof Poultry Biology*, 3:167-189

Correia G. M. G, Takata F. N., Medeiro J. P. (2000). Effect of organic selenium and zinc on the performance and egg quality of Japanese quails. *Revista brasileira de ciencia avicola*, 29 (5):1440-1445

Cortinas, L., Villaverde, C. Galobart, J., Baucells, M. D., Codony, R. and Barroeta, A. C. (2004). Fatty acid content in chicken thigh and breast as affected by dietary polyunsaturation level. *Poultry Sci*ence *Journal,* 83(11):55-64.

Coutts, J. A. and Wilson, G. C. (1990). Egg quality handbook, Queensland department of primary industries, Australia

Cunningham, F. E. and Sanford, P. E. (1974). A review of facts influencing egg yolk mottling. *World's Poultry Science Journal*, 30: 103-114

Daghir, N. J. (2009). Nutritional strategies to reduce heat stress in broiler and broder breeders. *Lohman Information*, 44 (1)6-15

Daghir, N. J., Marrion, W. W. and Balloun, S. L. (1960). Influence of dietary fat and choline on serum and egg yolk cholesterol in the laying chicken. *Poultry Science*, 39: 1459-1466

Damron, B. L., Johnson, W. L. and Kelly, L. S. (1986). Utilization of sodium bicarbonate by broiler chickens. *Poultry Science*, 65:782-785

Dawkin, M. (1983). Cage size and flooring preferences in litter-reared and cage-reared hens. *British Poultry Science*, 24(2): 177-182

Dawkin, M. (1989). Time budgets in Red Jungle fowl as a baseline for the assessement of welfare in domestic fowls. .*Applied Animal Behaviour Science*, 24:77-80

De Ketelaere, B., Bamelis, F., Kemps, B., Decuypere, B. and De Baerdemaeker, J. (2004). Non-destructive measurement of egg quality. *World's Poultry Science Journal*, 60 (3): 289-302

Deaton, J. W. and Quisenberry, D. (1965). Effect of amino acid supplemented of low protein corn and grain sorghum diets on the performance of egg production stock. *Poultry Science*, 43:1214-1219

DEFRA (2011). DEFRA code for the welfare of laying hens/Access date 5 December 2011

Dingle, N. M. and Henuk, Y. L. (1999). Formulating diets for laying hens without a vitamin and mineral premix gives less nutrient excesses. In: *Austrailian. Poultry Scienec Symposum* 11:185. University of Sydney, Sydney, NSW

Djukic-Stojcic, M., Peric, L., Bjedov, S. and Milosevic, N. (2009). The quality of table eggs produced in different housing systems. *Biotechnology in Animal Husbandry*, 25 (5-6):1103-1108

Domínguez, R.O., Marschoff, E. R., Guareschi, E. M., Repetto, M. G., Famulari, A. L. and Pagano, A. (2008). Biosynthesis and function of polyacetylenes and allied natural products. *Progress in Lipid Research*, 47 (4): 233–306.

Doyon, G., Bernier-Cardoyon, Bernier-Cardou, M., Hamilton, R. M. G., Castaigne, F. and Randall, C. J. (1986). Egg quality: 2. Albumen quality of eggs from five

commercial strains of White Leghorn hens during one year of lay. *Poultry Sci.ence,* 65:63-66.

Dudusola I. O. (2009). Effect of Storage Methods and Length of Storage on some Quality Parameters of Japanese quail eggs. *Tropicultura,* 27 (1): 45-48.

Duduyemi, O. A. (2005). Evaluation of egg-lating performance of low strain of bovan chickens (Bovan Brown and Bovan Nera) in the tropics. Proc. 1st Nigerian International Poultry Summit, Feb 20-25, 2005, Ota Ogun State Nigeria 33-35

Duncan, I. J. H. and Hughe, B. O. (1972). Free and operant) Free and operant feeding in domestic fowls. *Animal Behaviour,* 20:775-777

Duyck, S. K., Miles, R. D., Rossi, A. F. and Henry, P. R. (1990). Effect of time and storage conditions on interior egg quality from hens fed vanadium. *Poultry Science,* 69 (Supplement 10):164

Edward Jr., H. M., Marion, J. E. and Driggers, J. C (1962). Serum and cholesterol levels in mature hens as influenced by dietary protein and fat changes. *Poultry Sci*ence, 41:713-717

Elston, J. J. (2000). Laying hen behaviour: Effect of cage type and startle stimuli *Poultry Science Savoy*, 79(4):471-476

Emman, G. C. (1994). Effective energy: a concept of energy utilization applied across species. *British Journal of Nutrition,* 71: 801-802

Esonu B. O. (2006). Animal Nutrition and Feeding: A functional Approach. 2nd Edn Rukzeal and Ruksons Associates Memmory Press, Owerri, Nigeria

Etches, R. J. (1987). Calcium logistic in the laying hens. *Journal of Nutrition,* 117:619-628

Etches, R. J. (1996). Reproduction-Poultry. Wallingford: CAB Intectual: 378

Fahy, E., Subramaniam, S., Murphy, R., Nishijima, M., Raetz, C., Shimizu, T., Spener, F., Meer, G., Wakelam, M ., and Dennis, E. A. (2009). Update of the lipid maps comprehensive classification system for lipids. *Journal of Lipid Research*, 50(1): 9–14

FAO (2003). Egg marketing a guide for the production and sale of eggs. Food and Agriculture Organization of the United Nations Rome

FAO, (2000). World Watch List for Domestic Animal Diversity, Food and Agriculture Organization of the United Nations, 3rd Edition, Ed. Beate D. Scherf, Roma 2000: 364.

Faria, D. E., Junqueirao. M., Sakomura, N. K. and Santana A. E. (1999). Effect of different levels of manganese and phosphorus on the performance and eggshell quality of laying hens. *Revista Sociedade Brasileria Zo otecnia,* 28:105–112

Farooq, M., Mian, M., Ali, M., Durrani, F. Asghar, and Muqarrab, A. (2001). Egg traits of Fayoumi birds under subtropical conditions. *Sarhad Journal of Agriculture,* 17, 141-145

Farooqui, T. and Farooqui, A. (2011). Lipid-mediated oxidative stress and inflammation fromfats and cholesterol. *The Nutrition Source* Harvard School of Public Health

Fernandez, I. B., Cruz, V. C. and Polycarpo, G. V. (2011). Effect of dietary organic selenium and zinc on the internal egg quality of quail eggs for different periods and under different temperatures. *Rev. Brasilain Cienc Avic,* 13(1):234-255.

Filardi, R. S. (2005). Influence of different fat sources on the performance, egg quality, and lipid profile of egg yolks of commercial layers in the second laying cycle. *The Journal of Applied Poultry Research,* 14(2):258-264

Fisher, C. and Wilson, B. M. (1974). Responses to dietary energy concentration by growing chickens, In: *Energy Requirement of Poultry* (Morris, T. R and Freeman, B. M. Eds). Edinburgh. Constable 151-184

Fisher, L. J., Erfle, J. D., Lodge, G. A. and Sauer, F. D. (1973). Effects of propylene glycol or glycerol supplementation of the diet of dairy cows on feed intake, milk yield and composition, and incidence of ketosis. *Canadian Journal of Animal Science,* 53:289-296.

Franchinni, A., Sirri, F., Tallarico, N., Minelli, G., Iaffaldano, N. and Meluzzi, A. (2002). Oxidative stability and sensory and functional properties of eggs from laying hens fed supranutritional doses of vitamins E and C. *Poultry Science,* 81: 1744-1750

Franco, J. G. and Sakamoto, M. I. (2005).Qualidade de ovos: uma visão geral dos fatores que a influenciam. *Campinas Brasilian Cienc Avic,* 10(3)

Fraps, G. S. (1946). Composition and productive energy of poultry feeds and rations. *Texas Agricultural Experimental Station Bulletin,* 678-876

Fraser, A. F. and Bloom, D. M. (1990). Farm Animal Behaviour and Welfare, 3rd Edition, London, England: Bailliere Tindall p. vii

Freitas, E. R. (2000). Colesterol e ácidos graxos da gordura de frangos de corte alimentados com dietas contendo farelo da amêndoa da castanha de caju suplementado com enzimas In: Reunião Anual Da Sociedade Brasileira De-Zootecnia. *Viçosa. Anais Viçosa Journ.,* 37(4):261-278

Fromm, D. and Matrone, G. (1962). A rapid method for evaluating the strength of the vitelline membrane of the hen's egg yolk. *Poultry Science,* 41: 1516-1521

Galobart, J., Barroeta, A. C., Baucells, M. D., Cortinas, L. and Guardiola, F. (2001).α-Tocopherol transfer efficiency and lipid oxidation in fresh and spray-dried eggs enriched with n-3 polyunsaturated fatty acids. *Poultry Science,* 80:1496–1505

Galobart, J., Barroeta, A. C., Cortinas, L., Baucells, M. D. and Codony, R. (2002). Accumulation of α-tocopherol in eggs enriched with ω-3 and ω-6 polyunsaturated fatty acids. *Poultry Science Journal, 45*(11):567-588

Galobart, J., Sala, R., Rincon-Carruyo, X., Manzannilla, E. G., Vila, B. and Gasa, J. (2004). Egg yolk colour as affected by saponification of different natural pigmentation sources. *Journal of Applied Poultry Research,* 13:328-338

Gavril, R. and Usturoi, M. G. (2012).Effect of storage time and temperature on hen egg quality. *Seria Zootehnie,* 57:221-229

Gey, K. F. (1998). Vitamins E plus C and interacting co nutrients required for optimal health in adults. *Lancet Journal,* 6 (1): 8–11

Gezen, S.S., Eren, M. and Deniz, G. (2005). The effect of different dietary electrolyte balances on eggshell quality in laying hens. *Revue Medecine Veterinarie,* 156, (10):491-497

Glatz, P. C. (1993). Poultry Production in Hot Climates. Proceedings of the 9th Australian Poultry and Feed Convention, Gold Coast, Australia, 202-205

Gonzalez-Esquerra, R., and Leeson, S. (2000). Effect of feeding hens regular or deodorized menhaden oil on production parameters, yolk fatty acid profile, and sensory quality of eggs. *Poultry Science Journal,* 79:1597-1602

Gosler, A. G., Higham, J. P. and Reynolds, S. J. (2005). Why are birds' eggs speckled? *Ecology Lett*er, 8:1105-1113

Grashorn, M. A. (2005). Enrichment of egg and poultry meat with biologically active substances by feed modifications and effects on the final quality of the product. *Poultry Journal of food Nutrition Science,* 14(55):15-20

Grau, A., Guardiola, F., Grimpa, S., Barroeta, A. C. and Codony, R. (2001). Oxidative stability of special eggs. *Poultry Science Journal*, 81:30-33

Gregory, N. G. and Wilkins, L. J. (1989). Broken bones in domestic fowls: Handling and processing damage in end-of-lay battery hens. *British Poultry Sci*ence, 30:555-562.

Griffin, H. D. and Butterwith, S. C. (1988). Effect of *eschericia coli* endotoxin on tissue lipoprotein lipase activities in chickens. *British Poultry Science*, 29(2): 371-378

Grobas, S, Mendes J, Medel P, Lazaro R, Mateos, G. G. (1997). Influence of energy, linoleic acid and fat content of the diet on performance and weight ofegg components of brown layers. *Poultry Science Journal*, 76:256-258.

Grobas, S. (2001). Influence of source and percentage of fat added to diet on performance and fatty acid composition of egg yolk of two strains of laying hens. *Poultry Science Journal*, 80:1171-1179

Grobas, S., J., Mendez, C., Lopez Bote, C. De Blas and Mateos, G. G. (1999). Effect of vitamin E and A supplementation on egg yolk α-tocopherol concentration. *Poultry Science*, 81:376-381

Guesdon V. and Faure, J. M. (2004). Laying performance and egg quality in hens kept in standard or furnished cages. *Journal of Animal Research*, 53:45–57

Gunlu, A., Kirikci, K., Cetin, O. and Garip, M. (2003). Some external and internal quality characteristics of partridge (*A. graeca*) egg. *Food Agriculture and Environment*, 1: 197-199.

Gutteridge, J. M. (1995). Lipid peroxidation and antioxidants as biomarkers of tissue damage. *Clinical Chemistry*, 41:1819–1828

Halliwell, B. and Gutteridge, J. M. (1989).Free Radicals in Biology and Medicine 2nd ed.

Halliwell, B., Murcia, M. A., Chirico, S. and Aruoma, O. I. (1995). Free-radicals and antioxidants in food and *in-vivo*–what they do and how they work. *Critical Review of Food Science and Nutrition Journal*, 35:7–20.

Hamilton, R. M. G. (1982). Methods and factors that affect the measurement of egg shell quality. *Poultry Science*, 61: 2002-2039.

Hammershoj M. and Kjaer, L. (1999). Phase feeding for laying hens: Effect of protein and essential amino acids on egg quality and product. Acta Agriculturae Scandinavica section. *A-Animal Science,* 49-41

Hargreave, C. T. (1982). Layers nutrition in hot climates. *Poultry International*, 21:36-40

Harms, R. H., Russell G. B., Sloan D. R., (2000). Performance of four strains of commercial layers with major changes in dietary energy. *Journal of Applied Poultry Research,* 9:535–541

Hasin B. M., Ferdaus, A. J. M., Islam, M. A., Uddin, M. J. and Islam, M. S. (2006). Marigold and Orange Skin as Egg Yolk Color Promoting Agents. *International Journal of Poultry Science*, 5 (10): 979-987

Hassan, M. D., Rakibul, H. S., Choe, A., Yong, D., Jeong, I., Jong, H., Kyeong, S. and Ryu, D. (2013). Effect of dietary energy and protein on the performance, egg quality, bone mineral density, blood properties and yolk fatty acid composition of organic laying hens. *Italian Journal of Animal Science*, 12(10):234-239.

Haugh, R. R. (1937). The Haugh unit for measuring egg quality: US Egg Poulty Magazine. 43:552-555 and 572-573

Hayat, Z., Cherian, G., Pasha, T. N., Khattak, F. M. and Jabber, M. A. (2010). Oxidative stability and lipid components of eggs from flax-fed hens: Effect of dietary antioxidants and storage. *Poultry Science,* 89:1285-1292

Henry, P. R. (1995). Sodium and chloride bioavailability In: Ammernan, C. B., Baker, D. H. and Lewis, A. J. Eds.) *Bioavailability of Nutrients for Animals*, Academic Press, San Diego, California USA, 337-348

Hess, J. B. and Britton, W. M. (1989). The effect of dietary chloride or protein changes on eggshell pimpling and shell quality in late production leghorn hens. *Nutrition Reports International*, 40, 1107 -1115

Hetland, H., Choct, M. and Svihus, B. (2004). Role of insoluble non-starch polysaccharides in poultry nutrition. *World's Poultry Science Journal,* 60 (4): 415-422

Hidalgo, A., Rossi, M., Clerici, F. and Ratti, S. (2008). A market study on the quality characteristics of eggs from different housing systems. *Journal of Food Chemistry*, 106: 1031-1038

Hill, F. W. (1962). Some aspects of the physiology of food intake and digestion in chickens. In: *Nutrition of Pigs and Poultry* (Morgan, J. T. and Lewis, D. Eds.), London, Butterworth, 3-17

Hocking, P. M., Bain, M., Channing, C. E., Fleming, R. and Wilson, S., (2003). Genetic variation for egg production, egg quality and bone strength in selected and traditional breeds of laying fowl. *British Poultry Science,* 44: 365-373

Holland, K. G., Grunder, A. A. and Williams, C. L. (1980). Response to five generations of selection for blood cholesterol levels in White Leghorns. *Poultry Science Joural*, 59:1316-1326

House, J. D., Braun, K., Balance, D. M., O'Connor, C. P. and Guenter, W. (2002). The enrichment of eggs with folic acid through supplementation of the laying hen diet. *Poultry Science,* 2002: 81(9):1332-7

Hughes B. O., Dum, P. and McCorquodale, C. C. (1985). Shell strength of eggs from medium bodied hybrid hens housed in cages or on range outside pen. *British Poultry Science*, 26: 129-136

Hughes, B. O. (1975).Spatial preference in the domestic hens. *British Veterinary Journal,* 131 (5) 560-564

Humphrey, B. D. and Klasing, K. C. (2004). Modulation of nutrient metabolism and homeostasis by the immune system. *World's Poultry Science Journal,* 60 (1): 99-100

Hunton, P. (2005). Research on eggshell structure and quality: An historical overview. *Revista Brasileria de Ciencia Avicola,* 7 (2)

Huygheb, G., De Groote, G., Butter, E. A. and Morris, T. R. (1991). Optimum isoleucine requirement of laying hens and the effect of age. *British Poultry Science*, 32: 471-481

Ihsan, T. Tayeb (2012). Effect of storage temperature and length on egg quality parameters of laying hen. *Journal of Animal Science,* 1(2): 32-36.

Isikwenu, J.O., Okaplefe, C.S., Mmereole, F.U.C. (1999). Storability of chicken eggs under different storage conditions. Proceedings of the 26th Annual Nigeria Society for Animal Production Conference, 21-25: March, University of Ilorin, Ilorin, Nigeria.

Izat, A., Garder, A. and Mellor, D. (1985). The effect of age of bird and season of the year on egg quality. *Poultry Science,* 65:726-728

Jackson, N., Kirkpatrick, H. R. and Fulton, R. B. (1969). An experimental study of the utilization by the laying hen of dietary energy partially supplied as animal fat. *British Poultry Science,* 10: 115-126

Jacob, J. P., Miles, R. D. and Mather, F. B. (2000). Egg quality. University of Florida http//:edis.ifas.ufl.edu/pdffiles/PS/PS02000.PDF

Jin Y. H, Lee, K. T., Lee, W. I. and Han, Y. K. (2011). Effects of Storage Temperature and Time on the Quality of Eggs from Laying Hens at Peak Production. *Asian Australian Journal of Animal Science*, 24 (2): 279-284

Jin, L, and Craig, J. V. (1994). Some effects of cage and floor rearing on commercial white leghorn: Perspective on lipid index of eggs. *Nutrition bulletin*, 31(1): 21-27

Jones, D. R. (2006). Conserving and Monitoring Shell Egg Quality. Proceedings of the 18th Annual Australian *Poultry Science Symposium*, 157-165

Jones, D. R., Tharrington, J. B., Curtis, P. A., Anderson, K. E., Keener, K. M. and Jones, F. T. (1990). Physical quality and composition of retail shell eggs. *Poultry Science*, 81:727–733

Józefa, K., Zofia, S. and Beata, S. (2011). Effect of housing system on cholesterol, vitamin and fatty acid content of yolk and physical characteristics of eggs from Polish native hens. *Archiva geflügelk,* 75(3):151- 157.

Kamil, K., Mehmet, B., Emine, N., Mustafa, Ç., Abdullah, U., Erol, B. and Fethiye, Ç. (2012). Effects of rearing systems on performance, egg characteristics and immune response in two layer hen genotype. *Asian-Australian Journal of Animal Science*, 25(4):559–568

Kang, K. R., Cherian, G. and Sim, J. S. (1998). Tocopherols, retinol and carotenes in chicken egg and tissues as influenced by dietary palm oil. *Journal of Food Science*, 63: 592–596

Kang, K. R., Cherian, G. and Sim, J. S. (2001). Dietary palm oil alters the lipid stability of polyunsaturated acid-modified poultry product. *Poultry Science*, 80: 228-234.

Kato, A., Ibrahim H.R., Nakamura, S. and Kobayashi, K. (1994). New methods for improving the functionality of egg white proteins: In: Sim, J. S., Nakai, S. (eds): Egg Uses and Processing Technologies New Developments, *CAB International Wallingford* 250–267

Keener, K. M., LaCrosse, J. D., Curtis, P. A., Anderson, K. E. and Farkas, B. E. (2000). The influence of rapid air cooling and carbon dioxide cooling and subsequent

storage in air and carbon dioxide on shell egg quality. *Poultry Science*, 79:1067–1071

Kemps, B. J., Bamelis, F. R., De Katelaere, B., Mertens, K., Tona, K., Decuypere, E.M. and De Baerdemaeker, J. G. (2006). Visible transmission spectroscopy for the assessment of egg freshness. *Journal of the Science of Food and Agriculture*, 86: 1399-1406

Kershavarz, K. and Nakajima, S. (1993). Re-evaluation of calcium and phosphorous requirements of laying hens for optimum performance and egg shell quality. *Poultry Science*, 72: 144 - 153

Keshavarz, K. and Austic R. E.(1990). The effect of minerals on acid-base balance and eggshell quality. *Journal of Nutrition*, 120: 1360-1369.

Keshavarz, K. and Nakajima, S. (1995). The effect of dietary manipulations of energy, protein, and fat during the growing and laying periods on early egg weight and egg components. *Poultry Science*, 74:50–61

King'ori, A. M. (2011). Review of factors that influence egg fertility and hatchability in poultry. *International Journal of Poultry Science*, 10:483-492

Kirunda D. F. K. and McKee, S. R. (2001). Relating quality characteristics of age egg and fresh eggs to vitelline membrane strength as determined by a texture analyzer. *Poultry Science*, 79: 1189-1193

Klasing, K. C. (1984). Effect of inflammatory agents and interleukin-1 on iron and zinc metabolism. *American Journal of Physiology*, 247:R901-904.

Klasing, K. C., Laurin, D. E., Peng, R. K. and Fry, M. (1987). Immunologically mediated growth depression in chicks: Influence of feed intake, Corticosterone and Interleukin-1. *Journal of Nutrition*, 117:1629-1637

Knight, C. D., Klasing, K. C. and Forsyth, D. M. (1983). *E. coli* derxtraan-supplement pigs. *Journal of Animal Science*, 57: 387-395.

Koelkebeck, K. W (2003). What is egg quality and conserving it? *University of Illinois Extension Publications*, August

Koelkebeck, K. W. (1999). What is egg quality and conserving it? University of Illinois.http//www.traill.uiuc.edu/poultrynet/paperDisplay.cfm?ContentID=522

Koh, T. S., Peng, R. K. and Klasing, K. C. (1996). Dietary copper level affects copper metabolism during lipopolysaccharide-induced immunological stress in chicks. *Poultry Science*, 75:867-872

Kokantnur, M., Rand, R. T., Kummerow, F. A. and Scott, H. M (1958). Effects of dietary protein and fat on changes of serum cholesterol in mature birds. *Journal of Nutrition*, 64:177-184

Koutsos, E. A. and Klasing, K. C. (2001). Interaction between the immune system, nutrition and productivity of animals: In: *Recent Advance in Animal Nutrition.* 2001. P. C.Gansworthy and J. Wiseman eds. Nottingham University Press, Nottingham, 173-190

Kovács, G., Schmidt, J., Dublecz, K., Wágner, L. and Farkas-Zele, E. (2000). Effect of feed composition on cholesterol content of the table egg. *Animal Science Journal*, 29: 25-41

Kralik, G., Ivanković, S., Bogut, I., Csapo, J. (2006). Effect of dietary supplementation with PUFA n-3 on the lipids composition of chicken meat. *Animal Science Journal*, 33: 129-139

Krawczyk, J. and Gornowicz, S. (2009). Effect of layer age and egg production level on changes in quality traits of eggs from hens of conservation breeds and commercial hybrids. *Annals of Animal Science Journal*, 9(2): 185-193

Kucuk, O. Sahin, N., Sahin, K., Gursu, M. F., Gulu, F., Ozcelik, M. and Issi, M. (2003). Egg production, egg quality, and lipid peroxidation status in laying hens maintained at a low ambient temperature (6°C) and fed a vitamin C and vitamin E-supplemented diet. *Czeche Vetetrinary Medicine, 48 (1–2): 33–40*

Kul, S. and Seker, I. (2004). Phenotypic correlation between some external and internal egg quality traits of Japanese quail (*Coturnix coturnix Japonica*). *Iinternational Journal of Poultry Sci*ence, 3: 400-405

Kutlu, H. R. and Forbes, J. M. (1993). Changes in growth and blood parameters in heat-stressed broiler chicks in response to dietary ascorbic acid. *Livestock Production Science*, 36: 335-350

Lahti, D. C. (2008). Population differentiation and rapid evolution of egg color in accordance with solar radiation. *Aukland*, 125:796-802

Lanari, M.C., Hewavitharan, A.K., Becu, C. and de Jong, S. (2004). Effect of dietary tocopherols and tocotrienols on the antioxidant status and lipid stability of chicken. *Meat Science*, 68: 155–162

Langweiler, M. B., Sheffy, E. and Schultz, R. D. (1983). Effect of antioxidants on the proliferative response of canine lymphocytes in serum from dogs with vitamin E deficiency. *Animal Journal of Vetetinary Research*, 44:5

Larbier, M. and Leclercq, M. (1997).Nutrition and Feeding of Poultry. Nottingham University Press, Loughborough GB, 305

Lauorin, D. E and Klasing, K. C. (1987). Effect of repetitive immunogen injections and fasting versus feeding on iron, zinc and copper metabolism in chicks. *Biology of Trace Element Research*, 14:153-165

Ledur, M. C., Liljedahl, L. E., McMillan, I., Asselstine, L. and Fairfull, R. W. (2002). Genetic effects of aging on egg quality traits in the first laying cycle of White Leghorn strains and strain crosses. *Poultry Science*, 81:1439-1447

Leeson, S. (2007). Vitamin requirement: is there basis for re-evaluating dietary specifications. *World`s Poultry Science Journal*, 63:255-266.

Leeson, S. and Caston, L. J. (2003). Vitamin Enrichment of Eggs. *The Journal of Applied Poultry Research*, 12:24-26

Leeson, S. and Summers, J. D. (2001). Scott`s Nutrition of the Chicken. 4th ed. University Books, Guelph, Ontario, Canada, 591

Leeson, S. and Summers, J. D. (2005). Commercial Poultry Nutrition 3rded. Nottingham University Press, UK, 398

Leshchinsky, T. V. and Klasing, K. C. (2001). Divergence of inflammatory response in two types of chickens. *Developmental and Comparative Immunology*, 25:629-638

Li-Chan, E. and Nakai, S. (1989). Biochemical basis for the properties of egg white. *Critical Review of Poultry Biology*, 2:21-59

Li-Chan, E. C., Powrie, W. and Nakai, S. (1995). The chemistry of egg and egg products: In: *Egg Science and Technology*, 4th ed., W. J. Staelman and J. O. Cotterill, Food Product Press, New York, 105-175

Lichovnikova, M. and Zeman, L. (2008). Effect of housing system on the calcium requirements of laying hens and eggshell quality. *Czech Journal of Animal Science*, 53:162–168

Lopez-bote, C. J., Arias, R. S., Rey, A. I., Castano, A., Isabel, B. and Thos, J. (1998). Effect of free range feeding on omega-3 fatty acid and alpha- tocopherol content and oxidative stability of eggs. *Animal Feed Science and Technology*, 72:33-40

Mabe I., Rapp C., Bain Mm. and Nys Y. (2003). Supplementation of a corn-soybean meal diet with manganese, copper, and zinc from organic or inorganic sources improves eggshell quality in aged laying hens. *Poultry Science*, 82:1903-1913

Majekodunmi, B. C. (2014). Mitigation of heat stress in broiler chickens using ascorbic acid and electrolytes. Ph.D Thesis, Department of Animal Science Ibadan, Ibadan, Nigeria: 185

Marion, J. E. and Edward Jr., H. M. (1962). The influence of various oils in diet on lipid metabolism of fat deficient laying hens. *Poultry Science*, 41:1662

Marks, H. l. and Pesti, G. M. (1984). The role of protein level and diet forms in water consumption and abdominal fat depot deposition of broilers. *Poultry Science*, 63:1617-1625

Marks, J. (1979). A guide to the vitamins: Their Roles in Health and Diseases. MTF, Medical and Tech. Publishing and Limited, England

Marnett, L. J. (1999). Lipid peroxidation-DNA damage by malondialdehyde, *Mutation Research*, 8424(1-2):83–95

Martins, A., Dunnington, E. A., Gross, W. B., Briles, W. E., Briles, R. W. and Siegel, P. B. (1990). Production traits and alloantigen systems in line of chickens selected for high or low antibody responses to sheep erythrocytes. *Poultry Science*, 69:871-878

Mas, A. and Arola, L. (1985). Cadmium and lead toxicity effects on zinc, copper, nickel and iron distribution in the developing chick embryo. *Comparative Biochemistry and Physiology*, 80C: 185-188

Mashaghi, S., Jadidi, T., Koenderink, G. and Mashaghi, A. (2013). Lipid Nanotechnology. *International Journal of Molecular Science*, 13 (14): 4242–4282

Maynard, L. A., Loosli, J. K., Hintz, H. F. and Warner, R. G. (1979).Animal lipid in human nutrition. Tata McGraw-Hill Publishing Co. Ltd., 132.

McDonald, S. (1996). The complete blood count. URL http://www.oldworld avaries.com

McDowell L. R. (2000). Vitamins in animal and human nutrition Second Edition. Iowa State University Press, ISBN 0-8138-2630-6, 395

McDowell, L. R. (1989). Vitamin B 12. In:McDowell LR Vitamins in animal nutrition. San Diego, Academic Press, 323-346

McDowell, L. R. (2005). Vitamins in animal nutrition In: McDowell, L.R. (Ed.) Comparative aspects to human nutrition, Introduction and historical considerations.London. Academic Press, 3-12

McGee, H. (2004) .McGee on Food and Cooking: Eggs, Hodder and Stoughton, 20, ISBN 0-340-83149-9

Meluzzi, A., Tallarico, N., Manfreda, G., Sirri, F. and Franchini, A. (2000). Effect of dietary vitamin E on the quality of table eggs enriched With N-3 Long Chain Fatty Acids. *Journal of Poultry Science,* 79:539-545

Mertens, K., Bamelis, F., Kemps, B., Kamers, B., Verhoelst, E., De Ketelaere, B., Bain, M., Decuypere, E. and De Baerdemaeker, J. (2006). Monitoring of eggshell breakage and eggshell strength in different production of chains of consumption eggs. *Poultry Science,* 85: 1670-1677

Miles, R. D. (2001). Trace minerals and avian embryo development. *Ciência Animal Brasileira,* 2:1-10

Milinsk, M. C. (2003). Fatty acid profile of egg yolk lipids from hens fed diets rich in n-3 fatty acids. *Food Chemistry,* 83(2):287-292

Miller, E. R. (1995). Potassium bioavailability In:C. B. Ammernan, D. H. Baker, and A. J. Lewis (eds.). *Bioavailability of Nutrients for Animals,* Academic Press San Diego California US, 295-301

Minelli, G., Sirri, F., Folegatti, E., Meluzzi, A. and Franchini, A. (2007). Egg quality traits of laying hens reared in organic and conventional systems. *Italian Journal of Animal Science,* 6(1):728–730

Mmereole, F. U. C and Omeje, S. I. (2005). Genotypexseasion interaction effect on the laying mortality rates of the Nigerian local chicken, the Barred Plymouth Rock and the crosess. Proc. 1st Nigerian International Poultry Summit, Feb 20-25, 2005, Ota Ogun State Nigeria 28-31

Mohammed, A., Al-Harthi, S., Ahmed, A. and El-Deek, W. (2013). Effect of different dietary concentrations of brown marine algae (*Sargasum dentifebium*) prepared by different methods on plasma and yolk lipid profiles, yolk total carotene and lutein plus zeaxanthin of laying hens. *Italian Journal of Animal Science,* 11(4):234-239

Mohan, B., Kadirvel,R., Bhaskaran, M. and Notarajan, A. (1991). Effect of probiotic supplementation on serum/yolk cholesterol and on egg shell thickness in layers. *British Poultry Science,* 36: 799-803

Mohiti-Asli, M., Shariatmadari, F., Lotfollahian, H. and Mazuji, M. T. (2008). Effect of supplementing layer hen diets with selenium and vitamin E on egg quality, lipid

oxidation and fatty acid composition during storage. *Candian Journal of Animal Science,* 88(44):475-483

Mone, P. E., Warner, W. D., Poling C. E. and Rice, E. E. (1959). Influence of dietary fat and protein on serum cholesterol of cholesterol fed chicks. *Journal of American Oil Chemistry Society,* 36:141-142

Monria, K. N., Salahuddin, M. and Miah, G. (2003). Effect of breed and holding period on egg quality characteristics of chicken. *International Journal of Poultry Science,* 2:261-263

Morgin, P. (1981). Recent advances in dietary anion-cation balance: Application in poultry. Proceedings Nutrition Society, 40:285-294

Mori, A. V., Mendonca Junior., C. X. and Almeida, C. R. M. (2003). Supplementing hen diets with Vitamins A and E affects egg yolk retinol and *á*-tocopherol levels. *Journal of Applied Poultry Research,* 12:106-114

Morris, T. R. (1968). The effect of dietary energy level on the voluntary caloric intake of laying birds. *British Poultry Science,* 9: 285-295

Morris, T. R. (1985). The manipulation of egg size and egg quality. *African Journal of Animal Science,* 15:120–122

Morris, T. R. (2004). Nutrition of chicks and layers. *World's Poultry Science Journal,* 60(1): 5-18

Morrissey P.A., Brandon, S., Buckley, D., Sheehy, P. and Frigg, M. (1997). Tissue content of α-tocopherol and oxidative stability of broilers receiving dietary α-tocopherol acetate supplement for various periods post-slaughter. *British Poultry Science,* 38: 84–88

Murakami, A. E., Saleh, E. A., England, J. A., Dickey, D. A., Watkins, S. E. and Waldroup, P. N. (1997). Effect of level and source of sodium on performance of male broiler to 56 days. *Journal of Applied Poultry Research,* 6:123-136

Nam, K. (1997). Influence of dietary supplementation with linseed and vitamin E on fatty acids, a-tocopherol and lipid peroxidation in muscles of broiler chicks. *Animal Feed Science and Technology,* 66(114): 149-158

Nascimento, V. P., Cranstoun, S. and Solomon, S. E. (2014). Relationship between shell structure and movement of Salmonella enteritidis across the eggshell wall. *British Poultry Science,* 33: 37-48

Natalie, G. (2009). Factors affecting egg quality in the commercial laying hen: A review of Egg Producers Federation of New Zealand (Inc)/Poultry Industry Association of New Zealand 96 D Carlton Gore Road, Newmarket, 1023, Auckland

National Research Council (NRC) (1994). Nutrient requirements of poultry, 9th revised ed., National Academie Press, Washington, DC 155

Neijat M., House, J. D., Guenter, W. and Kebreab, E. (2011). Calcium and phosphorus dynamics in commercial laying hens housed in conventional or enriched cage systems. *Poultry Science*, 90 (10):2383-2396

Neospark, J. (2012).Eggshell deffects and dietary essentials. Downloaded from the internet on 1/07/2012

Nicol, C. J. (1986). Non-exclusive spatial preference in the laying hens. *Applied Animal Behaviour Science*, 15 (3):33-50

Njoya, J. and Picard, M. (1994). Climatic adaptation of laying hens. *Tropical Animal Health Production*, 26: 180-186

Nworgu, F. C. (2006). Prospects and Pitfalls of Agricultural Production in Nigerian. Blessed Publication, Ibadan, Nigeria, 79-96

Nys, Y. (1995). Influence of nutritional factors on eggshell quality at high environmental temperature. Proceedings VI European Symposium on the Quality of Eggs and Egg Products, Zaragoza, Spain, 209-220

Nys, Y., Gautron, J., Garcia-Ruiz, J. M. and Hincke, M. T. (2004). Avian eggshell mineralization: biochemical and functional characterization of matrix proteins.*Comptes Rendus Palevol*, 3: 549–62

Nys, Y., Hincke, M., Arias, J. L., Garcia-Ruiz, J. M. and Solomon, S. E. (1999).Avian eggshell mineralization. *Poultry Avian Biology Rev*iew, 10: 142-166

Nys, Y., Mayel-Afashar, S., Bouillon, R., Van-Baelen, H. and Lawson, D. E. M. (1989). Increases in calbindin D28K mRNA in the uterus of the domestic fowls induced by sexual maturity and shell formation. *General Comparative Endocrinology*, 76: 322-329

Nys, Y., Parkers, C. O. and Thomasset, M. (1986). Effect of suppression and resumption of shell formation and parathyroid hormone on uterine calcium binding protein, carbonic anhydrase activity and intestinal calcium absorption in hens. *General Comparative Endocrinology*, 64: 293-299

Obadasi, A. Z., Miles, R. D., Balaban, M. O. and Portier, K. M. (2007). Changes in Brown Eggshell Color as the Hen Ages. *Poultry Science*, 86: 356-363

Obanu, Z. A. and Mpieri, A. A. (1984). Efficiency of dietary vegetable oils in preserving the quality of shell eggs under ambient tropical conditions. *Journal Science Food and Agriculture*, 35:1311–7

Obidi, J. A., Onyeanusi, B. I., Relwot, P. L., Ayo, J. O. and Dzenda, T. (2008). Seasonal variation in seminal characteristics of Shikabrown breeder cocks. *International Journal of Poultry Science*, 7(2):1219-1223

Oduguwa, O. O. (1991). Protein utilization by broiler hickens fed on three commerciaql premixes at the starter aqnd finisher phase. Ph.D. Thesis in the Department of Animal Scoence, University of Ibadan, Ibadan Nigeria

Oduguwa, O. O. and Ogunmodede, B. K. (1995). Growth and protein utilization by broiler chicks fed three commercial micronutrients mixture. *International Journal of Animal Science*, 10: 170-175

Oduguwa, O. O., Oduguwa, B. O. Fanimo, A. O. and Dipeolu, M. A. (2000). Potency of two proprietary micronutrients premixes for broiler chicken at marginally deficient protein content. *Archiva Zootechnical*, 49: 433- 444

Oduguwa, O. O., Ogunmodede, B. K. and Fanimo, A. O. (1996). Comparative efficacy of three commercial micronutrients premixes for rearing broilers at two physiological phases. *Pertankan Journal of Tropical Agriculture*, 19:81-86

Ogunmodede, B. K. (1974). Dietary zinc and protein utilization by growing chickens. *Nigerian Journal of Animal Producvtion, 1:198-203*

Ogunmodede, B. K. (1975). Comparative effects of copper and Terramycin on performance of broiler chicks and growers. *Nigerian Journal of Animal Production*, 2(2):199-203

Ogunmodede, B. K. (1977). Riboflavimn requirements of starting chickens in a tropical environment. *Poultry Science*, 56:231-234

Ogunmodede, B. K. (1978). Biotin requirement of broiler chicks fed maize-groundnut cake or guinea corn-groundnut cake ration. *African Journal of Agricultural Science*, v:47-53

Ogunmodede, B. K. (1981a).Vitamin A requirement of broiler chicks in Nigeria. *Poult. Sci.*, 60:116-121

Ogunmodede, B. K. (1982). Folic acid requirement of broiler chicks in a humid tropical environment. *Nigerian Journal of Nutritional Science,* 3 (1):31-39

Ogunmodede, B. K. (1991). Vitamin nutrition by poultry in warm climates. Paper presented at a conference organized by Roche Nigeria Limited, 6th March

Ogunmodede, B. K.(1981b). The supplementation of groundnut based broiler chick rations with ptridoxine. *British Poultry Science,* 32(3):153-164

Oguntunji, A. O. and 0Alabi, O. M. (2010). Influence of high environmental temperature on egg production and shell quality – A review. *World's Poultry Science Journal,* 66:739-750

Ogunwole A. O., Kolade, E. O. and Taiwo, B. A. (2012). Performance and Carcass Characteristics of Broilers Fed Five Different Commercial Vitamin-Mineral Premixes in Ibadan.*Nigeria International Journal of Poultry Science,* 11 (2): 120-124, 2012ISSN: 1682-8356

Ogunwole, O. A. (2009). Implications of nutritional additives in the diets of commercial growing and laying chickens.*Science and Technology* In: Panoy Link 3rd Ed., A publication of the Poultry Association of Nigeria (PAN), Oyo State chapter

Ogunwole, O. A., Ojelade, A. Y. P., Oyewo, M. O. and Essien, E.A. (2015a). Proximate composition and physical characteristics of eggs from laying chickens fed different proprietary vitamin-mineral premix under two rearing systems during storage. *International Journal of Food Science and Nutrition Engineering,* 5 (1): 59-67. DOI: 10.5923/j. food. 20150501.08.

Ogunwole, O. A., Oso, Y. A. A., Omotoso, R. R., Majekdunmi, B. C., Ayinde, B. O. and Oikei, I. (2013). Performance, carcass characteristics and meat physic-chemical properties of broiler chickens fed graded levels of supplemented ascorbic acid. *Agriculture and Biology Journal of North America,* 4 (4): 485-49

Ogunwole, O.A., Ojelade A.Y.P., Essien, E.A., Oyewo, M.O. (2015b). Lipid profile of eggs from laying chickens fed five proprietary vitamin-mineral premixes under two rearing systems as influenced by duration of storage. *Food and Public Health,* 5(1):10-16. doi: 10.5923/j.fph.20150501.02.

Ojedapo, L.O. (2013). Effect of two housing systems (Cages vs Deep Litters) on external and internal egg characteristics of commercial laying birds reared in derived savanna zone of Nigeria. *Transnational Journal of Science and Technology,* 3(7):25-34

Okeudo, N., Onwuchekwa, C. and Okoli, I. (2003). Effect of oil ttreatment and length of storage on the internal quality, organoleptic attributes and microbial profile of chicken eggs. *Tropical Animal and Production,* 6:63-70

Okeudo, Ndukwe James., Ezetoha Uchechi., Akomas Chinenye and Akanno, Everistus Chima (2005). Egg quality of *Gallus domesticus* under domestic storage in Nigeria. *Animal Research International,* 2(2):319 – 321

Okoli C. G, Okorondu, U. V. and Opera, M. N. (2006). Environmental and public health issues of animal food product delivery system in Imo state Nigeria. *Online/Journal of Health Allied Science,* 5(2):1-11

Okoli I. C. and Abi Udedibe (2003). Effect of oil treatment and storage on egg quality. *Journal of Agricultural Rural Develop,* 1:55-56

Olomu, J. M. (2011). Monogastric Animal Nutrition: Principles and Practices (2nd Ed.,). St. Jackson Publishing, Benin City, Nigeria

Optimun Vitamin Nutrition (O.V.N.) (2010). Improving the nutritive value of eggs by feeding laying hens.*An Optimum Vitamin Nutrition* (OVN™)

Oviedo-Rondón, E. O., Murakami, A. E., Furlan, A. C., Moreira, I. and Macari, M. (2001). Sodium and chloride requirement of young broiler chickens fed corn-soyabean diets (one to twenty-one day of age). *Poultry Science,* 80:592-598

Pan, S. (2005). Processing of gluten based bioplastics. *Biochemistry and Engineering Journal,* 26

Panda, P. C. (1996). Shape and Texture: In a Textbook on egg and poultry technology, 57

Pandey, N. K., Mahapatra, C. M., Verma, S. S. and Johari, D. C. (1986). Effect of strain on physical egg quality characteristics in White Leghorn chickens. *International Journal of Poultry Science*, 21:304–307.

Park, S. Y., Kim, W. K., Birkhold, S. G., Kubena, L.E., Nisbet, D. J. and Ricke, S. C. (2004). Induced moulting issues and alternative dietary strategies for egg industry in the United State. *World's Poultry Science Journal,* 60 (2):196-209

Parkinson T. L (1966). Solid contents of eggs. *Poultry Science*, 45:221-226.

Parmentier, H. K., Neuwland, M. G., Rijke, E., De Vries Reilingh, G. and Scrama, J. W. (1996). Divergent antibody responses to vaccine and divergent body weight of chicken lines selected for high and low humoral responsiveness to sheep red blood cells. *Avian Disease,* 40:634-644.

Pasquoal Carrazzoni de Menezes1, Evilda Rodrigues de Lima1, Juliana Pinto de Medeiros, Wanessa Noadya Ketruy de Oliveira1, Joaquim Evêncio-Neto (2012). Egg quality of laying hens in different conditions of storage, ages and housing densities. *Revista Brasileira de Zootecnia*, 41(9): 2064-2069

Pavlovski, Z., Hopic, S. and Lukic, M. (2001). Housing systems for layers and egg quality. *Biotechnology in Animal Husbandry,* 17:197-201

Pavlovski, Z., Masic, B. and Apostolov, N. (1981). Quality of eggs laid by hens kept on free range and in cages. In: proceedings of first European Symposium by World Poultry Science Association, 231-235

Pavlovski, Z., Škrbić, M., Lukić1, D., Vitorović, S. and Lilić, V. P. (2012). Shell quality–everlasting problem in Today Poultry Science. *Biotechnology in Animal Husbandry*, 28 (3):393-404

Pistikova, V., Hovorka, M., Vecerel, V., Strakova, E. and Suchy, P. (2006). The quality comparison of eggs laid by laying hens kept in battery cage and in a deep litter system. *Czech. Journal of Animal Science*, 51:318-325

Ponnampalam, E. N. (2011). Differential effects of natural palm oil, chemically–and enzymatically–modified palm oil on weight gain, blood lipid metabolites and fat deposition in a pediatric pig model. *Nutrition Journal*, 10(1):1-7

Popova-Ralcheva, S., Snedkova, V., Valchev, G. and Bozakova, N. (2009). The effect of the age and genotypte on morphological egg quality of parent stock hens. *Archiva Zootechinoa*, 12:24-30

Potts, P. L., Wasburn, K. W. and Hale, K. K. (1974). Shell evaluation of white and brown egg strains by deformation, breaking strength, shell thinckness and specific gravity 2 Stepwise regression analyses of egg characteristics on methods of assessing shell strength. *Poultry Science*, 53:2167- 2174

Price, E. O. (1984). Behavioural aspects of animal domestication. *The Quaterly Review of Biology*, 59 (1): 1-32

Pryor, W. (1991). The antioxidant nutrients and disease prevention. *American Journal of Clinical Nutrition*, 53: 391–393

Puthpongsiriporn, U., Scheideler, S. E., Sell, J. L. and Beck, M. M. (2001). Effect of vitamin E and C supplementation on performance *in vitro* lymphocytes proliferation and antioxant statu of laying hens during heat stress. *Poultry Science*, 80:1190-1200

Qi, G. and Sim, J. (1998). Natural tocopherol enrichment and its effect in n-3 fatty acid modified chicken eggs. *Journal of Agriculture and Food Chemistry*, 46:1920-1926

Quirino, B. J., de Sousa, F. G., Perazzo, C. R., Queiroga, R. E., Walter, E. and Luveia de Souza (2009). Effect of different metabolizable energy and soybean oil levels in the diet of laying hens on the egg chemical composition and lipid profile. *Research Brasil Zootechology*, 38(4):685-689

Qureshi, M. A. and Havenstein, G. B. (1994). A comparison of the immune performance of a 1991 commercial broiler with a 1957 randombred strain when fed ‘‘typical’’ 1957 and 1991 broiler diets. *Poultry Science*, 73:1805-1812

Raji, A. O., Aliyu, J., Igwebuike, J. U. and Chiroma, S. (2009). Effect of storage methods and time on egg quality traits of laying hens in a hot dry climate. *ARPN Journal of Agricultural Biology Science*, 4 (4):1-7

Raven, P. and Walker, G. (1980). Food and Agricultural Organization of United Nation. Reitman, S. and S Frankel. *American Journal of Clinical Pathology*, 1957:28-56

Reid, J., Watson, R. D., Cochran, J. B. and Sprows, D. H. (1951). Sodium γ-Resorcylate in Rheumatic fever. *British Medical Journal*, 321-326 (PMCID: PMC2069708)

Repetto, M., Ossani, G., Monserrat, A. and Boveris, A. (2010). Oxidative damage: *The Review of Biochemistry*, 72(13):774-778

Rizzi, L., Simioli, G., Martelli, G., Paganelli, R. and Sardi, L. (2006). Effects of organic farming on egg quality and welfare of laying hens. Proceeding of European Poultry Conference, Verona, Italy

Roberts, Juliet R. (2004). Factors affecting egg internal quality and egg shell quality in laying hens. *Journal of Poultry Science*, 41:161-177

Roland, D. A. (2000). Nutrition and feeding for optimum egg shell quality. XXI World’s Poultry Congress, Montreal, Canada (CD Proceedings)

Rolon, A., Burhr, R. J. and Cunningham, D. L. (1993). Twenty-four-hour feed withdrawal and limited feeding as alternative methods for induction of moult in laying hens. *Poultry Science*, 72: 776-785

Roque, L. and Soares, M. C. (1994). Effects of eggshell quality and broiler breeder age on hatchability. *Poultry Science*, 73:1838-1845

Rose, Christmas, Ross, A. Harms, R. H. and Sloan, D. R. (1997). The absence of vitamins and trace minerals and broiler performance. *Journal of Applied Poultry Research,* 4: 407-410

Rossi, M. (2007). Influence of the laying hen housing systems on table egg characteristics. Proc. XVIII European Symp on the Quality of Poultry Meat and XII Eur. Prague, Czech Republic, 49-51

Roura, E., Homedes, J. and Klasing, K. C. (1992). Prevention of immunologic stress contributes to the growth-permitting ability of dietary antibiotics in chicks. *Journal of Nutrition*, 122:2383-2390

Ruxton, C., Reed, S., Simpson, M. and Millington, K. J. (2007). The health benefits of omega-3 polyunsaturated fatty acids: A review of the evidence. *Journal of Human Nutrition Diet,* 20:275–285

Safaa H. M., Serrano M. P., Valencia D. G., Frikha M., Jiménez-Moreno, E., Mateos G.G. (2008). Productive Performance and Egg Quality of Brown Egg-Laying Hens in the Late Phase of Production as Influenced by Level and Source of Calcium in the Diet. *Poultry Science*, 87:2043-2051

Sahin, K. and Küçük, O. (2001). Effects of vitamin C and vitamin E on performance, digestion of nutrients, and carcass characteristics of Japanese quails reared under chronic heat stress (34°C). *Journal of Animal Physiology and Animal Nutrition, 85,* 335–342

Samli, H. E., Agma, A. and Senkoylu, N. (2005). Effect of storage time and temperature on egg quality in old laying hens. *Journal of Applied Poultry Research,* 14: 548-553

Santos, C. O. (1998). Efeito da adicao de oleos poliinsaturados a racao nos niveis de lipidios plasmaticos e de colesterol no ovo de galinhas poedeiras [dissertacao], Sao Paulo (SP): Universidade de Sao Paulo

SAS Institute Inc. (2012). SAS STAT User's Guide Release 6.08, SAS Institute Inc., Cary, NC

Savory, C. J., Wood-Gush, D. G. M. and Duncan, I. J. H. (1978). Feeding behaviour in apopulation of domestic fowls in the wild. *Apllied Animal Ethology,* 4:13-27

Scatolini A. M. (2007). Mn, Zn e Se associados a moléculas orgânicas na alimentação de galinhas poedeiras no segundo ciclo de produção [dissertation]. Jaboticabal (SP): Universidade Estadual Paulista

Scientific Panel on Animal Health and Welfares (2005). Opinion of the Scientific Panel on Animal Health and Welfares as a request from Commission related to the welfare aspect of various system of keeping laying hens. *The EFSA Journal* 197:1.23. www.efsa.europa.eu/EFSA/Scientific_Opinion/lh_opinion1.pdf

Scott, M. L., Nesheim, M. C. and Young, R. J. (1982). Nutrition of the chicken 3rd Ed. M.L Scott and Associates, Ithaca, NY, USA, 119

Scott, T. A. and F. G. Silversides (2000). The effect of storage and strain of hen on egg quality. *Poultry Science*, 79:1725-1729.

SDC-ESSA (1970). Ecology and Animal Health. (Leif Norrgen and Jeffy M. Levengood Eds.), Baltic University Press, Amazon

Sell, J. L., Arthur, J. A. and Williams, I. L., (1982). Adverse effects of dietary vanadium contributed by dicalcium phosphate on egg albumin quality. *Poultry Science,* 61: 1541

Senkoylu N., Samli, H. E., Akyurek, H., Agma, A. and Yasar, S. (2005). Performance and Egg Characteristics of Laying Hens Fed Diets Incorporated with Poultry By-Product and Feather Meals. *Journal of Applied Poultry Research*, 14:542–547

Seven P. T. (2008). The Effects of Dietary Turkish Propolis and Vitamin C on Performance, Digestibility, Egg Production and Egg Quality in Laying Hens under Different Environmental Temperatures. *Asian-Australian Journal of Animal Science,* 21:1164-1170

Shafey, T.M. and Cham, B. E. (1994). Altering fatty acid and cholesterol contents of eggs for human comsuption. Washington: *CAB International*, 374-385

Shawkat, Md Ali (2002). Study on the effect of feed supplementation to laying hen under the rural condition of Bangladesh (M.Sc Thesis), The Royal Veterinary and Agricultural University, Dyrlægevej, 1870 Frederiksberg C., Denmark.

Short, F. J. (2001). Egg shell density in furnished cages: Effect of dustbath and perch provision. *British Poultry Science,* 41 (suppl.1):77-78

Siegel, H. S., Henken, A. M., Verstegen, M. W. A. and Van Der Hel, W. (1982).Heat production during the induction of an immune response to sheep red blood cells in growing pullets.*Poultry Science* 61:2296-2300

Silversides, E. G. (1994). The Haugh unit correction for egg weight is not adequate for comparing eggs from chickens of different lines and ages. *Journal of Applied Poultry Research,* 3:120-126

Silversides, F. G. and Budgell, K. (2004). The relationship among measures of egg albumen height, pH and whipping volume. *Poultry Sci*ence, 83:1619-1623

Silversides, F. G. and Scott, T. A. (2001). Effect of storage and layer age on quality of eggs from two lines of hens. *Poultry Science,* 80:1240-1245

Silversides, F. G., Korver, D. R. and Budgell, K. L. (2006). Effect of Strain of Layer and Age at Photostimulation on Egg Production, Egg Quality, and Bone Strength. *Poultry Science,* 85:1136-1144

Simčič, M., Stibilj, V. and Holcman, A. (2009). The cholesterol content of eggs produced by the Slovenian autochthonous Styrian hen. *Food Chemistry*, 114, 1-4.

Simopoulos, A. P. (2000). Human requirement for n-3 polyunsaturated fatty acids. *Journal of Poultry Science*, 79:961-970

Singh, K. S. and Panda, B. (1988).Nutrition and quality of poultry products. *Poultry Nutrition*, 159-161

Singh, R., Cheng, K. M. and Silversides, F. G. (2009). Production performance and egg quality of four strains of laying hens kept in conventional cages and floor pens. *Poultry Science*, 88:2 56–264

Sinha, P. and Giri, A. K. (1989). Consumption of Livestock Products-Analysis and Comparison of Data of NSS 32nd and 38th Round Livestock Economy of India. Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi

Skinner, J. T., Waldroup, A. L. and Waldroup, P. W. (1992). Effect of removal of vitamin and mineral supplements from grower and finisher diets on live performance and carcass composition of broilers. *Journal of Appled Poultry Research*, 1:280-286

Škrtić, G., Kralik, Z., Gajčević, I., Bogut, D. and Hanžek, Z. (2007). The Increase of the N-3 Pufa Content in Eggs. *Danish Journal*, 22(4):23–34

Smith, D. M. Jr., Milelke, H. W. and Heneghan, J. B. (2008). Sub-clinical lead feeding studing in male rats, Archiva Environment al *Contamination and Toxicology* 55(3):518-528

Smith, J. A., Ackerman, A. S., Jensen, E. J. and Toon, O. B. (2006). Role of deep convection in establishing the isotopic composition of water vapor in the tropical transition layer. *Geophys. Research Letter*, 33, L06812, doi:10.1029/2005GL024078.

Song K. T., Choil, S. C. and Oh, H. R. (2002). A comparison of egg quality of pheasant, chukar, quail and guinea fowl. *Asian-Australian Journal of Animal Science*, 13(7): 986-990

Stadelman, W. J. (1977). Quality identification of shell eggs: In *Egg Science and Technology*. 2nd ed., W. J. Stadelman and D. J. Cotterill, eds. AVI Publishing Company Inc., Westport, CT

Stadelman, W. J. (1995). Quality identification of shell eggs In: Egg Science and Technology, 4th rev. W. J. Stadelman and O. J. Cotterill eds. Food Products Press, Binghamton New York, 39-66

Stadelman, W. J. and Coterill, O. J. (1995). Egg Science and Technology, 4th rev. W. J. Stadelman and O. J. Cotterill eds. Food Products Press, Binghamton New York, 39-66

Stamler, J., Pick, R. and Katz, L. N. (1958). Effect of dietary protein and carbohydrate levels on cholesterolemia and athrogenesis in cockerels on a high-fat, high-cholesterol mash. *Circulation Research*, 6: 447-451

Supić, B., Cmiljanić, R., Savić, S., Milošević, N., Kočiš, I. and Jakobčić, Z. (1997). Uticaj vitamina C na proizvodnju i kvalitet ljuske jaja za konzum, I Jugoslovenski medjunarodni kongres o stočarstvu. *Biotehnologija u stočarstvu,* 3(4):177-186

Surai, P. (2003). Natural antioxidants in avian nutrition and reproduction. Nottingham University Press, England

Suttle N. F. and Jones, D. G. (1989). Recent developments in trace element metabolism and function. Trace elements, disease resistance and immune responsiveness in ruminant. *Journal of Nutrition*, 119:1055-1061

Tabidi M. H. (2011). Impact of storage period and quality on composition of Table egg. *Advance Environmental Biology*, 5(5):856-861

Takahashi, K., Ohta, N. and Akiba, Y. (1997). Influence of dietary methionine and cysteine on metabolic responses to immunological stress by *Escherichia coli* lipopolysacchar*ide* injection, and mitogenic response in broiler chickens. *British Journal of Nutrition*, 78:815-821

Tanaka, T. and Hurnik, J. F. (1992). Comparison of behavior and performance of laying hens housed in battery cages and an aviary. *Poultry Science*, 71:235–243

Tao, X. and Xin, H. (2003). Temperataure-humidity-velocity-indexfor market size broiler. Proc. of the ASAE 46:491-497

Tappel, A. L. (1968). Will antioxidant nutrients slow aging processes? *Geriatrics,* 23

Tauson, R., Wahlström, A. and Abrahamsson, P. (1999). Effect of two floor housing systems and cages on health, production, and fear response in layers. *Journal of Applied Poultry Research*, 8, 152–159

Taylor, T. G. (1965). Dietary phosphorus and egg shell thick-ness in the domestic fowl. *British Poultry Science,* 6:79-87

Tebesi T., Madibela, O. R and Moreki, J. C. (2012). Effect of storage time on internal and external characteristics of Guinea fowl (*Numida meleagris*) eggs. *Journal Animal Science Advance,* 2(6):534-542

Tharrington, J. B., Curtis, P. A., Jones, F. T. and Anderson, K. E. (1999). Comparison of physical quality and composition of eggs from historic strains comb White Leghorn chickens. *Poultry Science,* 78: 591-594

Thornton, P. A. (1962). The effect of environmemtal temperature and oxygen uptake by the chicken. *Poultry Science,* 41:1053-1062

Tilki, M. and Inal, S. (2004). Quality traits of goose eggs, 1 Effect of goose age and storage time of eggs. *Archiva Gefl ügelk.*, 68:182-186

Toney, J. and Bergquist, D. H. (1983). Functional egg products for the cereal foods industries. *Cereal Foods World*, 28, 445–447.

Toussant, M. J. and Latshaw, J. D. (1999). Ovomucin content and composition in chicken eggs with different interior. *Journal of Food Science and Agriculture,* (79):1666-1670

Tufft, L.S., Nockels, C. F. and Fettman, M. J. (1988). Effect of *Escherichia coli Escherichia coli* on iron, copper, and zinc metabolism in chicks. *Avian Disease,* 32:779-786

Tyler, C. (1961). Shell strength: Its measurement and its relationship to other factors. *British Poultry Science,* 2, 3-19.

U.S. Poultry and Egg Association (2012). Poultry Industry frequently asked questions. Retrieved June 21 htt://www.uspoultry.org/economic_data

United States Department of Agriculture (USDA) (2000). United States standards, grades, and weight classes for shell eggs. *Agricultural Research Note, RN* 23 12/99

Untied Egg Producer (2003). European Union Standards: Housing, space, feed and water. 2003-09-03

Untied Egg Producer (2009). European Union Standards: Housing, space, feed and water. *Washington Post*, 2003-09-03 Retrieved 2009-07-30

Usturoi M. G., Boişteanu, P. C., RaduRusu, R. M., Pop, I. M., Doliş, M. G. and Al. Usturoi (2010). Influence of husbandry technologies with horizontal disposing on the performances achieved by the Lohmann Brown hybrid. International Conference on Animal Science and Veterinary Medicine, Tokyo-Japonia, Academic Science Research, Cemal Ardil Editor-in-Chief, 88

Van den Bran, D., Parmentier, H. and Kemp, B. (2004). Effects of housing system (outdoor vs cages) and age of laying hens on egg characteristics. *British Poultry Science*, 45 (6):745-752

Van Niekerk (2014). Egg quality: Low Input Breeds Technical Note. Download at www.lowinputbreeds.org

Van-Elswky, M. E., Hargis, B. M., Willians, J. D. and Hargis, P. S. (1994). Dietary menhaden oil contributes to hepatic lipidosis in laying hens. *Poultry Science*, 73(5):653-662

Vansudevan, D. M., Sreekumari, S. and Kanna, Vaidyanathan (2011). Textbook of Biochemistry for Medical Students (6th ed.,) Jaypee Brothers Medical

Vargas, R. and Naber, E. (1984). Relationship between dietary fiber and nutrient density and its effect on energy balance, egg yolk cholesterol and hen performance. *Journal of Nutrition*, 114:645–652

Verma, S. V. S., Gowda, S. K. and Elangovan, A. V. (1998). *Animal Feed Science and Technology*, 76(1-2):169-175

Vicenzi, E. (1996). Fadiga de gaiola e qualidade da casca do ovo. Aspectos nutricionais. Anais do 6º Simpósio Técnico de Produção de Ovos, *São Paulo, SP. Brasil*, 77-91

Victor G Stanley, Dacian Nelson and Milton B Daley (2013). Evaluation of Two Laying Systems (Floor vs. Cage) on Egg Production. Quality, and Safety, *Agrotechnology*, 2:1http://dx.doi.org/10.4172/2168-9881.1000109

Vidal, F. T., Ana, L., Virgínia, K. G., Abreu, E., Freitas, M. A., Sousa Neto, S. and Jorge, F. (2013). Egg quality and yolk lipid composition of laying hens fed diets containing cashew nut meal. *Journal of Food Science and Technology Campinas*, 33(1): 172-179

Vitorović D., Pavlovski Z., Nikolovski J., Djurdjević Z., Todorović M. (1995). Kvalitet ljuske i dalje aktuelan problem avremenog živinarstva. IV Medjunarodni simpozijum "Novi pravci razvoja stočarstva", *Beograd, Biotehnologija u stočarstvu,* 3(6):301-306

Vits, A., Weizenburger, D., Hamann, H. and Distl, O. (2005). Influence of different small group systems on production traits, egg quality and bone breaking strength of laying hens. First communication: Production traits and egg quality. *Zuchtungskunde,* 77:303–323

Vogt, X., Dewar, W. A., Sauveur, B. and Simons, P.C.M. (1984). Mineral requirements and recommendations for adult birds. *World's Poultry Science Journal*, 40:183

Voslářová, E., Hánzálek, Z., Večerek, V., Stráknsá, E. and Scchý, P. (2006). Comparison between laying hens performance in the cage system and the deep litter system on adiet free from animal protein. *Acta Veterinaria Brno.,* 76:219-225

Vulleumier, J. P. (1968). The Roche Yolk Colour Fan-An instrument for measuring Yolk Colour. *Poultry Science,* 48:767-783

Waimaleongora-Ek, P., Garcia, K. M., No, H. K., Prinyawiwatkul, W. and Ingram, D. R. (2009). Selected quality and shelf-life of eggs coated with mineral oil with different viscosities. *Journal of Food Science*, 74:423–S429

Walker, A. W. and Hughes, B. O. (1998). Egg shell colour is affected by laying cage design. *British Poultry Science,* 39(5):696-699

Wang, X. L., Zheng, J. X., Ning, Z. H., Qu, L. J., Xu, G. and Yang, N. (2009). Laying performance and egg quality of blue-shelled layers as affected by different housing systems. *Journal of Food Science and Technology*, 4 (2): 45-48.

Washbourn K. (1982). Incidence, cause and prevention of egg shell breakage in commercial production. *Poulttry Science*, 61:2005-2012

Wheeler, R. S. and James Jr., E. C. (1950). The problem of wet poultry house litter: The influence of total chicken. *Poultry Science*, 29:496-500

Whitehead C. C. (1996). Nutrition and bone disorders in poultry, Proceedings of XX World's Poultry Congress. New Delhi II, 161-171

Whitehead, C. C. (1998). Vitamin interactions and requirements in poultry: In 7th International Symposium on Animal Nutrition, Kaposvar, Hungary 3-31

Whitehead, C. C. and Fleming, R. H. (2000). Osteoporosis in cage layers. *Poultry Science,* 79:1033-1041

William, K. C. (1992). Some factors affecting albumen quality with particular reference to Haugh unit score. *World's Poultry Science Journal,* 48:5-16

Wilson, H. R. (1991). Interrelationships of egg size, chick size, post hatching growth and hatchability. *World's Poultry Science Journal*, 47:5–20

Wong, Y. C., Herald, T. J. and Hachmeister, K. A. (1996). Evaluation of mechanical and barrier properties of protein coatings on shell eggs. *Poultry Science*, 75:417–22

Wuryastuti, H., Stowe, H. D., Bull, R. W. and Miller, E. R. (1993). Effect of vitamin E and selenium on immune response peripheral blood, colostrum and milk leukocytes of sows. *Journal of Animal Science*, 71: 2464

Yoruk M. A., Gul, M. and Hayirli, A. (2004). Laying performance and egg quality of hens supplemented with sodium bicarbonate during late laying period. *International Journal of Poultry Science,* 3:272-278

Yosefi, S., Braw-Tal, R. and Bar, A. (2003). Intestinal and eggshell calbindin and bone ash of laying hens as influenced by age and moulting. *Comparative Biochemistry and Physiology*, 136: 672-683

Zaghini A., Martelli G., Roncada P., Simioli M., Rizzi L., (2005). Mannanoligosaccharides and aflatoxin B1 in feed for laying hens: effects on egg quality, aflatoxin B1 and M1 residues in eggs, and aflatoxin B1 levels in liver. *Poultry Science,* 84:825-32

Zamani, A., Bahmani, H. and Pourreza, J. (2005). Effect of different levels of manganese and zinc supplement on the performance traits and egg quality of laying hens. *Pakistan. Journal of Biological Science*, 8:1035-1040

Zdunczyk, Z., Gruzauskas, R., Semaskaite, A., Juskiewicz, J., Raceviciute-Stupeliene, A. and Wroblewska, M. (2011). Fatty acid profile of breast muscle of broiler chickens fed diets with different levels of selenium and vitamin E. *Archiva Geflügelk*, 75:264-267

Zeidler, G. (2002). Shell Egg Quality and Preservation. In: Bell DD, Weaver WD, editors. Commercial chicken meat and egg production 5th ed., Norwell, Mass.: Kluwer Academic Publishers, 1190–1217

Zemková, Ĺ., Simenová, J., Lichovníkoá, M. and Somerlíková, K. (2007). The effects of housing systems and age of hens on the weight and cholesterol concentration of the egg. *Czech Journal of Animal Science*, 52(4): 110-115

Zhang, M. H., Lin, W. Y., Klein, S. A., Bacmeister, J. T., Bony, S., Cederwall, R. T., Del Genio, A. D., Hack, J. J., Loeb, N. G., Lohmann, U., Minnis, P., Musat, I., Pincus, R., Stier, P., Suarez, M. J., Webb, M. J., Wu, J. B., Xie, S. C., Yao, M. S. and Zhang, J. H. (2005). Comparing clouds and their seasonal variations in 10 atmospheric general circulation models with satellite measurements. *Journal of Geophysics Research*, 110, D15S02, doi:10.1029/2004JD005021.

Ziggers, G. W., Driessen, P. H. and Bloemer, J. M. (2011). Dynamics and Innovation in Food Networks. Proceedings of the 5th International European Forum, Innsbruck-Igls, AustriaGriffin, H. D. and Butterwith, S. C. (1988). Effect of eschericia coli endotoxin on tissue lipoprotein lipase activities in chickens. *British Poultry Science*, 29:2, pages ...

2012-2017 www.latlong.net//place/ibadan-nigeria-178.html