Optimal energy to total Lys ratios for broiler performance from day old to 35 days of age

FRANSCOIS CROTS

Submitted in partial fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY
In Poultry Nutrition

University of Kwazulu-Natal

Supervisor: Dr. Mariana Ciacciariello

July 2017

Abstract

A literature review revealed that broiler performance was influenced by varying energy to total lysine ratio's. A study was conducted with the aim of the experiments to study the response of broilers to varying total Lys and energy (ME) levels and their responses to the interaction between these two nutrients. The study comprised four independent experiments. Experiment 1 was conducted during the day-old to 7-day period. Experiment 2 were conducted during the 7 to 14-day period. Experiment 3 during the 14 to 28-day period and Experiment 4 during the 28 to 35-day period. In the period leading up to Experiments 2, 3 and 4, birds received the same starter and/or grower feed. In all four experiments, Ross 308 mixed male and female broilers were fed one of twelve rations. In Experiment 1, the dietary treatments consisted of four total Lys levels (1.77, 1.60, 1.40 and 1.30%) and three metabolisable energy (ME) levels (2700, 2900 and 3100 kcal ME/kg). The use of the dietary dilution technique, the combination of total Lys and energy levels, resulted in various ME: total Lys levels (1525, 1638, 1751, 1688, 1813, 1938, 1929, 2071, 2214, 2077, 2231, and 2385). In Experiment 2, the dietary treatments consisted of four total Lys levels (1.77, 1.60, 1.40 and 1.30%) and three ME levels (2700, 2900 and 3100 kcal ME/kg). The use of the dietary summit/dilution technique, the combination of total lysine and energy levels, resulted in various ME: total Lys levels (1525, 1638, 1751, 1688, 1813, 1938, 1929, 2071, 2214, 2077, 2231, and 2385). In Experiment 3, the dietary treatments consisted of four total Lys levels (1.56, 1.38, 1.23 and 1.08%) and three ME levels (2800, 3000 and 3200 kcal ME/kg). The use of the dietary summit/dilution technique, the combination of total Lys and energy levels, resulted in various ME: total Lys levels (1795, 1923, 2051, 2029, 2174, 2319, 2276, 2439, 2602, 2593, 2778 and 2963). In Experiment 4, the dietary treatments consisted of four total Lys levels (1.42, 1.25, 1.11 and 0.96%) and three ME levels (2800, 3050 and 3300 kcal ME/kg).

The use of the dietary summit/dilution technique, the combination of total Lys and energy levels, resulted in various ME: total Lys levels (1972, 2148, 2324, 2240, 2440, 2640, 2523, 2748, 2973, 2917, 3177 and 3438). Each one of the 12 treatments was replicated eight times.

In Experiment 1, the ADG (average daily gain) improved with an increase in the total Lys. However, ADG were not statistically influenced with an increase in the level of energy. The point of maximum response for ADG was achieved at 1.48% total Lys. However, ADFI (average daily feed intake) tended to be less responsive with two treatments only consuming differently compared to the other nine treatments. FCR (feed conversion ratio) were significantly improved with higher ME levels. For ADG, the point of maximum response were 1.60% total Lys, 3100 kcal/kg, thus implying a ME: total Lys ratio of 1938. For ADFI, the point of maximum response were 1.40% total Lys, 2700 kcal ME/kg, thus implying a ME: total Lys ratio of 1929 while, for FCR, the point of maximum response were 1.77% total Lys, 3100 kcal/kg, thus implying a ME: total Lys ratio of 1751.

In Experiment 2, the ADG of the broilers improved with an increase in total Lys content. The quadratic regression revealed that the point of maximum response for ADG was achieved at 1.61% total Lys and that 1.64% total Lys was needed for FCR. ADFI increased significantly when the total Lys was reduced below 1.60% total Lys while ADG was significantly improved when the energy was increased from 2700 to 2900 kcal ME/kg ME levels. FCR was significantly reduced for each increased level in energy. For ADG, the point of maximum response were 1.77% total Lys, 3100 kcal/kg, thus implying a ME: total Lys ratio of 1751. For FCR, the point of maximum response were 1.60% total Lys, 3100 kcal/kg, thus implying a ME: total Lys ratio of 1751.

In Experiment 3, the ADG of the broilers increased from 1.08 to 1.38% total Lys and was significantly reduced at the highest level of 1.56%. The quadratic regression revealed that the point of maximum response for ADG was achieved at 1.37% and that 1.34% total Lys was needed for FCR. There was no significant difference in ADFI between total Lys treatments. ADFI were significantly lower and FCR significantly higher when the energy was reduced from 3000 to 2800 kcal ME/kg. For ADG, the point of maximum response were 1.38% total Lys, 2800 kcal, thus implying a ME: total Lys ratio of 2029 while, for FCR, the point of maximum response were 1.56% total Lys, 3200 kcal ME/kg, thus implying a ME: total Lys ratio of 2051.

Broilers' breast meat percentage was significantly more and abdominal fat percentage significantly lower at the highest test level of total lysine of 1.56%. The broilers' breast meat and protein percentage was significantly more and abdominal fat and carcass fat percentage significantly lower at the highest test level of total lysine. Energy levels had no significant effect on broiler carcass breast meat, abdominal fat, protein and fat (%) parameters. It would appear that the response of broilers carcass breast meat (%), abdominal fat (%) as well as that carcass protein (%) and fat (%) due to varying ME: total Lys is influenced more by the total lysine (%) level and to a lesser extent due to the energy level in the feed.

In Experiment 4, the ADG were not significantly different for the four total Lys levels. The quadratic regression revealed that the point of maximum response for FCR was 3250 kcal ME/kg. ADFI were significantly lower with higher total Lys treatments while FCR was significantly improved when the total Lys was increased from 1.11 to 1.25% and further significantly improved when the total Lys was increased to 1.42%. Increasing the energy from 2800 to 3300 kcal resulted in significantly lower ADG, ADFI and FCR.

The pellet durability was significantly reduced when the energy was increased from 2800 to 3300 kcal/kg. This was probably responsible for the significantly lower ADG, ADFI and higher FCR at 3300 kcal ME/kg vs. the 2800 kcal ME/kg treatments. For ADG, the point of maximum response were 1.42% total Lys, 2800 kcal/kg, thus implying a ME: total Lys ratio of 1972. For FCR, the point of maximum response were 1.42% total Lys, with 3050 kcal implying a ME: total Lys ratio of 2148.

Broilers' breast meat percentage was significantly more at the highest test level of total Lys of 1.42% as compared to the lowest total Lys of 0.96%. Considering all the carcass data observations in the response of broiler fed varying ME: total Lys levels, it would appear that the total Lys levels have a more pronounced effect on the body carcass composition compared to the energy level of the diet.

The statistical evaluation of the response of broilers (0–7 days of age) to varying levels of ME: total Lys ratio gave rise to the following equations for ADG, ADFI and FCR:

$$ADG = 1.0000 + -0.9979 \text{ x (ME: total Lys ratio)} + 0.9919 \text{ x (ME: total Lys ratio}^2\text{)}$$

$$(R^2 = 0.37) \quad [2.3]$$

$$FCR = 0.2493608 + 0.000893\text{x (ME: total Lys ratio)} + -0.0000001833 \text{ x (ME: total Lys ratio}^2\text{)}$$

$$(R^2 = 0.39) \quad [2.4]$$

$$ADFI = 4.2932566 + 0.0099603 \text{ x (ME: total Lys ratio)} + 0.0003807 \text{ x (ME: total Lys ratio}^2\text{)}$$

$$(R^2 = 0.08) \quad [2.5]$$

The statistical evaluation of the quadratic response of the broilers (7-14 days of age) to varying levels of ME: total Lys ratio yielded in the following equations for ADG, ADFI and FCR:

$$ADG = -3.108766 + 0.0389846 \times (ME: total\ Lys\ ratio) + -0.00001038 \times (ME: total\ Lys\ ratio^2)$$

$(R^2 = 0.40)$ [3.3]

$$FCR = 2.0683794 + -0.000729 \times (ME: total\ Lys\ ratio) + 0.00000022155 \times (ME: total\ Lys\ ratio^2)$$

$(R^2 = 0.39)$ [3.4]

$$ADFI = 11.619103 + 0.0367668 \times (ME: total\ Lys\ ratio) + -0.000008828 \times (ME: total\ Lys\ ratio^2)$$

$(R^2 = 0.17)$ [3.5]

The statistical evaluation of broilers (14-28 days of age) ADG, ADFI and FCR revealed a quadratic response to varying levels of ME: total Lys ratio:

$$ADG = 33.606973 + 0.0302445 \times ME: total\ Lys + -0.00000684 \times ME: total\ Lys^2$$

$(R^2 = 0.43)$ [4.3]

$$FCR = 4.043189 + -0.002338 \times ME: total\ Lys + 0.00000049807 \times ME: total\ Lys^2$$

$(R^2 = 0.31)$ [4.4]

$$ADFI = 221.00755 + -0.112043 \times ME: total\ Lys + 0.0000233 \times ME: total\ Lys^2$$

$(R^2 = 0.22)$ [4.5]

The statistical analysis of the response of the broilers (28–35 days) to total Lys for ADG, ADFI and FCR revealed a quadratic response to varying levels of ME: total Lys ratio:

$$ADG = 136.87248 + -0.045396 \times ME: total\ Lys\ ratio + 0.0000077407 \times ME: total\ Lys\ ratio^2$$

$(R^2 = 0.30)$ [5.3]

$$FCR = 0.1641301 + 0.0011762 \times ME\text{: total Lys ratio} + -0.0000001848 \times ME\text{: total Lys ratio}^2$$

$$(R^2 = 0.37) \quad [5.4]$$

$$ADFI = 127.57202 + 0.003209 \times ME\text{: total Lys ratio} + 0.00000059861 \times ME\text{: total Lys ratio}^2$$

$$(R^2 = 0.07) \quad [5.5]$$

It may thus be concluded that there is no single ME: total Lys ratio for the modern broiler to achieve maximum or optimum ADG or FCR. On the other hand, it would appear that the ratio changes as the broiler ages but also that there is an optimum ME: total Lys ratio for each total Lys ratio.

An interesting comparison that can be drawn is that the R^2 of the ADG and FCR regressions obtained from feeding the 0-21 days old broilers a range of varying ME: total Lys varied significantly between the literature review and that obtained from this study. For ADG the literature review produced a R^2 of 0.89 and for FCR the R^2 was 0.85. Experiment 2 that was conducted from 7 to 14 days produced a ADG of R^2 of 0.40 and for FCR the R^2 was 0.39. A lower R^2 indicate that the model does not describe the data set that well. A possible explanation for this anomaly is that the literature review employed a wider range of ME: total Lys values (1641 to 6275) than that what was applied in this study (1525 to 2385). The literature review presented energy values that ranged from 2796 ME kcal/kg to 3274 ME kcal/kg while for the same period of evaluation in this study (7-14 days), the energy values varied only between 2700 and 3100 ME kcal/kg. Additionally, the literature reviewed employed a total Lysine that varied between 0.5 to 1.85% while in Experiment 2, the total Lys levels varied only between 1.30 and 1.77%. It can be postulated that if wider ME: total Lysine ranges were employed the R^2 would have been higher. However, based on the trial facility design and commercial application desired form this study, more treatments were not possible.

The prediction regressions of broilers’ response to varying ME: total Lys ratios do however have two major limitations. Firstly, the model makes no provision for environmental conditions. If the environmental temperature increases above a threshold value, the ME: total Lys ratios would probably increase until the bird is capable of consuming an amount of energy without increasing its heat production. Secondly, the model makes no provision for economic conditions. If the price of energy sources were high relative to protein sources, then a diet with a higher ME: total Lys ratio than the biological ME: total Lys ratio could be formulated. Conversely, if the price of protein sources were low relative to energy sources, a diet with a lower ME: total Lys ratio than optimum biological ME: total Lys ratio could be formulated. Another important concept to understand is that although the ME: total lysine regressions present commercial application, one must remember that that the same ME: total lysine ratio can be achieved with various combinations of energy and lysine. For example, a diet with 3000 kcal ME/kg and 1.50% total Lys will provide a ratio of 2000. However, the ratio of ME: total Lys of 2000 can also be achieved with 2900 kcal ME/kg and 1.45% total Lys. The economical optimum is thus a combination of lysine, energy and the ME: total lysine ratio, all balanced in relation to the prevailing prices of energy and protein resources.

General introduction

During the past five decades, intensive genetic selection for growth rate in broilers has resulted in a maximum daily rate of BW gain – at least 2.5 times that which was achieved in the 1950s (Havenstein *et al.*, 2003). Although primary breeding companies, i.e. Aviagen (2014) and Cobb-Vantress (2012) update their broiler nutrition specifications on a regular basis, it can only be applied as a guideline and not as the only standard specification. The reason for the guideline is due to the fact that at any given time, the optimum cost and coincidently optimum diet specification for broiler production at various countries at the same given time is not the same. Based on the prevailing meat and raw material cost, nutritionist's would adapt their dietary specifications to ensure that broiler meat is produced at the lowest cost optimum.

Commercial nutritionist has a range of tools that they can apply to get a better understanding of the cost optimum. Some of the tools include, linear/empirical models which is commercially available as the Brazilian tables (Rostagno, 2005) and growth models/mechanistic which is commercially available as the EFG model (2017). However, none of these models have been accepted as the standard practise by commercial nutritionist's. There is multitude of reasons for thus phenomena, but the general reasons commonly provide is that "the models are to complex" or that "the predictions of the model and the actual sometimes to differ too much" or even that "the modern broilers nutritional requirement has so many complexities that it cannot be simplified by in any of the current models". My view is that we need to get a better understanding of what is the fundamental drivers of these models and to which extent can variables affect the prediction. The objective of this thesis was thus to get a better understanding of how the modern broilers would react to a given range of ME: total Lys ratios at various ages and to integrate the findings of this study possible later into a present day mechanistic model.

Acknowledgements

I would like to express my gratitude towards Dr. Mariana Ciacciariello, Prof. John Brake and Gerrit Burgers who all provided me with insight, support and allowed me latitude to investigate the identified area of interest. Additionally, I would like to sincerely like to thank the staff at Ukulinga farm, without their assistance the trail would not have been possible.

"He gives power to the weak and strength to the powerless. Even youths will become weak and tired, and young men will fall in exhaustion. But those who trust in the Lord will find new strength. They will soar high on wings like eagles. They will run and not grow weary. They will walk and not faint" (Isaiah 40:27)

Declaration

I declare that the field study hereby submitted for the DOCTOR in PHILOSOPHY at the Department of Wildlife Sciences, University KwaZulu-Natal, is my own independent work and that I have not previously submitted this work, either in whole or in part, for a qualification at another university or at another faculty of this university. I also hereby cede copyright of this work to the University of KwaZulu-Natal.

Signature Date: 2017/07/23

I, Dr. Mariana Ciacciariello, chairperson of the supervisory committee, approve the release of this thesis for examination.

Signature Date:

Table of contents

Abstract……………………………………………………………………………. 1

General introduction ………………………………………………………………… 8

List of figures ……………………………………………………………………... 16

List of tables ………………………………………………………………………. 20

List of abbreviations ………………………………………………………………… 24

Chapter 1: ……………………………………………………………………... 25

Literature Review…………………………………………………………………. 25

1.1 Abstract…………………………………………………………………………... 25

1.2 Introduction……………………………………………………………………… 25

1.3 Materials and methods…………………………………………………………… 26

1.4 Results and discussion…………………………………………………………… 28

1.5 Conclusion and applications…………………………………………………….... 32

Chapter 2: ……………………………………………………………………… 35

The response of mixed sex broilers to varying dietary Energy: total Lys ratios fed from day old to 7 days of age………………………………………………………………… 35

2.1 Abstract…………………………………………………………………………... 35

2.2 Introduction……………………………………………………………………… 36

2.3 Material and methods…………………………………………………………… 39

2.4 Experimental diets……………………………………………………………… 39

2.4.1 Crumble size and quality index…………………………………………………... 40

2.5 Data collection…………………………………………………………………… 40

2.6 Statistical analysis……………………………………………………………….... 41

2.7 Results……………………………………………………………………………. 41

2.7.1 The effect feed particle size and the interaction with the ME: total Lys ratio………. 41

2.7.2 Animal performance……………………………………………………………… 45

2.7.2.1 The effect of feeding varying energy (ME): total Lys ratio's to mixed sex broilers from day old to 7 days of age……………………………………………………….. 45

2.7.2.2 The effect of feeding varying total Lys levels on mixed sex broilers from day old to 7 days of age………………………………………………………………………. 48

2.7.2.3 The effect of feeding varying energy (ME) levels on mixed sex broilers from day old to 7 days of age…………………………………………………………………… 51

2.8 Discussion………………………………………………………………………... 55

2.9 Conclusion……………………………………………………………………….. 56

Chapter 3: ……………………………………………………………………………. 58

The response of mixed sex broilers to varying dietary Energy: total Lys ratios fed from 7 days to 14 days of age………………………………………………………………... 58

3.1 Abstract…………………………………………………………………………... 58

3.2 Introduction………………………………………………………………………. 59

3.3 Material and methods……………………………………………………………. 60

3.4 Experimental diets……………………………………………………………….. 60

3.4.1 Pellet hardness and quality index……………………………………………… 61

3.5 Data collection…………………………………………………………………… 64

3.6 Statistical analysis………………………………………………………………... 64

3.7 Results……………………………………………………………………………. 65

3.7.1 Feed texture: Pellet quality index and hardness………………………………. 65

3.7.2 Animal performance…………………………………………………………..... 66

3.7.2.1 The effect of feeding varying energy (ME): total Lys ratio's to mixed sex broilers 66 from 7 to 14 days of age…………………………………………………………………...

3.7.2.2 The effect of feeding varying total Lys levels on mixed sex broilers from 7 to 14 days of age………………………………………………………………………………… 70

3.7.2.3 The effect of feeding varying energy (ME) levels on mixed sex broilers from 7 to 14 days of age………………………………………………………………………... 73

3.8 Discussion……………………………………………….. 76

3.9 Conclusion……………………………………………………………….............. 79

Chapter 4: ……………………………………………………………………………. 80

The response of mixed sex broilers to varying dietary Energy: total Lys ratios fed from 14 days to 28 days of age………………………………………………………………. 80

4.1 Abstract…………………………………………………………………………...... 80

4.2 Introduction……………………………………………………………………… 81

4.3 Material and methods……………………………………………………………. 84

4.4 Experimental diets………………………………………………………………….. 84

4.4.1 Pellet quality index hardness………………………………………………….... 85

4.5 Data collection…………………………………………………………………… 88

4.6 Body carcass and chemical analyses………………………………………………….. 88

4.7 Statistical analysis……………………………………………………………….... 88

4.7 Results……………………………………………………………………………. 89

4.7.1 Feed texture: pellet durability index and hardness………………………………….. 89

4.7.2 Animal performance………………………………………………………………… 89

4.7.2.1 The effect of feeding varying energy (ME): total Lys ratio's to mixed sex broilers from 14 to 28 days of age………………………………………………………………. 91

4.7.2.2 The effect of feeding varying total Lys levels on mixed sex broilers from 14 to 28 days of age………………………………………………………………………………… 95

4.7.2.3 The effect of feeding varying energy (ME) levels on mixed sex broilers from 14 to 28 days of age……………………………………………………………………... 98

4.7.2.4 Carcass composition………………………………………………………. 100

4.7.2.5 Body carcass protein and fat composition of 14 to 28 day old mixed sex broilers fed varying ME: total Lys ratio's…………………………………………………….. 104

4.8 Discussion……………………………………………………………………... 107

4.9 Conclusion…………………………………………………………………….. 109

Chapter 5: ……………………………………………………………………. 111

The response of mixed sex broilers to varying dietary Energy: total Lys ratios fed from 28 days to 35 days of age…………………………………………………………….. 111

5.1 Abstract………………………………………………………………………... 111

5.2 Introduction……………………………………………………………………. 112

5.3 Material and methods…………………………………………………………. 114

5.4 Experimental diets…………………………………………………………….. 115

5.4.1 Experimental diets…………………………………………………………... 115

5.4.2 Pellet hardness and quality index…………………………………………… 116

5.5 Data collection………………………………………………………………… 118

5.6 Carcass evaluation…………………………………………………………….. 118

5.7 Statistical analysis……………………………………………………………... 119

5.8 Results…………………………………………………………………………. 119

5.8.1 Feed texture: pellet harness and pellet quality index………………………... 119

5.8.2 Animal performance………………………………………………………… 121

5.8.2.1 The effect of feeding varying energy (ME): total Lys ratio's to mixed sex broilers from 28 to 35 days of age…………………………………………………………. 121

5.8.2.2 The effect of feeding varying total Lys levels on mixed sex broilers from 28 to 35 125 days of age………………………………………………………………………………

5.8.2.3 The effect of feeding varying energy (ME) levels on mixed sex broilers from 28 to 35 days of age…………………………………………………………………………... 126

5.8.2.4 Carcass composition………………………………………………………………. 131

5.8.2.5 Body carcass protein and fat composition of 28 to 35 day old mixed sex broilers fed varying ME: total Lys ratio's……………………………………………………….. 134

5.9 Discussion…………………………………………………………………………... 138

5.10 Conclusion………………………………………………………………………… 142

General discussion………………………………………………………………………… 144

References……………………………………………………………………………. 153

LIST OF FIGURES

Figure 1.1 Live weight gain as a function of Energy (ME): total Lys…………………….. 32

Figure 1.2. Feed intake as a function of Energy (ME): total Lys…………………………. 33

Figure 1.3. FCR as a function of Energy (ME): total Lys………………………………… 33

Figure 2.1: The effect of feeding day old to 7 days of age mixed sex broilers varying amounts of dietary ME: total Lys ratios on Average Daily Gain (ADG)…………………. 47

Figure 2.2: The effect of feeding day old to 7 days of age mixed sex broilers varying amounts of dietary ME: total Lys ratios on Average Daily Feed Intake (ADFI)…………. 47

Figure 2.3: The effect of feeding day old to 7 days of age mixed sex broilers varying amounts of dietary ME: total Lys ratios on Feed Conversion Ratio (FCR)………………. 48

Figure 2.4: The effect of feeding 7day old to 7 days of age mixed sex broilers varying amounts of dietary total Lys ratios on Average Daily Gain (ADG)………………………. 50

Figure 2.5: The effect of feeding day old to 7 days of age mixed sex broilers varying amounts of dietary total Lys ratios on Average Daily Feed Intake (ADFI)………………. 50

Figure 2.6: The effect of feeding day old to 7 days of age mixed sex broilers varying amounts of dietary total Lys ratios on Feed Conversion Ratio (FCR)……………………. 51

Figure 2.7: The effect of feeding day old to 7 days of age mixed sex broilers varying amounts of energy (ME) on the Average Daily Gain (ADG)…………………………….. 52

Figure 2.8: The effect of feeding day old to 7 days of age mixed sex broilers varying amounts of energy (ME) on the Average Daily Feed Intake (ADFI)……………………... 53

Figure 2.9: The effect of feeding day old to 7 days of age mixed sex broilers varying amounts of energy (ME) on the Feed Conversion Ratio (FCR)………………………… 53

Figure 3.1: The effect of feeding 7 to 14 days of age mixed sex broilers varying amounts of dietary ME: total Lys ratios on Average Daily Gain (ADG)…………………………... 67

Figure 3.2: The effect of feeding 7 to 14 days of age mixed sex broilers varying amounts of dietary ME: total Lys ratios on Average Daily Feed Intake (ADFI)…………………… 68
Figure 3.3: The effect of feeding 7 to 14 days of age mixed sex broilers varying amounts of dietary ME: total Lys ratios on Feed Conversion Ratio (FCR)……………………… 68
Figure 3.4: The effect of feeding 7 to 14 days of age mixed sex broilers varying amounts of dietary total Lys ratios on Average Daily Gain (ADG)……………………………… 71
Figure 3.5: The effect of feeding 7 to 14 days of age mixed sex broilers varying amounts of dietary total Lys ratios on Average Daily Feed Intake (ADFI)……………………… 71
Figure 3.6: The effect of feeding 7 to 14 days of age mixed sex broilers varying amounts of dietary total Lys ratios on Feed Conversion Ratio (FCR) …………………………... 72
Figure 3.7: The effect of feeding 7 to 14 day of age mixed sex broiler varying amounts of energy (ME) levels on Average Daily Gain (ADG)……………………………………. 74
Figure 3.8: The effect of feeding 7 to 14 day of age mixed sex broiler varying amounts of energy (ME) levels on Average Daily Feed Intake (ADFI)……………………………. 74
Figure 3.9: The effect of feeding 7 to 14 day of age mixed sex broiler varying amounts of energy (ME) levels on Feed Conversion Ratio (FCR)…………………………………. 75
Figure 4.1: The effect of feeding 14 to 28 days of age mixed sex broilers varying amounts of dietary ME: total Lys ratios on Average Daily Gain (ADG)…………………. 92
Figure 4.2: The effect of feeding 14 to 28 days of age mixed sex broilers varying amounts of dietary ME: total Lys ratios on Average Daily Feed Intake (ADFI)…………. 93
Figure 4.3 The effect of feeding 14 to 28 days of age mixed sex broilers varying amounts of dietary ME: total Lys ratios on Feed Conversion Ratio (FCR)……………………… 93
Figure 4.4: The effect of feeding 14 to 28 days of age mixed sex broilers varying amounts of dietary total Lys ratios on Average Daily Gain (ADG)………………………. 95
Figure 4.5: The effect of feeding 14 to 28 days of age mixed sex broilers varying 96

amounts of dietary total Lys ratios on Average Daily Feed Intake (ADFI)……………….
Figure 4.6: The effect of feeding 14 to 28 days of age mixed sex broilers varying amounts of dietary total Lys ratios on Feed Conversion Ratio (FCR)……………………. 96
Figure 4.7: The effect of feeding 14 to 28 day of age mixed sex broiler varying amounts of energy (ME) levels on Average Daily Gain (ADG)……………………………………. 98
Figure 4.8: The effect of feeding 14 to 28 day of age mixed sex broiler varying amounts of energy (ME) levels on Average Daily Feed Intake (ADFI)…………………………. 98
Figure 4.9: The effect of feeding 14 to 28 day of age mixed sex broiler varying amounts of energy (ME) levels on Feed Conversion Ratio (FCR)…………………………………. 99
Figure 4.10: The effect of feeding 14 to 28 days of age mixed sex broilers varying amounts of dietary total Lys ratios on carcass protein (%)……………………………….. 104
Figure 4.11: The effect of feeding 14 to 28 days of age mixed sex broilers varying amounts of dietary total Lys ratios on carcass fat (%)…………………………………….. 104
Figure 5.1: The effect of feeding 28 to 35 days of age mixed sex broilers varying amounts of dietary ME: total Lys ratios on Average Daily Gain (ADG)…………………. 123
Figure 5.2: The effect of feeding 28 to 35 days of age mixed sex broilers varying amounts of dietary ME: total Lys ratios on Average Daily Feed Intake (ADFI)…………. 123
Figure 5.3: The effect of feeding 28 to 35 days of age mixed sex broilers varying amounts of dietary ME: total Lys ratios on Feed Conversion Ratio (FCR)………………. 124
Figure 5.4 The effect of varying total Lys levels on Average Daily Gain (ADG) of broilers from 28 days to 35 days……………………………………………………... 126
Figure 5.5 The effect of varying total Lys levels on Average Daily Feed Intake (ADFI) of broilers from 28 days to 35 days…………………………………………………….. 126
Figure 5.6 The effect of varying total Lys levels on Feed Conversion Ratio) FCR of broilers from 28 days to 35 days……………………………………………………... 127

Figure 5.7: The effect of feeding 28 to 35 day of age mixed sex broiler varying amounts of energy (ME) levels on Average Daily Gain (ADG)………………………………………. 128

Figure 5.8: The effect of feeding 28 to 35 day of age mixed sex broiler varying amounts of energy (ME) levels on Average Daily Feed Intake (ADFI)……………………………. 129

Figure 5.9: The effect of feeding 28 to 35 day of age mixed sex broiler varying amounts of energy (ME) levels on Feed Conversion Ratio (FCR)…………………………………. 129

Figure 5.10: The effect of feeding 28 to 35 days of age mixed sex broilers varying amounts of dietary total Lys ratios on carcass protein (%)……………………………….. 133

Figure 5.11: The effect of feeding 28 to 35 days of age mixed sex broilers varying amounts of dietary total Lys ratios on carcass fat (%)…………………………………….. 135

LIST OF TABLES

Table 1.1: Selected data sets with varying energy (ME) and total Lys (%)………………. 30

Table 1.2: Descriptive statistics of the main variables of the broiler experiments………... 31

Table 2.1: The Raw material composition of the basal dilution and summit diet composition fed to mixed sex broilers from day old to 7 days of age…………………….. 42

Table 2.2: The nutrient composition of the basal and summit dilution diets fed from day old to 7 days mixed sex broilers…………………………………………………….... 43

Table 2.3: The effect of varying total Lys and energy (ME) levels in diets on crumble quality……………………………………………………………………………... 44

Table 2.4: The effect of varying total Lys (%) levels in diets on crumble quality and pellet hardness (2700, 2900 and 3100 ME kcal/kg)………………………………………. 44

Table 2.5: The effect of varying energy (ME) in diets on crumble quality……………….. 45

Table 2.6: The effect on the performance of day old to 7 days of age mixed flock broilers due to the feeding of varying ME: total Lys ratio’……………………………………... 46

Table 2.7: The effect on the performance of day old to 7 days of age mixed flock broilers to the feeding of varying total Lys levels (2700, 2900 and 3100 ME kcal/kg)…………… 49

Table 2.8: The effect on the performance of day old to 7 days of age mixed flock broilers due to the feeding of varying energy (ME) levels (1.3, 1.4, 1.6, 1.77% total Lys)……….. 52

Table 3.1 The raw material composition of the basal dilution and summit diet………….. composition fed to mixed sex broilers from 7 days to 14 days. 61

Table 3.2 The nutrient composition of the basal dilution and summit diet fed to mixed sex broilers from 7 to 14 days……………………………………………………….... 62

Table 3.3: The effect of varying total Lys and energy levels (ME) in diets and the effect thereof on pellet durability index (PDI) and pellet hardness……………………………… 64

Table 3.4: The effect of varying total Lys (%) levels in diets and the effect thereof on 65

pellet durability index (PDI) and pellet hardness……………………………………………

Table 3.5: The effect of varying energy (ME) levels in diets and the effect thereof on pellet durability index (PDI) and pellet hardness…………………………………………… 65

Table 3.6: The effect on the performance of 7 to 14 days of age mixed flock broilers due to the feeding of varying ME: total Lys ratio's…………………………………………… 66

Table 3.7: The effect on the performance of 7 to 14 days of age mixed flock broilers to the feeding of varying total Lys levels (2700, 2900 and 3100 ME kcal/kg)……………… 70

Table 3.8: The effect on the performance of 7 to 14 days of age mixed flock broilers due to the feeding of varying energy (ME) levels (1.30, 1.40, 1.60; 1.77% total Lys)………... 73

Table 4.1 The raw material composition of the basal dilution and summit diet composition fed to mixed sex broilers from 14 days to 28 days of age…………………... 85

Table 4.2 The nutrient composition of the basal dilution and summit diet fed to mixed sex broilers from 14 to 28 days of age……………………………………………………….. 86

Table 4.3: The effect of varying total Lys and energy levels (ME) in diets on the pellet durability index (PDI) and pellet hardness………………………………………………... 89

Table 4.4: The effect of varying total Lys (%) levels in diets on the pellet durability index (PDI) and pellet hardness………………………………………………………… 89

Table 4.5: The effect of varying energy (ME) levels in diets on the pellet durability index (PDI) and pellet hardness…………………………………………………………… 90

Table 4.6: The effect on the performance of 14 to 28 days of age mixed flock broilers due to the feeding of varying ME: total Lys ratio's……………………………………….. 92

Table 4.7: The effect on the performance of 14 to 28 days of age mixed flock broilers to the feeding of varying total Lys levels (2800, 3000 and 3200 ME kcal/kg)……………… 95

Table 4.8: The effect on the performance of 14 to 28 days of age mixed flock broilers due to the feeding of varying energy (ME) levels (1.08, 1.23, 1.38; 1.56% total Lys)…… 97

Table 4.9: The effects of feeding 14 to 28 days' mix sex broilers varying ME: total Lys ratio on carcass protein and fat composition (% of carcass weight at processing) ……… 100
Table 4.10: The effects of feeding 14 to 28 days' mix sex broilers varying total Lys (%) on broiler carcass protein and fat composition (% of carcass weight at processing) …… 101
Table 4.11: The effects of feeding 14 to 28 days' mix sex broilers varying ME on broiler carcass protein and fat composition (% of carcass weight at processing) ……………… 102
Table 4.12: The effects of feeding 14 to 28 day old mix sex broilers varying ME: total Lys ratios and the composition of broiler carcass protein and fat………………………… 103
Table 4.13: The effects of feeding 14 to 28 day old mixed sex broilers varying total Lys (%) and the composition of broiler carcass protein and fat……………………………….. 105
Table 4.14: The effects of feeding 14 to 28 day old mixed sex broilers varying ME levels and the composition of broiler carcass protein and fat……………………………………. 106
Table 5.1 The raw material composition of the basal dilution and summit diet composition fed to mixed sex broilers from 28 days to 35 days of age…………………... 115
Table 5.2 The nutrient composition of the basal dilution and summit diet fed to mixed sex broilers from 28 to 35 days of age…………………………………………………….. 116
Table 5.3: The effect of varying total Lys and energy levels (ME) in diets and the pellet durability index (PDI) and pellet hardness…………………………………………….... 119
Table 5.4: Varying total Lys (%) levels in diets and the effect on the pellet durability index (PDI) and pellet hardness………………………………………………………… 119
Table 5.5: Varying energy (ME) levels in diets and the effect on the pellet durability index (PDI) and pellet hardness………………………………………………………… 120
Table 5.6: The effect on the performance of 28 to 35 days of age mixed flock broilers due to the feeding of varying ME: total Lys ratio's…………………………………….. 122
Table 5.7: The effect on the performance of 28 to 35 days of age mixed flock broilers to 125

the feeding of varying total Lys levels (2800, 3050 and 3300 ME kcal/kg)………………

Table 5.8: The effect on the performance of 14 to 28 days of age mixed flock broilers due to the feeding of varying energy (ME) levels (0.96, 1.11, 1.25; 1.42% total Lys)…… 128

Table 5.9: The effects of feeding 28 to 35 days' mix sex broilers varying ME: total lysine ratio on carcass protein and fat composition (% of live weight at processing) …………... 130

Table 5.10: The effects of feeding 14 to 28 days' mix sex broilers varying total Lys (%) on broiler carcass protein and fat composition (% of carcass weight at processing) …… 131

Table 5.11: The effects of feeding 28 to 35 days' mix sex broilers varying ME on broiler carcass protein and fat composition (% of carcass weight at processing) ……………… 132

Table 5.12: The effects of feeding 28 to 35 day old mix sex broilers varying ME: total Lys ratios and the composition of broiler carcass protein and fat………………………… 134

Table 5.13: The effects of feeding 28 to 35 day old mixed sex broilers varying total Lys (%) and the composition of broiler carcass protein and fat………………………………. 135

Table 5.14: The effects of feeding 28 to 35 day old mixed sex broilers varying ME levels and the composition of broiler carcass protein and fat……………………………………. 136

LIST OF ABBREVIATION

AA	Amino Acids
ADFI	Average Daily Feed Intake
ADG	Average Daily Gain
AME	Apparent Metabolizable Energy
bw	Body Weight
BP	Balanced Protein
BWG	Body Weight Gain
Cal	Calorie
CP	Crude Protein
CP:ME	Crude Protein: Metabolizable energy ratio
d	Days
Dig. Lys	Digestible Lysine
FCR	Feed Conversion Ratio
FI	Feed Intake
g	Gram
hr	Hour/s
IAAP	Ideal Amino Acid Profiles
kcal/kg	Kilo Calorie per kilogram
Lys	Lysine
kg	Kilogram
ME	Metabolizable energy
ME:tot lys	Metabolizable Energy to total lysine ratio
NE	Net Energy
PDI	Pellet Durability Index
PROC GLM	General Linear Models Procedure
SEM	Standard Error of the Mean
SID	Standardised Ileal Amino Acid Digestibility
Tot Lys	Total Lysine

Chapter 1:

Literature Review

1.1 Abstract

Studies published between 1982 and 2011 dealing with the response of day old to 21 days of age broilers due varying energy (ME) to total Lys ratios were selected. Data were subjected to multiple-regression analysis to obtain regressions equations that would assist nutritionists in predicting ADG, FCR and ADFI for broilers fed a range of varying ME to total Lys ratios. The estimated prediction equation for ADG was: = 35.863 + -0.004157*(ME: total Lys ratio) (R^2 = 0.891), for FCR= 0.3543 + 0.0002790*(ME: total Lys ratio) (R^2 = 0.845) and ADFI: 31.572 + -0.001852*(ME: total Lys ratio) (R^2 = 0.378). The results of this study suggest that broilers ADG and FCR respond linearly towards the ME: total Lys ratio, while feed intake is less responsive towards changing ME: total Lys ratios.

1.2 Introduction

Even though numerous studies have clearly illustrated the effect of varying dietary energy to protein ratio on broiler performance (Morris *et al.,* 1987; Sterling *et al.,* 2003) and body composition (Fraps, 1943; Bartov *et al.,* 1973; Jackson *et al.,* 1982; Gous *et al*., 1990; Macleod, 1991), the recommendations of these publications have not found their way into commercial practice. Further, it has been suggested that the use of calorie to protein ratio for describing the relationship between these two important diet fractions is outdated and serves only to discuss the relationship in the most general of terms (Classen, 2013).

In commercial operations, modern broiler rations are formulated based on the ideal amino acid profile (IAAP), instead of the crude protein (CP) content. This approach has proven to achieve equivalent broiler production performance (Waldroup *et al.,* 1976; Han *et al.,* 1992; Kerr and Kidd, 1999; Dean *et al.*, 2006; Namroud *et al.,* 2008). However, this practise allows nutritionists to reduce feed costs without compromising animal performance. This practice resulted in major economic benefits to feed companies and integrated poultry producers, being applied commercially to great extents.

Although nutritionists would generally follow the recommendations of the primary breeder for the particular hybrid being fed variations of regional conditions will affect the final composition of a formulation. Each region and feed mill may use different feed specifications for different clients depending on the unique conditions prevalent at that economic time period (Tillman, 2013). However, there is a lack of scientific literature that could be applied commercially providing prediction regressions equations for a range of optimal energy: lysine (Lys) ratios required to maximize muscle deposition and to minimize the feed conversion ratio (FCR). In order to determine predicting equations for optimum ME: total Lys ratio for average daily gain (ADG), feed conversion ratio (FCR) and average daily feed intake (ADFI) data from published literature were collected and analysed using multiple regression analysis.

1.3 Materials and methods

A literature study has been conducted to evaluate the effect of varying dietary energy to Lys ratios on the performance of broilers from day old to 21 days of age. A selection criteria was established and a database was compiled with studies published between 1982 and 2011 investigating the effect of energy, protein or Lys on broilers raised from day old to 21 days of age. A total number of 30 articles, consisting of 58 experiments, were originally selected. Expression of total Lys was maintained in the analysis and, where needed, the total Lys of the experimental diets was calculated using the CVB (2001) matrix. However, based on the refined selection criteria for only day old to 21 days, only 8 articles were included in the final selection, consisting of 24 experiments in total (Table 1.1).

Publications reporting several experiments were dealt with by assigning a specific code to each experiment. In all publications, growth performance indicators were body weight gain (BWG) and feed conversion ratio (FCR). When missing, feed intake (FI) was calculated as the ratio of BWG to FCR. ADG and ADFI were then calculated by dividing BWG and ADFI by the trial duration in days. The ME: total Lys ratio was calculated by dividing the ME by the total Lys of the experimental diets.

The selected data sets (Table 1.1) included a range of variables, such as growth potential (year of the experiment, sex, strain) and diet CP, total Lys, ME, ME: total Lys, graded supplementation, diet dilution). All data included in the analysis were checked and at that stage decided whether or not they could be used in the further calculations. Multiple regression analysis was performed with Genstat for Windows 14th Edition. The experiment was introduced in the model as a fixed effect to ensure a proper prediction of coefficients of the model and an accurate description of the error of prediction (Sauvant *et al.*, 2008). Based on a graphical examination of the within-experiment response of each dependent variable (ADG, ADFI, FCR) to the independent variable (ME: total Lys) (Figure 1.1, 1.2 and 1.3), a linear model was adjusted as follows:

$$Yi = \propto i + xi(b) + ei \qquad (1.1)$$

where Y_i is the value of the dependent variable Y in the experiment with the level of ME: total Lys ratio, a as the overall intercept with the condition that the sum of the effect of each experiment a_i is equal to 0 and b are the linear coefficients, respectively, of the overall response to the ME: total Lys ratio and e_i is the residual error.

Four criteria were used to assess the accuracy of the model to predict observed values: the deviation of the intercept (a) from 0, the deviation of the slope (b) from 1, the coefficient of determination (R^2) and the residual variation expressed as root mean square error (RMSE).

Descriptive statistics for each variable in the dataset that was used for further calculations were generated (Table 1.2).

1.4 Results and discussion

The prediction equations are presented in Figures 1.1, 1.2 and 1.3. When ADG and FCR were considered in relation to ME: total Lys ratio, all data points resulted in a linear response. However, ADFI (Figure 1.2) tended to be more horizontally aligned. The results suggest that ADG and FCR have a linear relationship with the ME: total Lys ratio and that the prediction equation for ADG (0.891) and for FCR (0.845) indicates a high level of confidence in the regression obtained. The results indicate further that to achieve higher ADG or improved FCR, lower ratios of ME: total Lys would be required. However, the results show a curve linear response for ADFI with a lower R^2 (0.378) and that the slope of the ADFI is much lower than that of ADG and FCR. This indicates that the ME: total Lys ratio regression equations calculated can describe ADG and FCR with a high level of confidence, while the ADFI is not well defined by the regression. Feed intake is thus then likely to be less influenced by the ME: total Lys ratio, but likely more by other factors, i.e bulkiness of the feed, ability to loose heat, (Swatson, 2002) and gut capacity (Brickett *et al.* 2007 and Kamran *et al.* 2008).

There are a few examples in the literature where broilers received graded levels of balanced amino acid profiles at two or three dietary energy levels. Bellaver *et al*. (2002) fed diets with graded levels of an IAAP and with either 3000, 3100 and 3200 kcal ME/kg to male chickens from 1 to 21 days of age. It was concluded that the ME: dig. Lys (digestible lysine) ratio for maximum performance increased with decreasing content of the feed density. In other words, the trial data suggested that optimum dietary IAAP levels should be unchanged or even increased with decreasing dietary energy level to obtain maximum performance. The response to increasing IAAP on FCR was an improvement with both increasing dietary energy and increasing IAAP. Moreover, the results of the trial indicated that the plateau for FCR was not achieved suggesting a generally higher IAAP requirement for FCR were needed than that for weight gain which also has been documented in a literature review by Pack *et al*. (2003) for various single amino acids.

Plumstead *et al*. (2007) conducted two experiments with a similar design to that of Bellevar *et al*. (2002). The authors observed that there was significant interaction between ME and dig. Lys at 21 days of age on BW gain and FCR. Broiler 21 d BW increased and FCR decreased when the dietary ME was increased from 3000 to 3100 kcal/kg with no further improvement to 3200 kcal ME/kg. Increasing dietary ME or dig. Lys had no effect on feed intake to 21 days. These responses were similar to those of Bellevar *et al*. (2002). In both trials, the curves appeared to shift to the higher ME: dig. Lys ratios with decreasing energy supply.

Lemme (2007) proposed that there appears to be no optimum fixed or single ME: Lys ratio as sometimes indicated when recommendations are given in gram amino acid per unit dietary energy. It follows therefore that based on the results of this study, it would appear that the optimum energy: Lys ratio changes during the production phases or weight of the modern broiler. There is therefore not one single energy: Lys ratio, but rather a shifting optimum energy: Lys ratio that can be applied on a commercial basis which is supported by the constant changes in nutrient requirements throughout the life of the bird.

It has been hypothesized that the dietary crude protein (CP) and AA requirements should be expressed as a percentage of the dietary energy (ME) concentrations (NRC, 1984; CVB, 2001). This is based on the evidence that poultry adjust their feed intake to differences in dietary ME density to maintain ME intake (NRC, 1984; Leeson, Caston and Summers, 1996; Dozier *et al.*, 2006). However, there is also contradicting research that indicate the modern broiler is unable to adjust its feed intake due to different energy levels in its diet (Plumstead *et al.*, 2007; Latshaw, 2008; Delezie *et al*., 2010; Li, *et al*., 2010, Classen, 2013, Gous 2013). Therefore, from the results of this study, it would appear that the data support the view that the modern broiler from day old to 21 days is not able to adjust its FI to varying energy: total Lys levels.

Table 1.1: Selected data sets with varying energy (ME) and total Lys (%)

Day	CP%	total Lys %	ME (kcal/kg)	Ratio ME: tot. Lys	Sex	[1] Strain	[2]Technique	References
0-14	21.8-26.9	1.19-1.47	3000-3200	2041-2689	As hatched	C500	Dd	Plumstead et al. (2007)
0-14	21.8-26.9	1.19-1.47	3000-3200	2041-2689	As hatched	R308XR344	Dd	Plumstead (2007)
0-21	14.0	0.8-0.96	3107	3251-3893	Male	R1	Dd	Morris et al. (1987)
0-21	16.8	0.89-1.05	3107	2966-3491	Male	R1	Dd	Morris et al. (1987)
0-21	19.6	0.98-1.14	3107	2726-3164	Male	R1	Dd	Morris et al. (1987)
0-21	22.4	1.07-1.23	3107	2523-2893	Male	R1	Dd	Morris et al. (1987)
0-21	25.2	1.17-1.32	3107	2348-2665	Male	R1	Dd	Morris et al. (1987)
0-21	28.0	1.26-1.42	3107	2195-2470	Male	R1	Dd	Morris et al. (1987)
7-21	14.0	0.8-0.96	3107	3251-3893	As hatched	R1	Dd	Morris et al. (1987)
7-21	16.8	0.89-1.05	3107	2966-3491	As hatched	R1	Dd	Morris et al. (1987)
7-21	19.6	0.98-1.14	3107	2726-3164	As hatched	R1	Dd	Morris et al. (1987)
7-21	22.4	1.07-1.23	3107	2523-2893	As hatched	R1	Dd	Morris et al. (1987)
7-21	25.2	1.17-1.32	3107	2348-2665	As hatched	R1	Dd	Morris et al. (1987)
7-21	28.0	1.26-1.42	3107	2195-2470	As hatched	R1	Dd	Morris et al. (1987)
0-18	17.4-25.1	1.01-1.47	3000-3031	2041-3001	Male	RxR	Gi	Urdaneta-Rincón & Leeson (2008)
0-19	15-31.5	0.58-1.85	3035-3274	1641-5645	Male	-	Dd	Ajinomoto Hartland Inc. (1990)
0-21	21-23	1.25-1.35	3000-3200	2222-2560	Male	R308	Gi	Jafarnejad and Sadegh, (2011)
9-18	17.5-23.5	0.51-0.78	3200	4103-6275	As hatched	C x C	Dd	Sterling et al. (2003)
9-19	17-23	0.51-1.44	3200	2226-6275	Male	C x C	Gi	Sterling et al. (2003)
10-18	17-23	0.51-1.44	3200	2226-6275	As hatched	C x C	Dd	Sterling et al. (2003)
0-21	20	1.06-1.28	2796-3203	2334-2638	As hatched	-	Gi	Baghel & Pradhan (1989)
0-21	22	1.11-1.26	2796-3203	2364-2870	As hatched	-	Gi	Baghel & Pradhan (1989)
0-21	25	1.22-1.37	2796-3203	2095-2627	As hatched	-	Gi	Baghel & Pradhan (1989)
	12.5-30	0.5-1.29	3100	2396-6212	As hatched	H	Dd	Clark, Gous and Morris (1982)

[1]C = Cobb, R= Ross, H= Hybro
[2]Gi = Graded increase, Dd= Diet dilution

The use of prediction regressions of broilers response to varying ME: total Lys ratios does however have two major limitations. Firstly, the model makes no provision for environmental conditions. If the environmental temperature increases above a threshold value, the ME: total Lys ratios will likely increase until the bird is capable of consuming an amount of energy without increasing its heat production (Swatson, 2002).

Table 1.2: Descriptive statistics of the main variables of the broiler experiments

	n	Mean	Minimum	Maximum	SEM
Independent variables					
total Lys	122	1.12	0.49	1.85	0.21
ME: total Lys	122	2849	1641	6275	788
Dependent variables					
ADG* (g/d)	122	23.9	9.25	28.8	6.52
ADFI# (g/day)	122	11.8	26.3	30.4	14.3
FCR*# (g gain/g feed)	122	1.15	0.80	2.39	0.59

*ADG = average daily gain,

#ADFI = average daily feed intake

*#FCR = feed conversion ratio

Secondly, the model makes no provision for economic conditions. If the price of energy sources were high relative to protein sources, then a diet with a higher ME: total Lys ratio than the biological ME: total Lys ratio may be formulated. Conversely, if the price of protein sources was low relative to energy sources, a diet with a lower ME: total Lys ratio than optimum biological ME: total Lys ratio may be formulated. This would result in birds being fatter than their desired level due to excess energy available. This situation is often present in broilers at market age (Swatson, 2002). However, as the cost of protein sources remains high relative to energy sources this is the most profitable option. This is of course true, unless the consumer is prepared to pay more for a leaner chicken.

1.5 Conclusion and applications

1. For broilers 0-21 days of age, the meta-analysis provided the following ADG, FCR and ADFI of the ME: total Lys response regressions:

   ADG = 35.863 + -0.004157*(ME: total Lys ratio) (R^2 = 0.89) [1.1]

   FCR = 0.3543 + 0.0002790*(ME: total Lys ratio) (R^2 = 0.85) [1.2]

   ADFI = 31.572 + -0.001852*(ME: total Lys ratio) (R^2 = 0.34) [1.3]

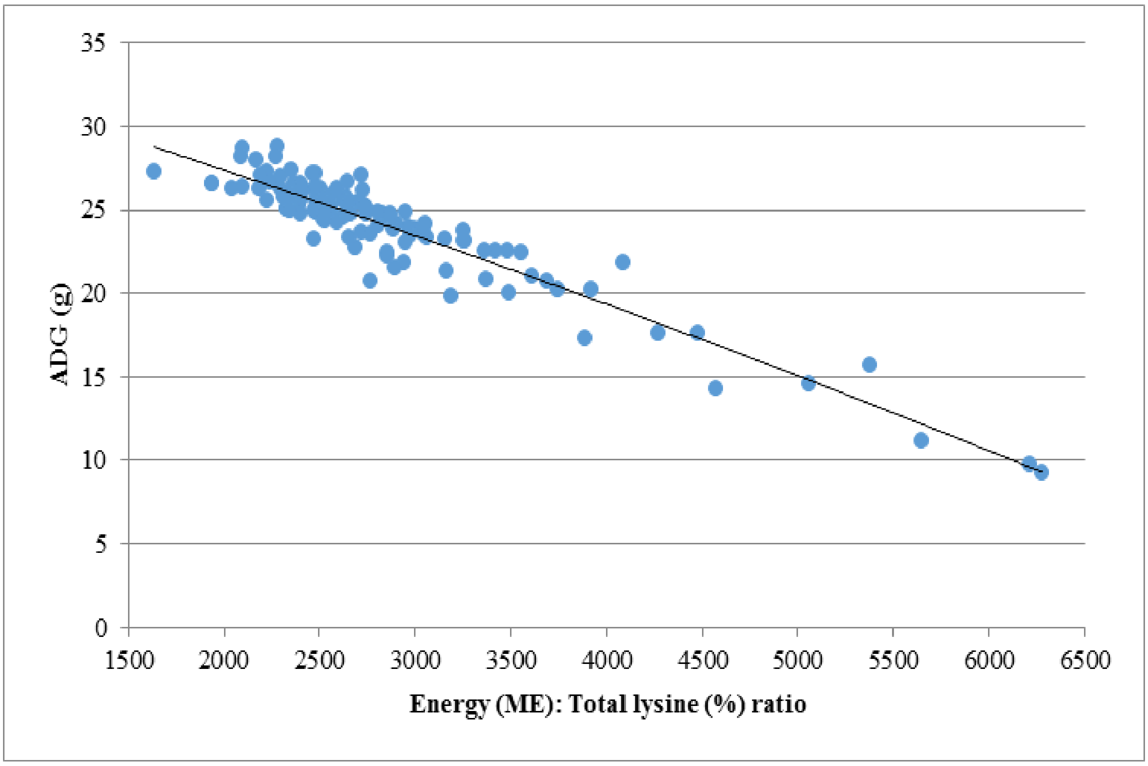

Figure 1.1 Live weight gain as a function of Energy (ME): total Lys

2. The response regression for ADG and FCR have a high R^2 coefficient and the slope of response appears to be responsive to varying ME: Lys ratios.

3. The response regression for ADFI have a low R^2 coefficient and the slope of response appears to be less responsive to varying ME: Lys ratios. Factors other than ME: total Lys is likely affecting FI i.e. bulkiness of the feed, ability to loose heat and gut capacity.

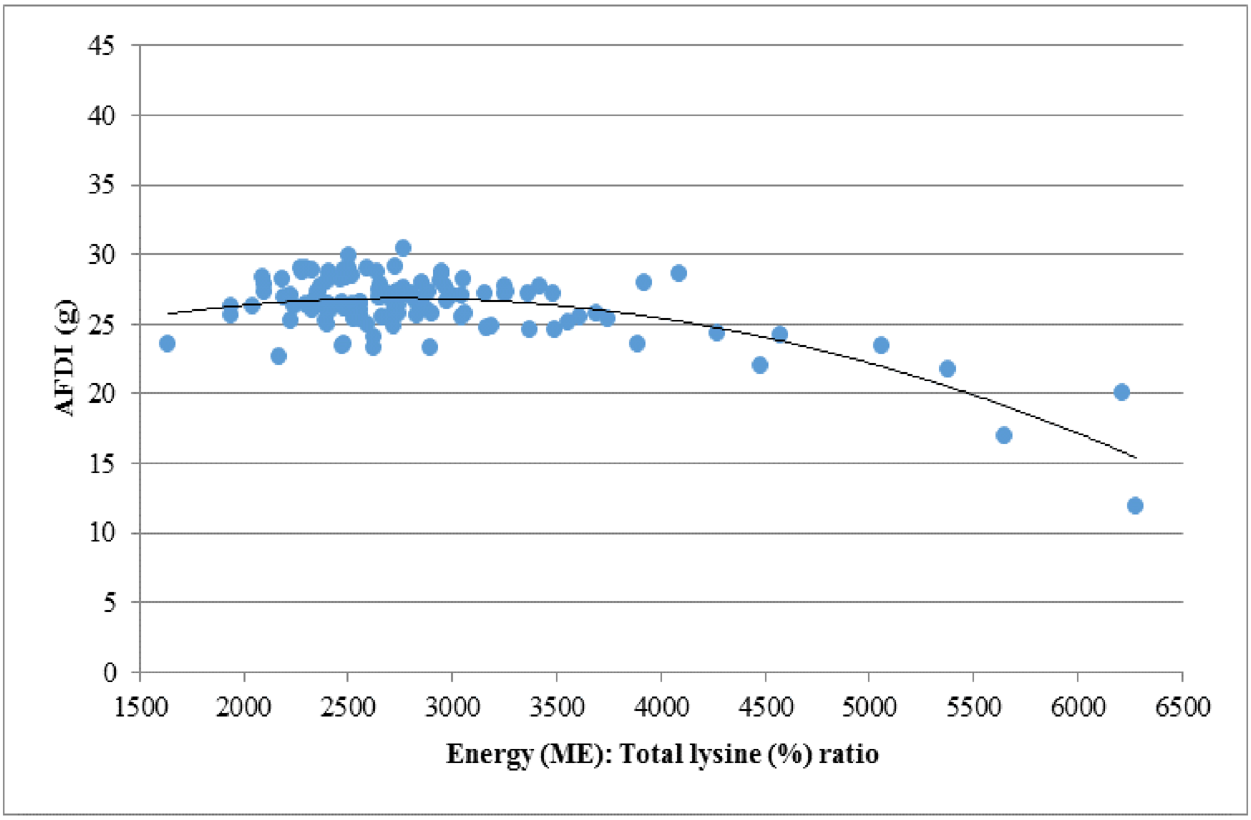

Figure 1.2. Feed intake as a function of Energy (ME): total Lys

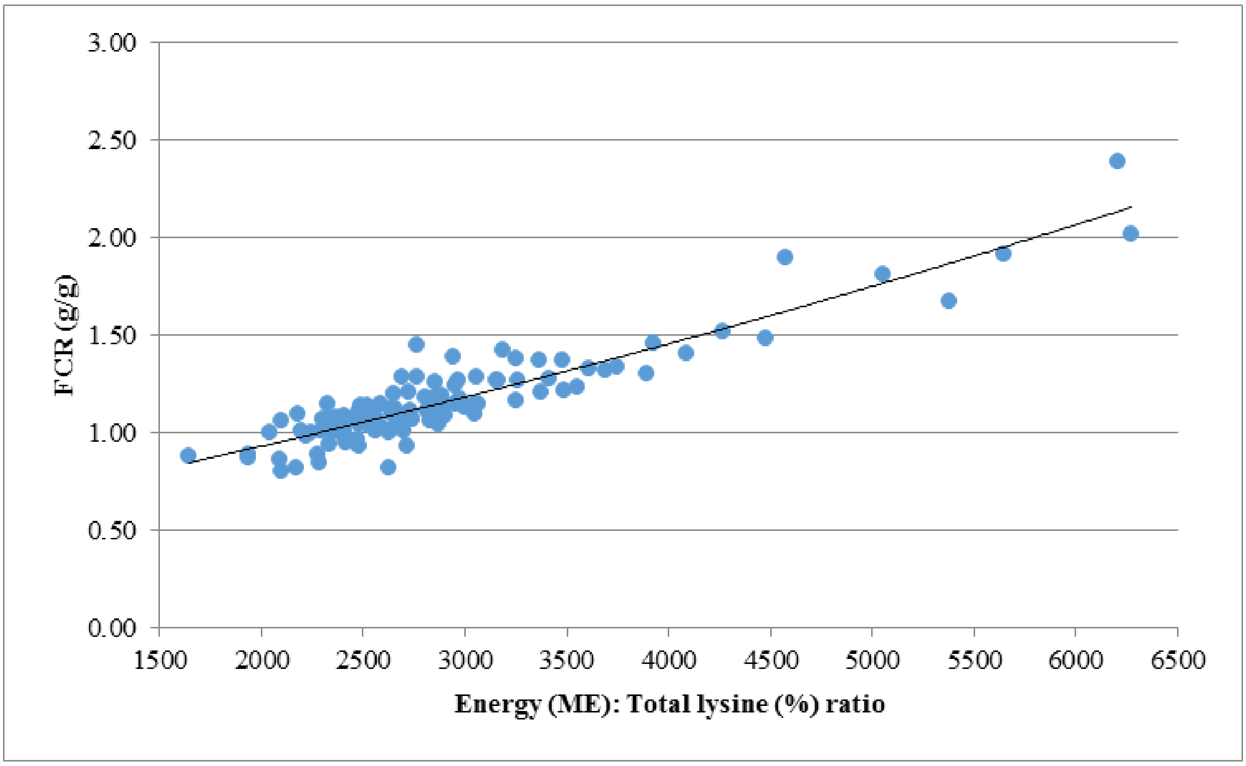

Figure 1.3. FCR as a function of Energy (ME): total Lys

4. The ME: total Lys response regressions have their limitations since they are not able to factor in environmental influences nor are they able to predict the economic optimal ME: total Lys ratios' due to changing energy or protein source prices.

5. It would be interesting and valuable to know what the ADG, FCR and ADFI prediction regressions equations for the latter stages of the broilers' production phases (21 to 35 and 35 to 42 days) were.

Chapter 2:

The response of mixed sex broilers to varying dietary energy: total Lys ratios fed from day old to 7 days of age

2.1 Abstract

A trial was conducted to study the response of broilers to varying total Lys and energy (ME) levels as well as their response to the ratio between these two nutrients. Five hundred and seventy-six Ross 308 mixed male and female broilers were fed one of twelve rations. The dietary treatments consisted of four total Lys levels (1.77, 1.60, 1.40 and 1.30%) and three ME levels (2700, 2900 and 3100 kcal ME/kg). The use of the dietary summit/dilution technique, the combination of total Lys and energy levels, resulted in various ME: total Lys levels (1525, 1638, 1751, 1688, 1813, 1938, 1929, 2071, 2214, 2077, 2231, and 2385). Each of the 12 treatments was replicated 8 times. Birds were fed the trial diets from day old to 7 days. It was found that ADG improved with an increase in total Lys content but there was no statistical effect of ME. The point of maximum response for ADG was achieved at 1.48% total Lys. However, ADFI tended to be less responsive, with two treatments only consuming differently from the other seven treatments. There was a significantly improvement in FCR with an increase in ME levels.

The point of maximum response for ADG were achieved with 1.60% total Lys and a ME value of 3100 kcal/kg, thus implying a ME: total Lys ratio of 1938. For ADFI, the point of maximum response were 1.40% total Lys, 2700 kcal ME/kg, thus implying a ME: total Lys ratio of 1929. For FCR, the point of maximum response were 1.77% total Lys, 3100 kcal ME/kg, thus implying a ME: total Lys ratio of 1751.

The statistical evaluation of the response of broilers (0–7 days of age) to varying levels of ME: total Lys ratio gave rise to the following equations for ADG= 1.0000 + -0.9979 x (ME: total Lys ratio) + 0.9919 x (ME: total Lys ratio^2) (R^2 = 0.37); FCR = 0.2493608 + 0.000893x (ME: total Lys ratio) + -0.0000001833 x (ME: total Lys ratio^2) (R^2 = 0.39) and ADFI = 4.2932566 + 0.0099603 x (ME: total Lys ratio) + 0.0003807 x (ME: total Lys ratio^2) (R^2 = 0.08).

2.2 Introduction

According to Classen (2013), dietary energy and amino acids comprise the largest components of poultry diets as well as the

most expensive. Consequently, an understanding of the relationship between these dietary components is of major significance. In addition, during the past five decades, intensive genetic selection for growth rate in broilers has resulted in a maximum daily rate of BW gain – at least 2.5 times that which was achieved in the 1950s (Havenstein *et al.*, 2003). Stimulated by the genetic selection of the modern broiler, considerable research has been conducted over the past 40 years with this research being directed at better defining the minimum intake of the dietary CP and amino acids required to support the improved genetic potential. In general, the Lys requirement hierarchy, as indicated by Leclercq (1998), would take the following order: weight gain < breast fillet < feed conversion < abdominal fat. Based on numerous variables that may affect the optimum Lys requirement, it was not surprising that published estimates of the requirement have varied accordingly.

In order to obtain optimum performance in broilers, it is essential that dietary feed supply sufficient amounts of energy as well as other essential nutrients (Lemme, 2007). The amount of nutrient necessary in a diet may be expressed either as an absolute value or as a ratio to another nutrient. Amino acid recommendations are sometimes not expressed only as a percentage of the diet or as gram per kg but as gram per kcal or ME (GfE, 1999; Rostagna *et al.,* 2005). The notion of expressing amino acid recommendations per unit of energy is based primarily on the assumptions that poultry are able to adjust their FI based on their energy level in order to maintain their energy intake. This would therefore mean that if the FI is decreased because of an increased dietary energy level, the concentration of amino acids also should be increased to the same degree in order to compensate for the reduced intake. According to Classen (2013), the use of the energy to protein ratio term and the interaction between these nutrients needs to be reviewed. Classen (2013) therefore suggested that it would be more relevant to describe the relationship of energy to the level of ideal digestible amino acids (most limiting amino acid) and the minimum levels of protein required to supply nitrogen for non-essential amino acid synthesis.

Literature has provided some evidence of the interaction between different energy and balanced amino acid levels in broiler performance from a day old to 7 days of age. Sklan and Noy (2003) provided one to 7-day old male broiler Ross birds with increasing balanced protein (BP) levels covering a range from approximately 5 to 11 g digestible Lys/ kg at dietary energy levels of either 3200 or 3000 kcal ME/kg. The birds responded linearly to increasing BP levels regardless of the dietary energy. Although FI did not differ between treatments weight gain was numerically lower with the lower energy level in the diet resulting in a shift of the feed conversion line to the lower ratio at higher dietary energy levels. Lemme (2007) argued that, if the concept of a constant optimum protein to energy ratio worked, the birds should have balanced their energy intake by FI, thus resulting in similar weight gains at both energy levels. However, when the dig. Lys to ME was calculated, the birds fed 3200 kcal ME/kg performed better in terms of the weight gain response when compared to the 3000 kcal ME/kg treatment.

Plumstead (2005) conducted two experiments. In Experiment 1, Cobb 500 male broilers were fed from a day old to 7 days a range of three energy levels (3000, 3100 and 3200 ME kcal/kg) and three levels of amino acid density (1.05, 1.13, 1.21, 1.29% Dig. Lys). It was found that the broilers' ADG did not improve significantly with an increase in amino acid density. In addition, no significant interaction was observed with an increase in energy. In Experiment 2, the same amino acid and energy treatments as in Experiment 1 were fed, but Ross 344 male broilers were utilized in the trial. The broilers' ADG was significantly increased, ADFI significantly decreased and FCR significantly reduced with an increase in balanced amino acid levels. However, much like in Experiment 1, energy level had no significant effect on ADG, ADFI or FCR. Similar results were found by Basurco *et al*. (2015) feeding Cobb 500 broilers from day old to 8 days a range of three energy levels (3000, 3050 and 3096 kcal/kg) and three levels of amino acid density (1.15, 1.28, 1.41% Dig. Lys). Average daily gain and ADFI improved significantly with an increase in amino acid density but not with an increase in energy level. However, contrary to the findings of the experiments conducted by Plumstead (2005), the FCR was significantly improved and the ADFI increased significantly with an increase in amino acid density. Although an increase in energy level did not result in a significant increase in ADG, the reductions in ADFI and FCR were, however, significant with an increase in energy level. It would appear that broilers 0 to 7 days' response to energy is different to older broilers and that they are unable to adjust their FI to an increase in energy, which differs from older birds (14 to 35 days). The aim of this experiment was thus to derive equations that could relate the body weight gain and feed conversion of broilers to varying total Lys and energy levels.

These equations would then permit commercial nutritionists to determine the most economical point for broilers during the day old to 7-day period.

2.3 Material and methods

Two hundred and eighty-eight Ross 308 day-old male and 288 Ross 308 day-old female broilers were randomly allocated to one of the twelve dietary treatments. The broilers were obtained from a commercial hatchery at 0 d of age. The broilers were placed in a brooding facility with battery cages containing six birds per pen (3 males and 3 females per pen). In total 96 cages were used. The cages were 0.8 m long x 0.5 m wide x 0.42 m high, giving a total floor area of 0.4 m^2. The stocking density for the trial was 15 birds/ m^2. A line feeder was available through the front side of the cage (0.8 m long) and water was provided by nipple drinkers (2 nipples per cage). Feed and water were available on an *ad libitum* basis. The temperatures were set to 34°C at 0 d and reduced by 1°C every day thereafter. The light intensity was set to 30 lux (23 hr light and 1 hr darkness).

2.4 Experimental diets

The dietary treatments consisted of a 3 x 4 factorial arrangement. Twelve diets were formulated in order to achieve three levels of ME (2700, 2900, and 3100 kcal ME/kg) and four total Lys amino acid levels (1.77, 1.60, 1.40, and 1.30%) in order to achieve a combination of energy: total Lys ratios (1525, 1638, 1751, 1688, 1813, 1938, 1929, 2071, 2214, 2077, 2231, and 2385). It is important to note that the same ME: total lysine ratio can be achieved with various combinations of energy and lysine. For examples a diet with 3000 kcal ME/kg and 1.50% total Lys will provide a ratio of 2000. However, the ratio of 2000 ME: total Lys can also be achieved with 2900 kcal ME/kg and 1.45% total Lys. Prior to the diet formulation, amino acid content of all ingredients was calculated as a percentage of the analysed CP content using standard procedure (CVB, 2001). Due to different digestible co-efficient reported in literature (GfE, 1999, CVB, 2001 and Rostagno, 2005) and in order to try to make the study as applicable as possible, it was decided to rather formulate the feed on a total lysine basis and not on a digestible basis. The ME content of the raw materials was calculated using the WPSA (1989) procedure. An inert filler (sand) was required in the diet formulation to allow the application of these principles across a wide range of ME and total Lys. In order to reduce the variation between the dietary treatments that may have originated as a result of the batching and mixing of ingredients, and to ensure uniform gradations of dietary total Lys within each ME level, a summit/dilution blending technique (Gous and Morris, 1983) was applied during the mixing of the diets. This technique consisted of first formulating and mixing basal diets 1, 4, 7, 10 and summit diets 3, 6, 9, 12. The four intermediate diets were then derived by blending the respective summit and dilution diets within each level of ME in the proportions presented in Table 2.1 and Table 2.2. Following the mixing and blending procedure all diets were subsequently analysed for proximate and amino acid composition. The diets were pelleted in a pellet mill (Simon-Brown California Hyflo 67) equipped with a 3 mm die where after the feed was subjected to a crumbling process.

2.4.1 Crumble size quality index

A sieve analysis was carried to measure the crumble size of diets 1 to 12. A mechanical shaker with two different sieves (2.36 mm and 1.4 mm) was used to shake the crumbled feed. The crumbled feed was placed on top of the mechanical shaker and shaken for 30 seconds. The feed for each sieve was weighed individually. The crumble quality of the various diets is presented in Table 2.3. Crumble size, expressed as a percentage, was then calculated by using the equation below:

$$\text{Retained crumbled feed in screen (\%)} = \frac{\text{Weight of feed in sieve after shake}}{\text{Weight of total feed}}$$

2.5 Data collection

Broilers were weighed on day old and 7 days and FI was calculated as the difference between the feed allocated at the beginning of the trial and the amount left over at the end of the trial. The average daily gain (ADG) and feed conversion ratio (FCR) could therefore be calculated. Mortality (%) was recorded on a daily basis. However, FCR was not corrected for the mortality.

2.6 Statistical analysis

JMP ® of SAS version 12.0.1 (SAS institute Inc. JMP Software, Cary, NC, 2015) was used to test for normality and the data was then analysed using Proc GLM (general linear models procedure) as a 3 (energy) x 4 (total Lys) factorial arrangement ($P \leq 0.05$). The mean differences were separated using Tukey's honest significant differences test (Tukey, 1991). For the purposes of this study, there were eight replications with 6 birds per replication and 12 treatments. Of the 6 birds per replication, 3 birds were male and 3 birds were females. The linear and quadratic regressions of JMP ® of SAS version 12.0.1 (SAS institute, 2015) were used to observe the treatments effect of weight, ADG, FI and FCR.

$$Y = b0 + b1\ E \qquad [2.1]$$

$$Y = b0 + b1E + b2\ E^2 \qquad [2.2]$$

Where Y predicted response, E = energy contentment of the diet, b0 = intercept and b1 and b2 = regression coefficient.

2.7 Results

2.7.1 The effect of feed particle size and the interaction with the ME: total Lys ratio

There was no interaction or trend between the various energy: total Lys ratios (Table 2.3); varying total Lys percentage (Table 2.4) and varying ME (Table 2.5) levels and feed particle size. All the treatments produced crumbles (>1.4 mm and 2.36 mm particles) of the same general standard. However, on a relative scale, the amount of fines (<1.4 mm) produced by all the treatments were more than the general commercially accepted standard of 25%.

Table 2.1: The raw material composition of the basal dilution and summit diet composition fed to mixed sex broilers from day old to 7 days of age

	Basal Diet 1	Summit Diet 3	Basal Diet 4	Summit Diet 6	Basal Diet 7	Summit Diet 9	Basal Diet 10	Summit Diet 12
Blending ratio	50	50	50	50	50	50	50	50
Ingredients	---------------------------------- Percentage (%) -------------------------------------							
Maize	39.5	47.1	42.5	51.5	47.1	55.2	51.0	58.7
Soy bean oilcake	37.7	19.8	28.3	17.9	24.8	16.2	20.9	14.6
Sunflower oilcake	0.00	3.00	5.00	3.00	5.00	3.00	5.00	3.00
Middlings	2.29	1.00	4.00	1.00	4.00	1.00	4.00	1.00
Corn gluten meal	6.00	6.00	6.00	6.00	4.79	6.00	3.74	6.00
Soy oil	1.00	2.00	1.00	2.00	1.00	2.00	1.00	2.00
Fishmeal (65%)	3.42	13.1	2.00	9.96	2.00	7.29	2.00	4.82
Full fat soya	2.00	6.00	3.00	6.00	2.00	6.00	2.00	6.00
L-lysine	0.28	0.12	0.37	0.17	0.34	0.21	0.32	0.25
DL-methionine	0.31	0.20	0.26	0.18	0.23	0.15	0.20	0.13
L-threonine	0.06	0.01	0.06	0.01	0.06	0.01	0.06	0.01
MCP[1]	0.92	0.00	1.19	0.34	1.28	0.72	1.36	1.08
Limestone	1.69	1.02	1.78	1.25	1.76	1.41	1.75	1.55
Salt	0.35	0.18	0.36	0.23	0.36	0.27	0.36	0.31
Sodium Bicarb.	0.11	0.11	0.14	0.12	0.14	0.14	0.15	0.15
Choline (60%)	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Pre-mix[2]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Filler (sand)	4.00	0.00	3.78	0.00	4.75	0.00	5.87	0.00

[1]MCP = Monocalcium phosphate

[2]Premix provided the following (per kg diet): Vitamin A, 13000 IA; vitamin D_3, 5000 ICU; vitamin E, 80.0 mg; vitamin B_{12}, 20.0 ug; riboflavin, 9.0 mg; niacin, 60.0 mg; d-pantothenate, 20.0 mg; menadione (K_3), 3.0 mg; folic acid, 3.2 mg; thiamine, 1.6 mg; pyridoxine, 4.0 mg; d-biotin, 250.0 ug; selenium (as Na_2SeO_3), 0.30; Manganese, 110 mg; zinc, 100 mg; iron, 70 mg; copper, 20 mg; iodine, 2.0 mg; cobalt, 0.5 g

Table 2.2: The nutrient composition of the basal and summit dilution diets fed from day old to 7 days mixed sex broilers

	Basal Diet 1	Summit Diet 3	Basal Diet 4	Summit Diet 6	Basal Diet 7	Summit Diet 9	Basal Diet 10	Summit Diet 12
Blending ratio	50	50	50	50	50	50	50	50
Calculated nutrients[1]								
Moisture (%)	9.62	9.60	9.60	9.70	9.50	9.70	9.50	9.80
ME [2], kcal/kg	2700	3100	2700	3100	2700	3100	2700	3100
CP, %	28.2	29.3	25.7	26.7	23.2	24.4	21.0	22.3
Lys [3], %	1.77	1.77	1.60	1.60	1.40	1.40	1.30	1.30
Met [3], %	0.80	0.81	0.71	0.72	0.64	0.64	0.58	0.58
Met & Cys [3], %	1.26	1.27	1.15	1.15	1.04	1.04	0.94	0.40
Thr [3], %	1.17	1.19	1.06	1.07	0.96	0.97	0.87	0.87
Val [3], %	1.35	1.39	1.22	1.26	1.10	1.14	1.00	1.01
Arg [3], %	1.78	1.78	1.61	1.61	1.45	1.47	1.31	1.31
SID. Lys [4], %	1.58	1.57	1.44	1.43	1.31	1.30	1.18	1.18
SID. SAA [4], %	1.12	1.11	1.02	1.00	0.92	0.92	0.83	0.83
SID. Thr [4], %	0.99	0.99	0.90	0.90	0.81	0.82	0.74	0.74
SID. Val [4], %	1.18	1.22	1.07	1.11	0.97	1.01	0.88	0.89
SID. Arg [4], %	1.61	1.56	1.46	1.42	1.31	1.29	1.18	1.18
Ca, %	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Avl. P %	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48
Na, %	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Cl, %	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28
DEB[5], Meq/kg	217	168	192	161	178	154	164	148
CP: ME ratio	54.3	61.6	63.2	65.1	67.4	66.5	61.8	55.9
ME: total Lys	1525	1751	1688	1938	1929	2214	2077	2385
Analysed nutrient composition								
CP, %	28.3	29.7	25.5	26.9	23.9	23.9	21.6	22.6
Lys, %	1.75	1.78	1.61	1.58	1.43	1.40	1.31	1.28

[1]Nutrient composition calculated from proximate analyses of all ingredients prior to formulation

[2]Energy content of ingredients calculated as per WPSA 3rd Ed, 1989

[3]total amino acid content of ingredients calculated as a percentage of the analysed CP content

[4]Calculated from standardised ileal digestibility coefficients applied to the calculated amino acid content (Evonik, 2014)

[5]DEB = dietary electrolyte balance (Na + K – CL)

Table 2.3: The effect of varying total Lys and energy (ME) levels in diets on crumble quality

Treatment	Total Lys (%)	Energy (ME) (kcal/kg)	Ratio (ME: total Lys)	>2.36 mm (%)	>1.4 mm (%)	<1.4 mm (%)
1	1.77	2700	1525	50	8	42
2	1.77	2900	1638	40	11	49
3	1.77	3100	1751	39	10	50
4	1.60	2700	1688	40	8	50
5	1.60	2900	1813	31	8	61
6	1.60	3100	1938	49	8	44
7	1.40	2700	1929	35	11	53
8	1.40	2900	2071	51	10	39
9	1.40	3100	2214	47	11	42
10	1.30	2700	2077	59	9	33
11	1.30	2900	2231	40	9	51
12	1.30	3100	2385	42	11	47
SEM				7.50	1.25	7.00
p > F						
ME: total Lys quadratic				0.56	0.80	0.75

[a-d] Means in the same column with no common superscript differ significantly ($p \leq 0.05$)

Table 2.4: The effect of varying total Lys (%) levels in diets on crumble quality and pellet hardness (2700, 2900 and 3100 ME kcal/kg)

Total Lys (%)	>2.36 mm (%)	>1.4 mm (%)	<1.4 mm (%)
1.77	43	10	47
1.60	40	8	52
1.40	44	11	45
1.30	47	10	43
SEM	0.05	0.10	0.06
p > F			
total Lys quadratic	0.79	0.32	0.60

[a-d] Means in the same column with no common superscript differ significantly ($p \leq 0.05$)

Table 2.5: The effect of varying energy (ME) in diets on crumble quality

Energy (ME) (kcal/kg)	>2.36 mm (%)	>1.4mm (%)	<1.4mm (%)
2700	46	10	45
2900	41	10	50
3100	44	10	45
SEM	0.06	0.01	0.05
p > F			
ME quadratic	0.64	0.77	0.58

[a-d] Means in the same column with no common superscript differ significantly ($p \leq 0.05$)

2.7.2 Animal performance

2.7.2.1 The effect of feeding varying energy (ME): total Lys ratios to mixed sex broilers from day old to 7 days of age

The response to varying energy: total Lys levels for ADG, ADFI, and FCR are presented in Table 2.6 and Figures 2.1, 2.2 and 2.3 respectively. The ADG of the broilers improved with an increase in total Lys content as well as with an increase in energy. However, ADFI tended to be less responsive with only Treatment 2 (1.60% total Lys; 3100 kcal/kg; 1638 ratio) consuming less and treatment 7 (1.40% total Lys; 2700 kcal/kg; 1929 ratio) significantly more when compared to the other treatments. No mortalities occurred during the 7-day trial period.

The statistical evaluation of the response of broilers (0–7 days of age) to varying levels of ME: total Lys ratio gave rise to the following equations for ADG, ADFI and FCR:

$$\text{ADG} = 1.0000 + -0.9979 \times (\text{ME: total Lys ratio}) + 0.9919 \times (\text{ME: total Lys ratio}^2) \quad (R^2 = 0.37) \ [2.3]$$

$$\text{FCR} = 0.2493608 + 0.000893x\ (\text{ME: total Lys ratio}) + -0.0000001833 \times (\text{ME: total Lys ratio}^2) \quad (R^2 = 0.39) \ [2.4]$$

$$ADFI = 4.2932566 + 0.0099603 \times (ME: total\ Lys\ ratio) + 0.0003807 \times (ME: total\ Lys\ ratio^2)$$

$$(R^2 = 0.08) \quad [2.5]$$

A linear regression for ME: total Lys ratio indicated that the point of maximum response for ADG was 1939 while lowest FCR was achieved at 1751. No area of maximum response in FCR could be calculated since plateaus had not been reached.

Table 2.6: The effect on the performance of day old to 7 days of age mixed flock broilers due to the feeding of varying ME: total Lys ratios

Treatment	Total Lys (%)	ME (kcal /kg)	Ratio[#]	Day-0 weight (g)	Day-7 weight (g)	ADG (g)	ADFI (g)	FCR (g/g)	Mortality (%)
1	1.77	2700	1525	46.8	128^{ab}	11.6^{ab}	14.1^{abc}	1.22^{bcd}	0.00
2	1.77	2900	1638	45.5	122^{abc}	11.0^{abc}	13.0^{c}	1.19^{cd}	0.00
3	1.77	3100	1751	46.5	126^{abc}	11.3^{abc}	13.3^{bc}	1.17^{d}	0.00
4	1.60	2700	1688	45.7	122^{abc}	10.9^{abc}	14.0^{abc}	1.29^{bcd}	0.00
5	1.60	2900	1813	45.7	122^{abc}	11.0^{abc}	13.6^{abc}	1.24^{bcd}	0.00
6	1.60	3100	1938	45.8	130^{a}	12.0^{a}	14.7^{ab}	1.23^{bcd}	0.00
7	1.40	2700	1929	46.5	124^{abc}	11.1^{abc}	14.8^{a}	1.32^{abc}	0.00
8	1.40	2900	2071	45.5	120^{bcd}	10.7^{bcd}	14.0^{abc}	1.31^{abcd}	0.00
9	1.40	3100	2214	45.8	119^{cd}	10.5^{bcd}	13.3^{bc}	1.27^{bcd}	0.00
10	1.30	2700	2077	45.0	111^{d}	9.50^{d}	13.6^{abc}	1.45^{a}	0.00
11	1.30	2900	2231	46.6	119^{bcd}	10.5^{bcd}	14.2^{abc}	1.35^{ab}	0.00
12	1.30	3100	2385	46.3	118^{cd}	10.3^{cd}	13.6^{abc}	1.32^{abc}	0.00
SEM				0.60	1.80	0.30	0.30	0.03	-
$p > F$									
Ratio quadratic				0.35	0.0001	0.0001	0.0002	0.001	-

[#] Ratio = ME: total Lys

[a-d] Means in the same column with no common superscript differ significantly ($p \leq 0.05$)

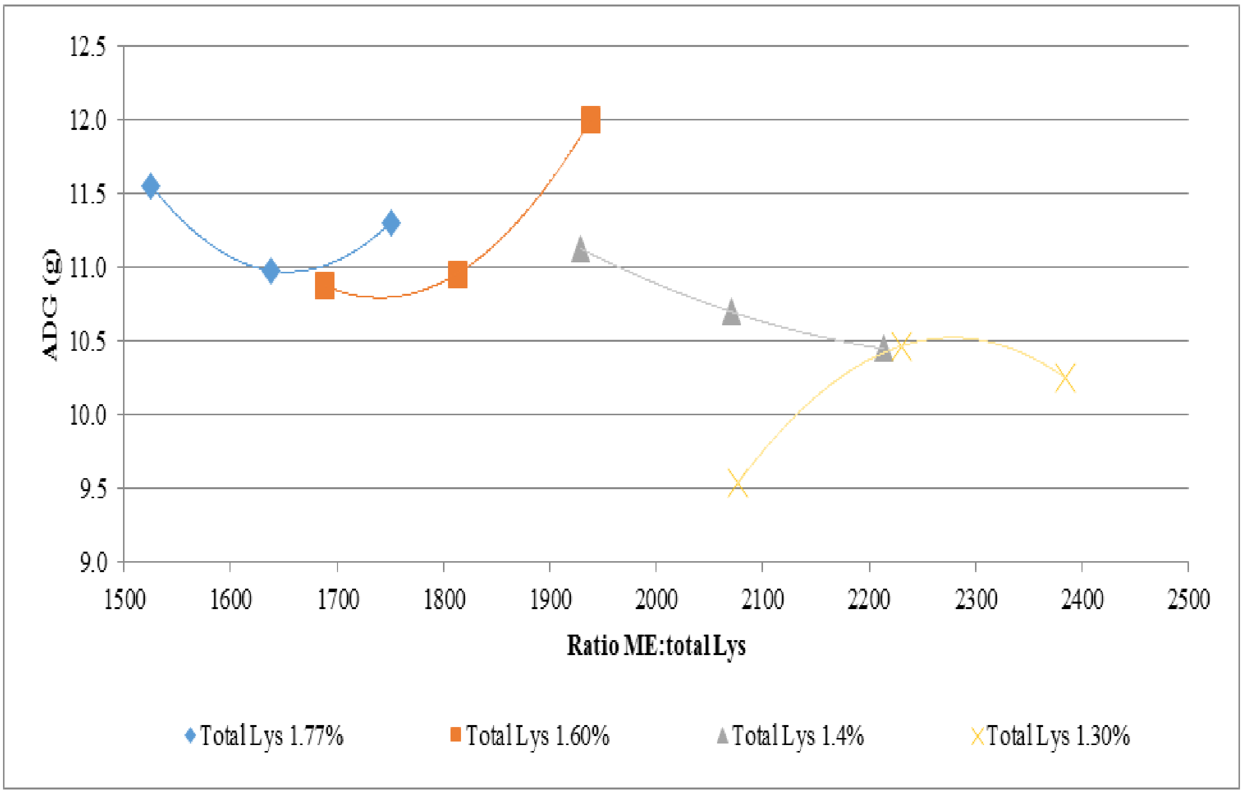

Figure 2.1: The effect of feeding day old to 7 days of age mixed sex broilers varying amounts of dietary ME: total Lys ratios on Average Daily Gain (ADG)

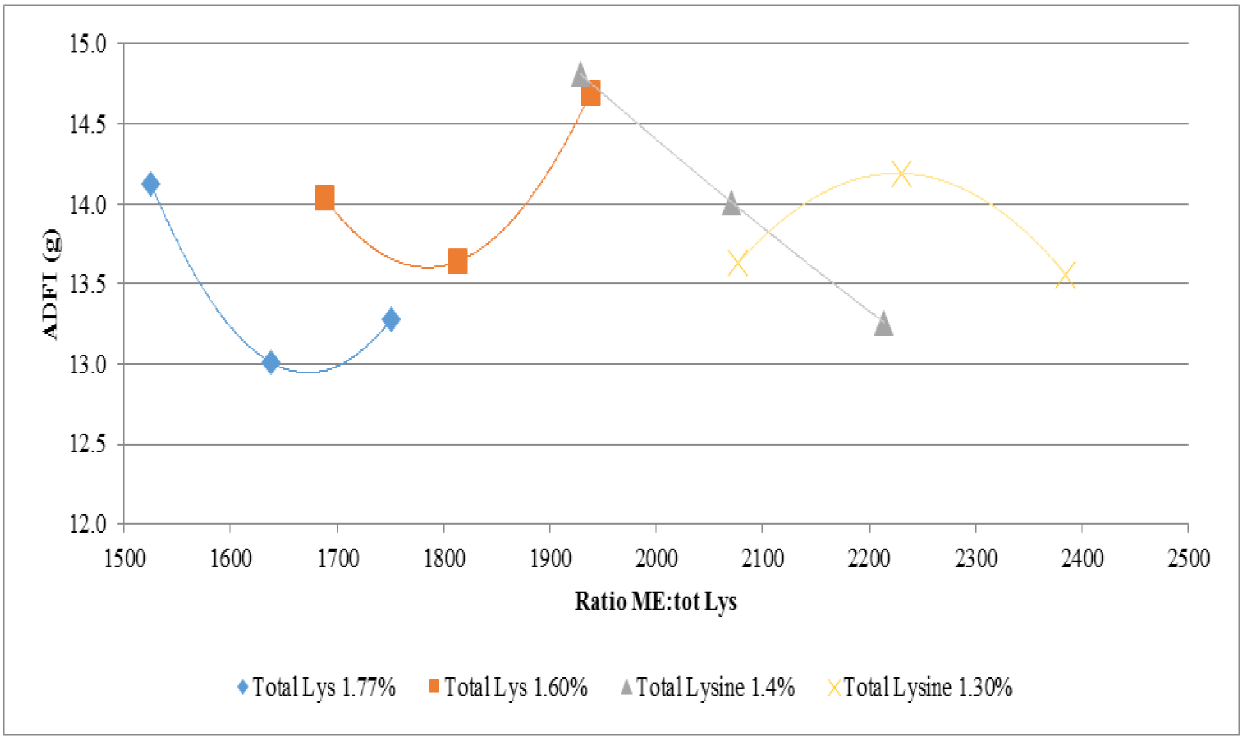

Figure 2.2: The effect of feeding day old to 7 days of age mixed sex broilers varying amounts of dietary ME: total Lys ratios on Average Daily Feed Intake (ADFI)

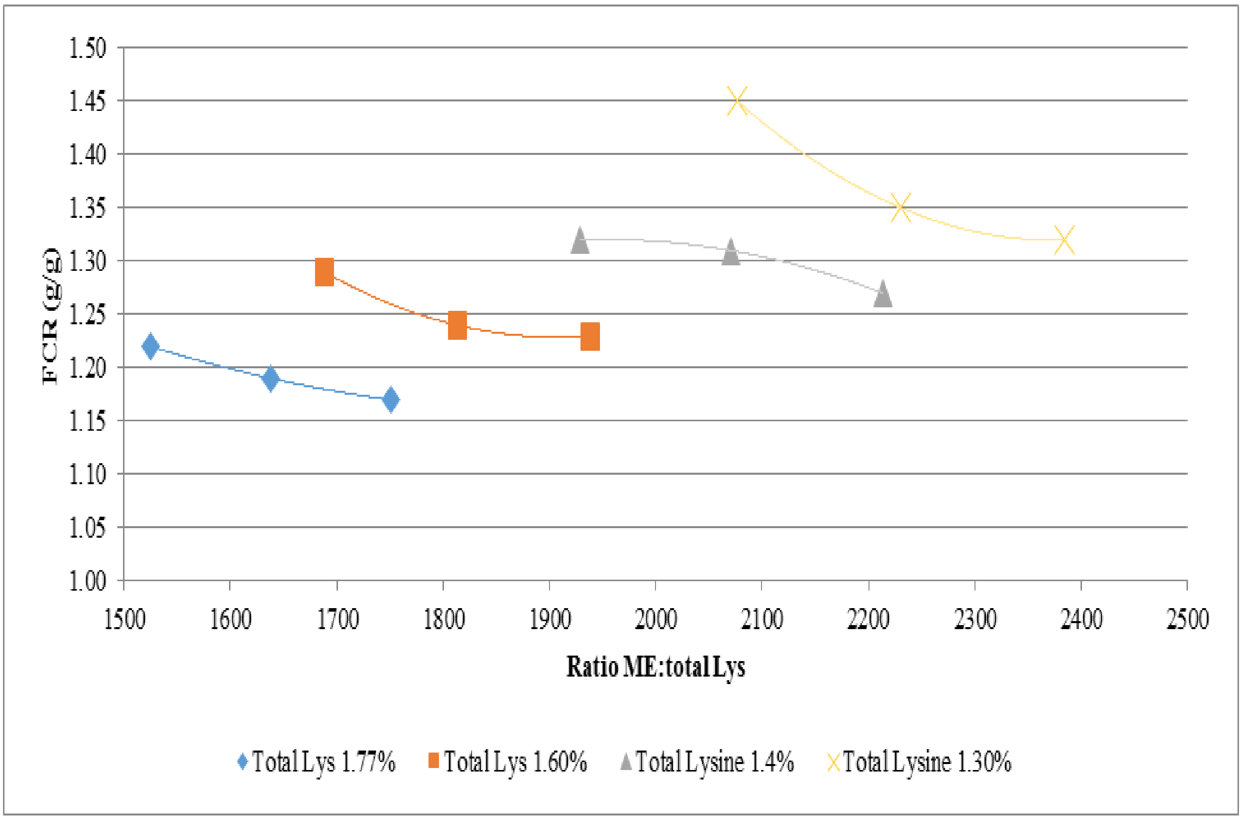

Figure 2.3: The effect of feeding day old to 7 days of age mixed sex broilers varying amounts of dietary ME: total Lys ratios on Feed Conversion Ratio (FCR)

2.7.2.2 The effect of feeding varying total Lys *levels on mixed sex broilers from day old to 7 days of age*

The responses of mixed sex broilers (0-7 days) to the feeding of varying levels of total Lys are depicted in Table 2.7 and Figures 2.4 (ADG), 2.5 (ADI) and 2.6 (FCR). The broilers' ADG increased significantly when the total Lys were increased from 1.40% to 1.60% but no further improvement were seen at the highest level of 1.77%. The FCR decreased with an increase in total Lys. The FCR were significant lower when the total Lys were increased from 1.40 to 1.77% total Lys. No significant differences in ADFI between the treatments were detected.

Table 2.7: The effect on the performance of day old to 7 days of age mixed flock broilers to the feeding of varying total Lys levels (2700, 2900 and 3100 ME kcal/kg)

Total Lys (g)	Day old weight (g)	7 Day weight (g)	ADG (g)	ADFI (g)	FCR (g/g)	Mortality (%)
1.77	46.3	125. ^{a}	11.3 ^{a}	13.5	1.20 ^{c}	0.00
1.60	45.7	125 ^{a}	11.3 ^{a}	14.1	1.25 ^{bc}	0.00
1.40	45.9	121 ^{a}	10.8 ^{b}	14.0	1.30 ^{b}	0.00
1.30	45.9	116^{b}	10.1^{b}	13.8	$^{1.38}$b	0.00
SEM	0.33	1.18	0.16	0.20	0.02	0.00
p>F						
tot. Lys Quadratic	0.66	0.10	0.0001	0.109	0.001	0.63

[a-d] Means with the same column with no common super script differ significantly $(p \leq 0.05)$

The statistical analysis of the response of mixed sex broilers (0-7 days) to total Lys for ADG, ADFI and FCR is indicated in the equations below:

ADG = -17.166+ 35.220 x total Lys + -10.930 x total Lys ratio^2 ($R^2 = 0.80$) [2.6]

FCR = 1.794 + -0.3387 x total Lys ($R^2 = 0.99$) [2.7]

ADFI = 12.88 + -16.961 * total Lys + 5.51 * total Lys ^{2} ($R^2 = 0.98$) [2.8]

According to the quadratic regression for total Lys, the point of maximum response for ADG was achieved at 1.48%. No point of maximum response in FCR could be calculated since the plateaus had not been achieved.

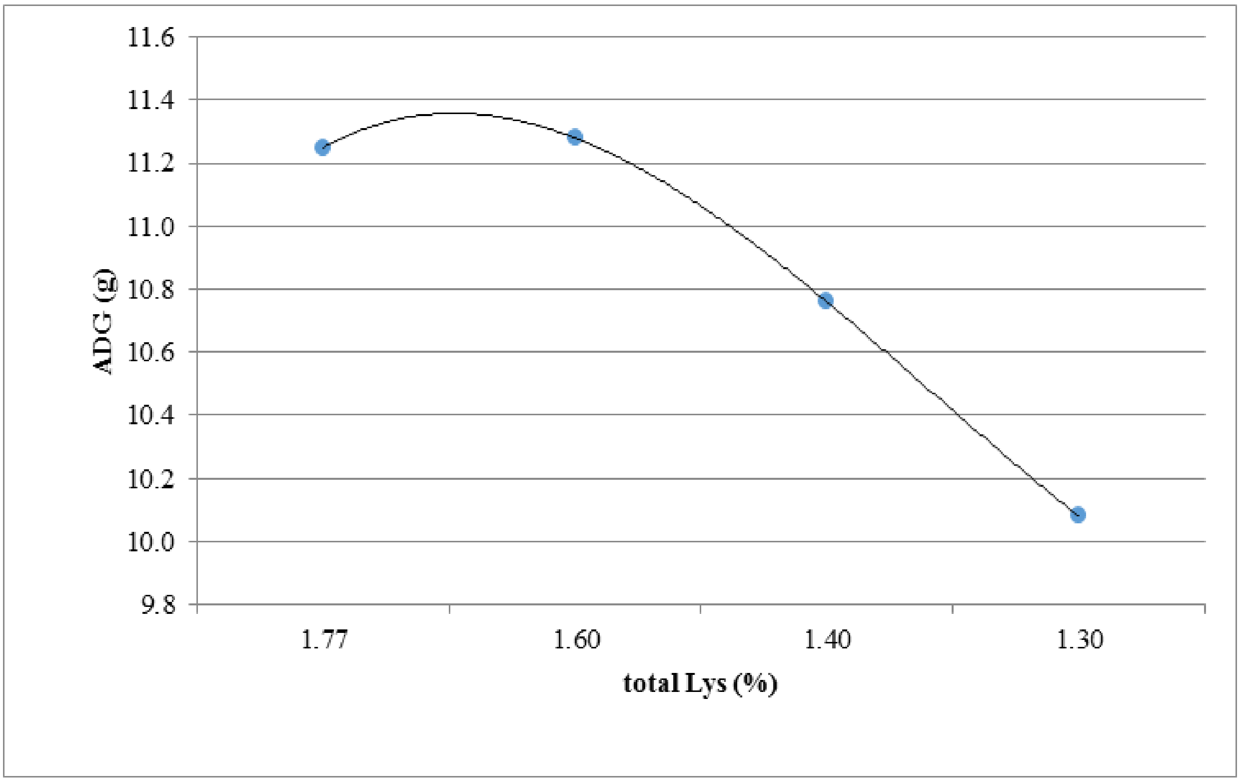

Figure 2.4: The effect of feeding 7day old to 7 days of age mixed sex broilers varying amounts of dietary total Lys ratios on Average Daily Gain (ADG)

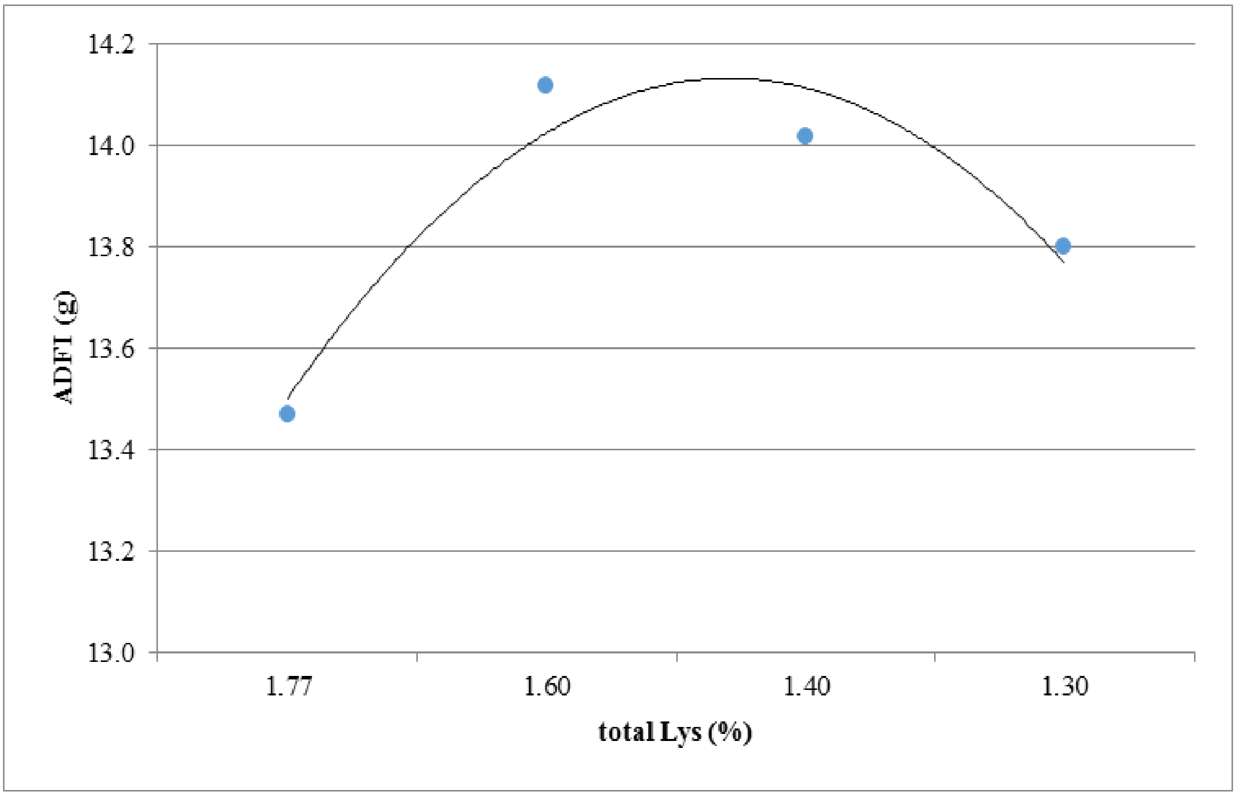

Figure 2.5: The effect of feeding day old to 7 days of age mixed sex broilers varying amounts of dietary total Lys ratios on Average Daily Feed Intake (ADFI)

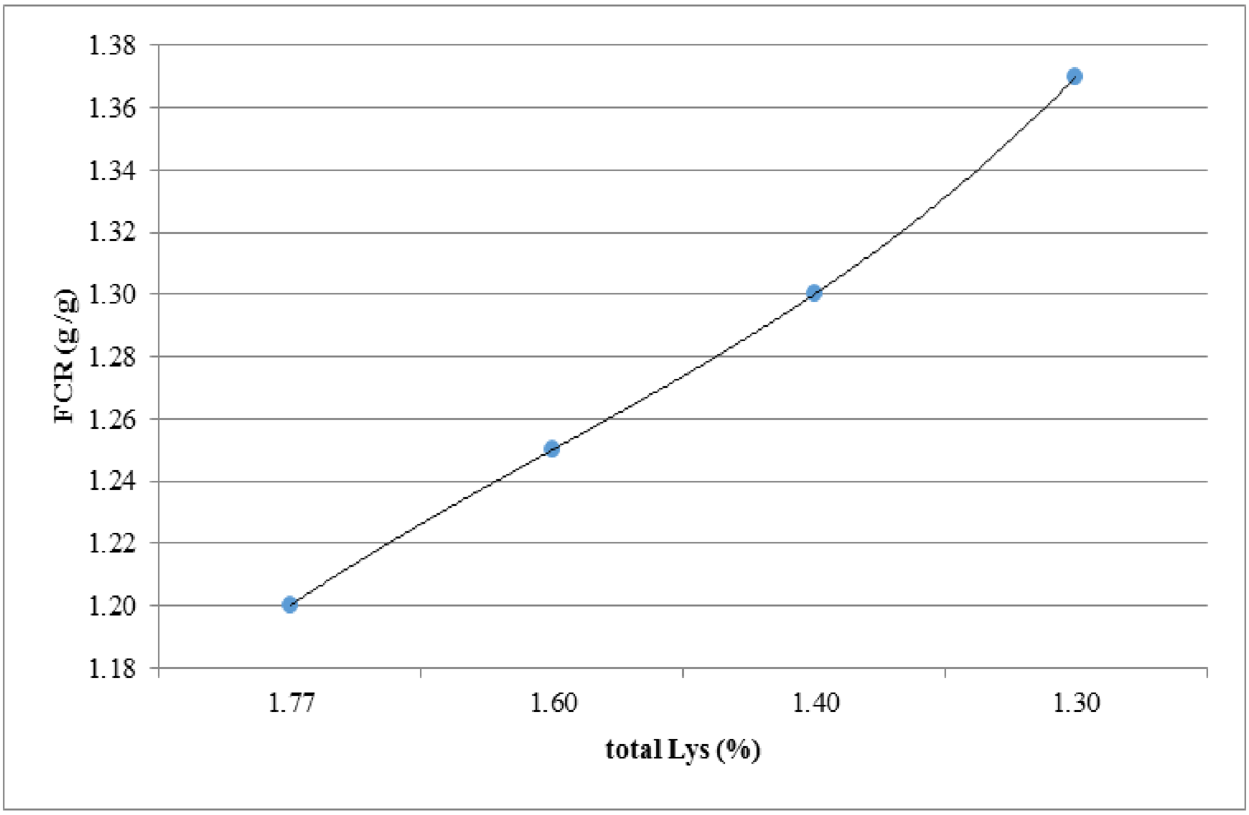

Figure 2.6: The effect of feeding day old to 7 days of age mixed sex broilers varying amounts of dietary total Lys ratios on Feed Conversion Ratio (FCR)

2.7.2.3 The effect of feeding varying energy (ME) levels on mixed sex broilers from day old to 7 days of age

The broilers' responses (0-7 days) to varying energy (ME) are depicted in Table 2.8 and Figures 2.7 (ADG), 2.8 (ADI) and 2.9 (FCR). Although the ADG and ADI tended to be the highest at the 3100 kcal, no significantly differences between treatments were observed. However, FCR improved significantly with higher ME levels while the highest ME (3100 kcal/kg) resulted in a significant improved FCR when compared to the lowest level (2700 kcal ME/kg).

The quadratic statistical analysis of the response of broilers (0-7 days) to energy (ME) for ADG, ADI and FCR is indicated in the equations below:

ADG = 36.4975 + -0.0183 x ME + 0.00000325 x ME ^{2} (R^2 = 0.89) [1.9]

$$FCR = 2.83875 + -0.0009 \times ME + 0.000000125 \times ME^2 \quad (R^2 = 0.92) \; [1.10]$$

$$ADFI = 3.31625 + 0.00975 \times ME + -0.000002125 \times ME^2 \quad (R^2 = 0.95) \; [1.11]$$

Table 2.8: The effect on the performance of day old to 7 days of age mixed flock broilers due to the feeding of varying energy (ME) levels (1.3, 1.4, 1.6, 1.77% total Lys)

ME (kcal/kg)	Day old weight (g)	7 Day weight (g)	ADG (g)	ADFI (g)	FCR (g/g)	Mortality (%)
2700	46.0	122	10.8	14.2	1.32 [a]	0.00
2900	45.8	121	10.8	13.7	1.28 [ab]	0.00
3100	46.1	123	11.0	13.1	1.25 [b]	0.00
SEM	0.28	1.18	0.16	0.15	0.018	0.00
p>F						
ME Quadratic	0.66	0.43	0.51	0.12	0.02	0.65

[a-d] Means in the same column with no common superscript differ significantly ($p \leq 0.05$)

In view of the fact that there were no significant differences in ADG in response to varying energy levels between the treatments, it is not possible to predict an optimum level of ME for ADG. In addition, since the FCR did not reach a plateau when the energy was increased from 2700 to 3100 kcal ME/kg it was not possible to predict an optimum level of ME for FCR.

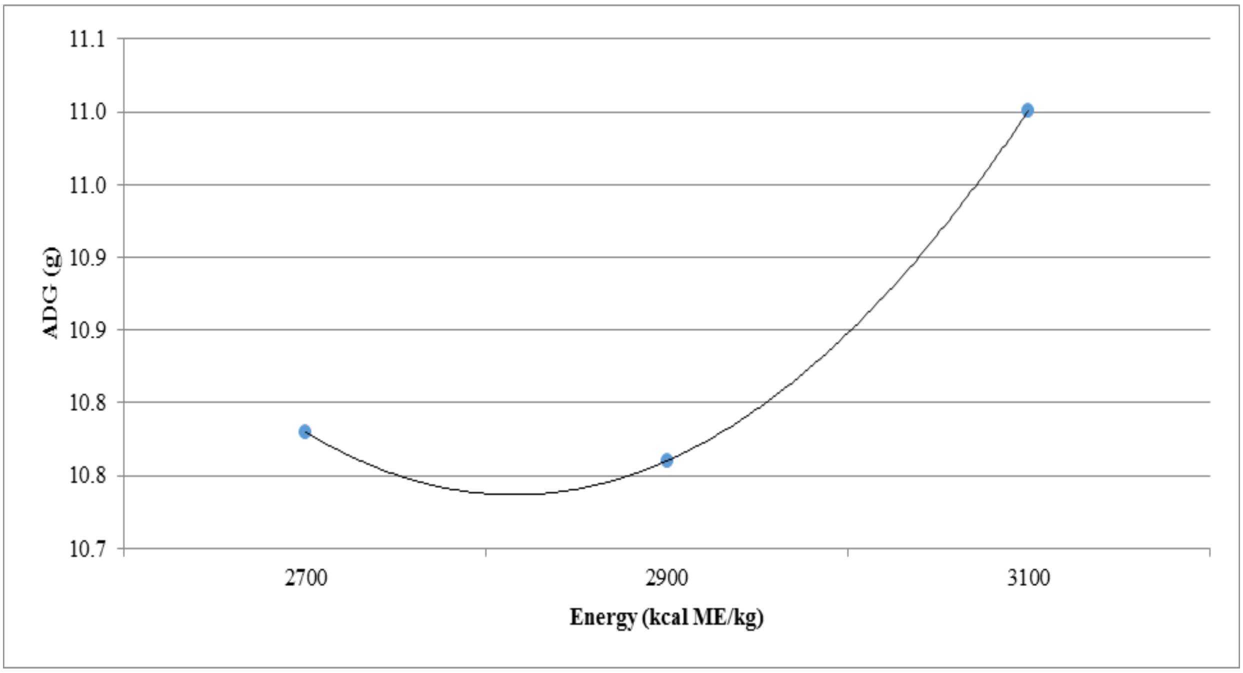

Figure 2.7: The effect of feeding day old to 7 days of age mixed sex broilers varying amounts of energy (ME) on the Average Daily Gain (ADG)

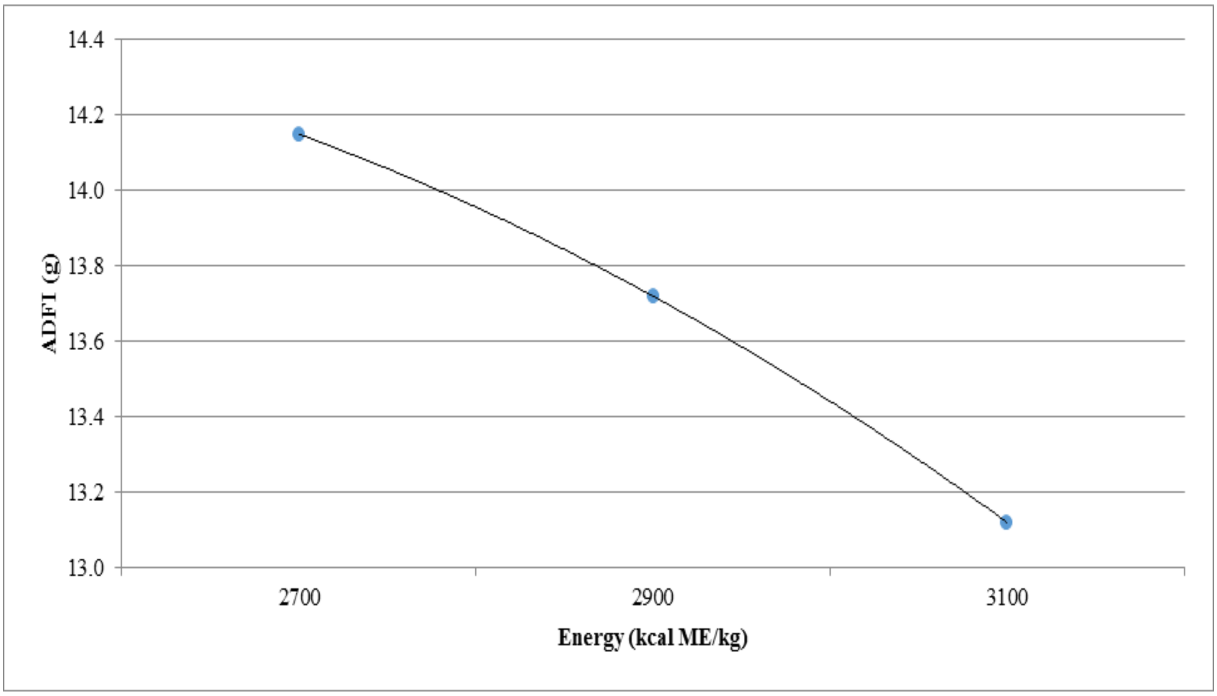

Figure 2.8: The effect of feeding day old to 7 days of age mixed sex broilers varying amounts of energy (ME) on the Average Daily Feed Intake (ADFI)

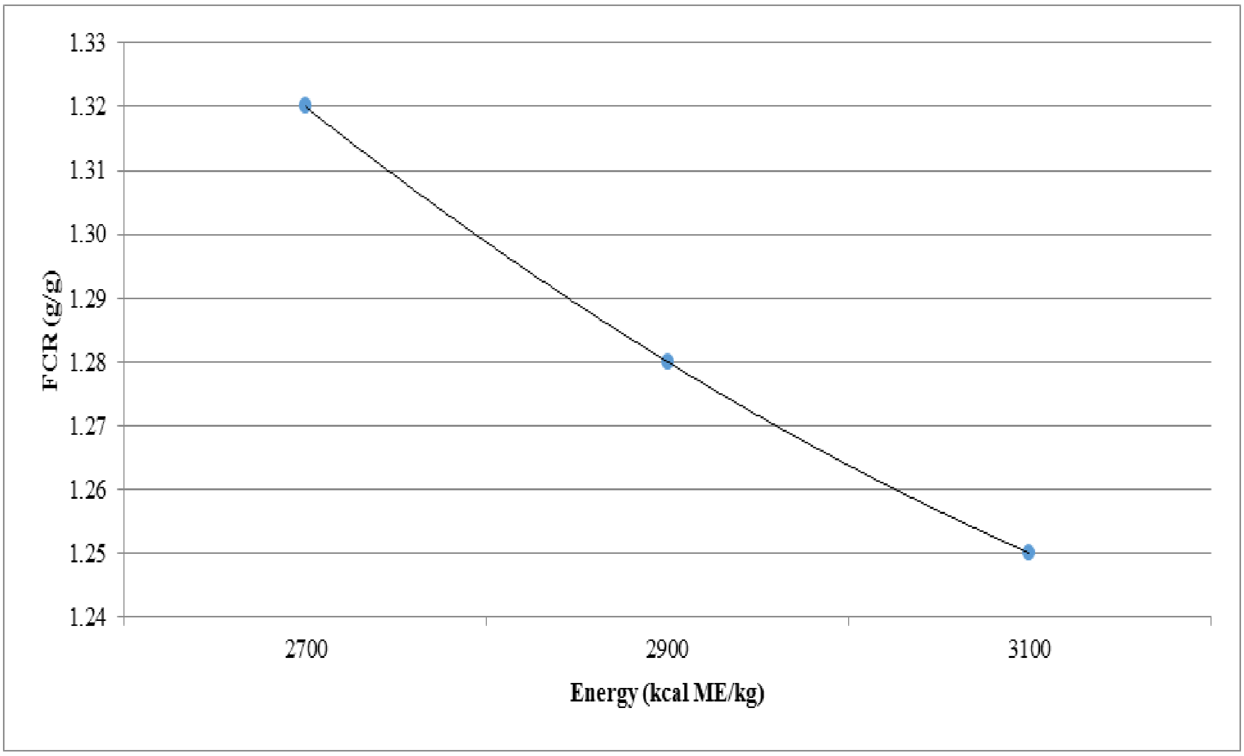

Figure 2.9: The effect of feeding day old to 7 days of age mixed sex broilers varying amounts of energy (ME) on the Feed Conversion Ratio (FCR)

2.8 Discussion

According to Lemme (2007), a typical way in which to determine optimum dietary nutrient levels is to find the point at which maximum performance is achieved. The literature highlights differences with respect to the estimated minimum Lys requirement of broilers that, in part, have been ascribed to differences in broiler strain (Acar *et al.*, 1991; Bilgili *et al.*, 1992), rate of breast meat accretion (Biligili *et al.*, 1992; Labadan *et al.*, 2001), method of determination of requirement (Mack *et al.*, 1999), preferred response variable (Baker *et al.*, 2002), environmental factors (Kidd and Fancher, 2001), and the form of the diet (Greenwood *et al.*, 2005). Several researchers have also suggested that the Lys requirement of broilers increased when the dietary CP of the diet was increased (Grau and Kamei, 1950; Morris *et al.*, 1987; Adebe and Morris, 1990).

Dozier and Payne (2012) conducted two experiments with Ross × Ross 708 and Hubbard × Cobb 500 female broilers from day 1 to 7 with the Dig. Lys ranging from 0.95% (1.21% on a total Lys basis) to 1.43% (1.21% on a total Lys basis). They concluded that the optimum broiler responses at 7 days were achieved at 1.35% digestible Lys (1.61% on total Lys basis) for ADG and 1.38% digestible lysine for FCR (1.64% total Lys). However, in an experiment conducted by Urdaneta-Rincón and Leeson (2008) with Ross males, the researchers concluded that the area of maximum response for ADG and FCR was achieved at 1.47%. The difference in responses may be explained by the fact that Urdaneta-Rincon and Leeson (2008) maximum level of treatment was, in fact, 1.47% total Lys while the maximum level could have been even higher as no plateau in response were observed.

Plumstead (2005) fed Ross 344 broilers from day old to 7 days in two experiments. In experiment 2, a significant improvement in ADG was observed when the total Lys was increased beyond 1.29% although no further significant response could be observed when the total Lys were increased to 1.38%. A quadratic regression revealed that the poimt of maximum response was achieved at a level of 1.46% total Lys. However, much like the experiment of Urdaneta-Rincón and Leeson (2008), it is difficult to state whether this was, indeed, the point of maximum response as 1.47% was the highest level of treatment. The results of this experiment in this trial indicates that the ADG at a total Lys level of 1.40% was significantly more than that of birds fed 1.30% and also that there was no statistical improvement beyond 1.40%. However, the quadratic equation indicated that the point of maximum response was 1.48% total Lys for ADG – a result similar to the results reported by Plumstead (2005) and Urdaneta-Rincón and Leeson (2008). Since higher levels of total Lys were tested than by Plumstead (2005) and Urdaneta-Rincón and Leeson (2008), it becomes apparent that, beyond 1.48% total Lys, no further improvement in ADG response could be expected. However, it was not clear from the results of this experiment, what the point of maximum response of FCR could be. Even at a level of 1.77%, the quadratic regression indicated that the point of maximum response had not been achieved and that the optimum point may still be beyond 1.77%.

The results from this trial indicated that ADG of broilers fed from day old to seven days was not significantly improved by ME levels with birds fed 2700 kcal ME/kg and 3100 kcal ME/kg. However, the FCR was significantly improved when the ME was increased from 2700 to 3100 kcal ME/kg. Taking into account the ME: total Lys ratios, the ADG and FCR of the birds tended to improve with a lower ratio. However, ADG and FCR were only significantly improved when the ME: total lysine ratio was lower than 2071.

2.9 Conclusion

The ADG of broilers improved with an increase in total Lys content but did not improve significantly with an increase in energy. The point of maximum response for ADG was achieved at 1.48% total Lys. The ADFI tended to be less responsive with only two of the treatments in this study consuming differently from the other seven treatments. The FCR was however, significantly improved with higher ME levels while the highest ME (3100 kcal ME/kg) resulted in a significant lower FCR when compared to the lowest energy level (2700 kcal ME/kg). With regards to ADG, the point of maximum response was 1.60% total Lys, 3100 kcal ME/kg, implying a ME: total Lys ratio of 1938 while, for FCR, the point of maximum response were 1.77% total Lys, 3100 kcal/kg, implying a ME: total Lys ratio of 1751. The statistical evaluation of the response of broilers (0–7 days of age) to varying levels of ME: total Lys ratio gave rise to the following equations for ADG= 1.0000 + -0.9979 x (ME: total Lys ratio) + 0.9919 x (ME: total Lys ratio^2) (R^2 = 0.37); FCR = 0.2493608 + 0.000893x (ME: total Lys ratio) + -0.0000001833 x (ME: total Lys ratio^2) (R^2 = 0.39) and ADFI = 4.2932566 + 0.0099603 x (ME: total Lys ratio) + 0.0003807 x (ME: total Lys ratio^2) (R^2 = 0.08).

Chapter 3:

The response of mixed sex broilers to varying dietary Energy: total Lys ratios fed from 7 days to 14 days of age

3.1 Abstract

A trial was conducted to study the response of mixed sex broilers to varying ME: total Lys levels, as well as the bird's response to the interaction between these two nutrients. Five hundred and seventy-six Ross 308 mixed male and female broilers were randomly fed one of twelve rations. The dietary treatments consisted of four total Lys levels (1.77, 1.60, 1.40 and 1.30%) and three ME levels (2700, 2900 and 3100 ME kcal/kg). The use of the dietary summit/dilution technique and the combination of total Lys and energy levels resulted in various ME: total Lys levels (1525, 1638, 1751, 1688, 1813, 1938, 1929, 2071, 2214, 2077, 2231, and 2385). Each one of the 12 treatments was replicated 8 times. Birds from day 7 to 14 days were fed the trial diets. The ADG of broilers improved with an increase in total Lys content. The quadratic regression revealed that the point of maximum response for ADG was achieved at 1.61% total Lys and that 1.64% total Lys was needed for minimum FCR. Average daily feed intake increased significantly when the total Lys was reduced below 1.60% total Lys. The ADG was improved significantly when the energy was increased from 2700 to 2900 kcal ME kcal/kg ME levels while the FCR was significantly reduced for each increased level in energy. The point of maximum response for ADG were 1.77% total Lys, 3100 kcal ME, implying a ME: total Lys ratio of 1751. On the other hand, for FCR, the point of maximum response were 1.60% total Lys, 3100 kcal/kg, implying a ME: total Lys ratio of 1938.

The statistical evaluation of the response of broilers (7–14 days of age) to varying levels of ME: total Lys ratio gave rise to the following equations for ADG = -3.108766 + 0.0389846 x (ME: total Lys ratio) + -0.00001038 x (ME: total Lys ratio^2) (R^2 = 0.40); FCR =2.0683794+ -

0.000729 x (ME: total Lys ratio) + 0.00000022155x (ME: total Lys ratio^2) (R^2 = 0.39); ADFI =11.619103+0.0367668 x (ME: total Lys ratio) + -0.000008828 x (ME: total Lys ratio^2) (R^2 = 0.17)

3.2 Introduction

The majority of literature reported on broiler response to amino acid and energy requirements report only the response of broilers to energy or Lys and not the interaction between these two nutrients (Vazquez and Pesti, 1997; Dozier and Payne, 2012). There are only few examples in the literature where broilers received graded levels of balanced amino acid profiles at two or three dietary energy levels. Plumstead (2005) fed Ross broilers from day old to 14 days a range of lysine (total lysine, 1.47, 1.38, 1.29, 1.20%) and energy levels (3000, 3100 and 3200 kcal ME/kg). ME: total Lys ratios varied between 2041 and 2667. Fourteen-day weight gain was the highest at the lowest ratios (2000 to 2400) and reduced considerably after 2400. The effect on FCR showed very much the same trend with the lowest value being achieved at the lowest ratios (2000 to 2400) and increasing after 2500. Relative to FCR, ADFI tended to be less responsive although birds at the lowest ratio consumed less feed than the birds fed at the highest ratio. The birds' ADG at the highest energy levels (3100 and 3200 kcal ME/kg) was significantly higher when compared to the lowest energy treatment (3000 kcal ME/kg) while FCR improved significantly when the energy was increased from 3000 to 3200 kcal ME/kg.

Urdaneta-Rincón and Leeson (2008) fed broilers from day old to 18 days with treatments consisting of a wider ME: total Lys levels (2000 to 3000), consisting of five total Lys levels but only one energy level (3000 kcal ME/kg) only. The broilers' ADG was significantly higher at the lower ME: total Lys ratios while FCR was also significantly improved. In a trial conducted by Basurco *et al.* (2015), Cobb 500 broilers from 8 days to 18 days were fed three energy levels (3054, 3100 and 3147 kcal/kg) and three levels of amino acid density (1.10, 1.22, 1.34% dig.

Lys). The broilers' ADG, FCR and ADFI improved significantly with an increase in amino acid density. However, an increase in energy level had no significant effects on ADFI. However, ADG was increased and FCR reduced significantly with an increase in energy from 3054 to 3100 kcal/kg as well as from 3100 to 3147 kcal/kg. The aim of this experiment was to derive equations that could relate ADG and FCR of broilers to varying energy and total Lys levels. It was anticipated that these equations would enable commercial nutritionist to determine the most economical point for broilers during the 7 to 14 day period.

3.3 Material and methods

A total of 288 Ross 308 day-old male and 288 Ross 308 day-old female broilers were sexed at the hatchery and reared separately on a commercial starter feed. The commercial starter feed was offered from 0-7 days old (2900 kcal ME/kg, 220 g CP/kg) on an *ad-libitum* basis. At 7 days of age, male and female birds were randomly allocated to one of the 12 dietary treatments. The broilers were placed in a brooding facility containing battery cages with six birds per pen. A total of 96 cages were used. The cages were 0.8 m long x 0.5 m wide x 0.42 m height, thus giving a total floor area of 0.4 m^2. The stocking density for the trial was 15 birds/ m^2. A line feeder was available through the front side of the cage (0.8 m long) and water was provided by nipple drinkers (2 nipples per cage). Feed and water were available on an *ad-libitum* basis.

The temperatures were set to 28°C at 7 d and reduced by 1°C every day thereafter. The light intensity was set at 30 lux (23 hr light and 1 hr darkness).

3.4 Experimental diets

Dietary treatments consisted of a 3 x 4 factorial arrangement. Twelve diets were formulated to contain three levels of ME (2700, 2900, and 3100 kcal ME/kg) and four total Lys AA levels (1.77, 1.60, 1.4, and 1.3%) in order to achieve a range of ME: total Lys ratios (1525, 1638, 1751, 1688, 1813, 1938, 1929, 2071, 2214, 2077, 2231, and 2385). It is important to note that the same ME: total lysine ratio can be achieved with various combinations of energy and lysine.

For examples a diet with 3000 kcal ME/kg and 1.50% total Lys will provide a ratio of 2000. However, the ratio of 2000 ME: total Lys can also be achieved with 2900 kcal ME/kg and 1.45% total Lys. Prior to the diet formulation, the total AA of all ingredients was calculated as a percentage of the analysed CP content using standard procedure (CVB, 2001). Due to different digestible co-efficient reported in literature (GfE, 1999, CVB, 2001 and Rostagno, 2005) and in order to try to make the study as applicable as possible, it was decided to rather formulate the feed on a total lysine basis and not on a digestible basis. The metabolisable energy (ME) content of the raw materials was calculated by WPSA (1989) procedure. An inert filler (sand) was required in the diet formulation to allow for the application of these principles across a wide range of ME and lysine. In order to reduce the variation between the dietary treatments that may have arisen as a result of batching and mixing of ingredients, and also to ensure uniform gradations of dietary total Lys within each ME level, a summit/dilution blending technique (Gous and Morris, 1983) was used during the mixing of the diets. This technique consisted of first formulating and mixing basal diets 1, 4, 7, 10 and summit diets 3, 6, 9, 12. The four intermediate diets were then derived by blending the respective summit and dilution diets within each level of ME in the proportion as shown in Table 3.1 and Table 3.2. Following the mixing and blending procedure all the diets were then analysed for proximate and amino acid composition. The diets were pelleted in a pellet mill (Simon-Brown California Hyflo 67) equipped with a 3 mm die.

3.4.1 Pellet hardness and quality index

A representative sample from each one of the twelve treatments was subjected to a feed texture quality test. The pellet durability index (PDI) was measured by weighing 500 grams of the diet pellets and then placing the sample in a rolling drum (300 x 300 x 125 mm) (ASAE Standards, 1987). The rolling drum was rotated at 50 revolutions per minute. After 10 minutes, the pellets and fines were removed from the drum and sieved in a 2.36 mm and 1.4 mm screen.

The PDI, expressed as a percentage, was then calculated by using the equation below:

$$\text{PDI (\%)} = \frac{\text{Mass of pellets after rotation}}{\text{Mass of pellets before rotation}}$$

Table 3.1 The raw material composition of the basal dilution and summit diet composition fed to mixed sex broilers from 7 days to 14 days

	Basal Diet 1	Summit Diet 3	Basal Diet 4	Summit Diet 6	Basal Diet 7	Summit Diet 9	Basal Diet 10	Summit Diet 12
Ingredients	Percentage (%)							
Maize	39.5	47.1	42.5	51.5	47.1	55.2	51.0	58.7
Soy bean o/c	37.7	19.8	28.3	17.9	24.8	16.2	20.9	14.6
Sunflower o/c	0.00	3.00	5.00	3.00	5.00	3.00	5.00	3.00
Middlings	2.29	1.00	4.00	1.00	4.00	1.00	4.00	1.00
Corn gluten	6.00	6.00	6.00	6.00	4.79	6.00	3.74	6.00
Soy oil	1.00	2.00	1.00	2.00	1.00	2.00	1.00	2.00
Fishmeal	3.42	13.1	2.00	9.96	2.00	7.29	2.00	4.82
Full fat soya	2.00	6.00	3.00	6.00	2.00	6.00	2.00	6.00
L-lysine	0.28	0.12	0.37	0.17	0.34	0.21	0.32	0.25
DL-Methionine	0.31	0.20	0.26	0.18	0.23	0.15	0.20	0.13
L-Threonine	0.06	0.01	0.06	0.01	0.06	0.01	0.06	0.01
MCP[1]	0.92	0.00	1.19	0.34	1.28	0.72	1.36	1.08
Limestone	1.69	1.02	1.78	1.25	1.76	1.41	1.75	1.55
Salt	0.35	0.18	0.36	0.23	0.36	0.27	0.36	0.31
Sodium Bicarb.	0.11	0.11	0.14	0.12	0.14	0.14	0.15	0.10
Choline (60%)	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Pre-mix[2]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Filler (sand)	4.00	0.00	3.78	0.00	4.75	0.00	5.87	0.00

[1] MCP = Monocalcium phosphate

[2] Premix provided the following (per kg diet): Vitamin A, 13000 IA; vitamin D_3, 5000 ICU; vitamin E, 80.0 mg; vitamin B_{12}, 20.0 ug; riboflavin, 9.0 mg; niacin, 60.0 mg; d-pantothenate, 20.0 mg; menadione (K_3), 3.0 mg; folic acid, 3.2 mg; thiamine, 1.6 mg; pyridoxine, 4.0 mg; d-biotin, 250.0 ug; selenium (as Na_2SeO_3), 0.30; Manganese, 110 mg; zinc, 100 mg; iron, 70 mg; copper, 20 mg; iodine, 2.0 mg; cobalt, 0.5 g

Table 3.2 The nutrient composition of the basal dilution and summit diet fed to mixed sex broilers from 7 to 14 days

	Basal Diet 1	Summit Diet 3	Basal Diet 4	Summit Diet 6	Basal Diet 7	Summit Diet 9	Basal Diet 10	Summit Diet 12
Blending ratio	50	50	50	50	50	50	50	50
Calculated nutrients[1]				Percentage (%)				
Moisture (%)	9.62	9.60	9.60	9.70	9.50	9.70	9.50	9.80
ME [2], kcal/kg	2700	3100	2700	3100	2700	3100	2700	3100
CP, %	28.3	29.3	25.7	26.7	23.2	24.4	21.0	22.3
Lys [3], %	1.77	1.77	1.60	1.60	1.40	1.40	1.30	1.30
Met [3], %	0.80	0.81	0.71	0.72	0.64	0.64	0.58	0.58
Met & Cys [3], %	1.26	1.27	1.15	1.15	1.04	1.04	0.94	0.94
Thr [3], %	1.17	1.19	1.06	1.07	0.96	0.97	0.87	0.87
Val [3], %	1.35	1.39	1.22	1.26	1.10	1.14	1.00	1.01
Arg [3], %	1.78	1.78	1.61	1.61	1.45	1.47	1.31	1.31
SID. Lys [4], %	1.58	1.57	1.44	1.43	1.31	1.30	1.18	1.18
SID. SAA [4], %	1.12	1.11	1.02	1.00	0.92	0.92	0.83	0.83
SID. Thr [4], %	0.99	0.99	0.90	0.90	0.81	0.82	0.74	0.74
SID. Val [4], %	1.18	1.22	1.07	1.11	0.97	1.01	0.88	0.89
SID. Arg [4], %	1.61	1.56	1.46	1.42	1.31	1.29	1.18	1.18
Ca, %	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Avl. P %	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48
Na, %	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Cl, %	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28
DEB[5], Meq/kg	217	168	192	161	178	154	164	148
CP: ME ratio	54.3	61.6	63.2	65.1	67.4	66.5	61.8	56.9
ME: total Lys	1525	1751	1688	1938	1929	2214	2077	2385
Analysed nutrient composition								
CP, %	28.3	29.7	25.5	26.9	23.9	23.9	21.6	22.6
Lys, %	1.75	1.78	1.61	1.58	1.43	1.40	1.31	1.28

[1]Nutrient composition calculated from proximate analyses of all ingredients prior to formulation

[2]Energy content of ingredients calculated as per WPSA 3rd Ed, 1989

[3]Total amino acid content of ingredients calculated as a percentage of the analysed CP content

[4]Calculated from standardised ileal digestibility coefficients applied to the calculated amino acid content (Evonik, 2014)

[5]DEB = dietary electrolyte balance (Na + K – CL)

Ten pellets per treatments were subjected to a pellet hardness test as prescribed by Payne *et al.* (1987). The test consists of a pellet being held between the jaws of a manual Kahl pellet hardness tester. Pressure is exerted by screwing down by hand onto a coil spring. The pointer indicates the load in kilogrammes at which the pellets burst. The data obtained from the pellet hardness test was used to test for statistical significance between treatments.

3.5 Data collection

The broilers were weighed at 7 and 14 days of age and the FI calculated as the difference between the feed allocated at the beginning of the trial and the amount left over at the end of the trial. This, in turn, meant that average daily gain (ADG) and feed conversion ratio (FCR) could, therefore, be calculated. Mortality (%) was recorded on a daily basis. However, FCR was not corrected for the mortality.

3.6 Statistical analysis

JMP ® of SAS version 12.0.1 (SAS institute Inc. JMP Software, Cary, NC, 2015) was used to test for normality and the data was then analysed using Proc GLM (general linear models procedure) as a 3 (energy) x 4 (total Lys) factorial arrangement ($P \leq 0.05$). The mean differences were separated using Tukey's honest significant differences test (Tukey, 1991). For the purposes of this study, there were eight replications with 6 birds per replication and 12 treatments. Of the 6 birds per replication, 3 birds were male and 3 birds were females. The linear and quadratic regressions of JMP ® of SAS version 12.0.1 (SAS institute, 2015) were used to observe the treatments effect of weight, ADG, FI and FCR.

$$Y = b0 + b1\ E \qquad [3.1]$$

$$Y = b0 + b1E + b2\ E^2 \qquad [3.2]$$

Where Y predicted response, E = energy contentment of the diet, b0 = intercept and b1 and b2 = regression coefficient

3.7 Results

3.7.1 Feed texture: Pellet quality index and hardness

There was no interaction or trend between the various energy (ME): total Lys ratios (Table 3.3); varying total Lys percentage (Table 3.4) or varying energy (ME) (Table 3.5) levels and feed particle size. All the treatments produced pellets of the same general standard. In addition, there was no statistical difference ($P < 0.05$) between total Lys (%) and pellet durability and pellet hardness nor was there an interaction ($P < 0.05$) between energy (ME) and pellet durability and pellet hardness.

Table 3.3: The effect of varying total Lys and energy levels (ME) in diets and the effect thereof on pellet durability index (PDI) and pellet hardness

Treatment	total Lys (%)	ME (kcal/kg)	Ratio (ME: total Lys)	PDI (%)	Pellet hardness (kg)
1	1.77	2700	1525	94.6	6
2	1.77	2900	1638	97.0	9
3	1.77	3100	1751	97.4	4
4	1.60	2700	1688	97.5	7
5	1.60	2900	1813	96.7	7
6	1.60	3100	1938	92.2	3
7	1.40	2700	1929	94.6	6
8	1.40	2900	2071	97.4	4
9	1.40	3100	2214	93.6	5
10	1.30	2700	2077	97.2	5
11	1.30	2900	2231	98.0	6
12	1.30	3100	2385	91.4	5
SEM				2.17	
p>F					
PDI (%) quadratic				0.760	
SEM					1.55
p>F					
Pellet Hardness					0.89

$^{a-d}$ Means in the same column with no common superscript differ significantly ($p \leq 0.05$)

Table 3.4: The effect of varying total Lys (%) levels in diets and the effect thereof on pellet durability index (PDI) and pellet hardness

total Lys (%)	PDI (%)	Pellet hardness (kg)
1.77	96.3	6.0
1.60	95.4	5.5
1.40	95.2	5.0
1.30	95.5	5.5
SEM	1.50	-
p>F		
PDI (%) quadratic	0.95	-
SEM		1.07
p>F		
Hardness quadratic		0.89

a-d Means in the same column with no common superscript differ significantly ($p \leq 0.05$)

Table 3.5: The effect of varying energy (ME) levels in diets and the effect thereof on pellet durability index (PDI) and pellet hardness

ME (kcal/kg)	PDI (%)	Pellet hardness (kg)
2700	96.0	6.0
2900	97.3	6.5
3100	93.6	4.0
SEM	0.90	-
p>F		
PDI (%) quadratic	0.05	-
SEM		0.95
p>F		
Hardness quadratic		0.07

a-d Means in the same column with no common superscript differ significantly ($p \leq 0.05$)

3.7.2 Animal performance

3.7.2.1 The effect of feeding varying energy (ME): total Lys ratios to mixed sex broilers from 7 to 14 days of age

The response of feeding 7 to 14 days of age mixed sex broilers varying ratios of ME: total Lys is depicted in Table 3.6 (ADG, ADFI, FCR) and in Figures 3.1 (ADG), 3.2 (ADFI) and 3.3 (FCR). In general, ADG and FCR of the broilers improved with a decrease in ME: total Lys content. The highest response in 14 day weight and ADG were achieved with Treatment 3 (1.77%; total Lys; 3100 kcal ME/kg; 1751 ratio). The ADG of treatment 3 (1.77% total Lys; 3100 kcal ME/kg; 1751 ratio) was significantly higher when compared to the lower ME: total Lys ratios of treatments 1 (1.77% total Lys; 3100 kcal ME/kg; 1751 ratio), 10 (1.30% total Lys; 2700 kcal ME/kg; 2077 ratio) and 12 (1.30% total Lys; 3100 kcal ME/kg; 2385 ratio).

Table 3.6: The effect on the performance of 7 to 14 days of age mixed flock broilers due to the feeding of varying ME: total Lys ratios

Treatment	total Lys (%)	ME (kcal/kg)	Ratio ME: tot Lys	7 Day (g)	14 Day (g)	ADG (g)	ADFI (g)	FCR (g/g)	Mortality (%)
1	1.77	2700	1525	165	380^{b}	30.7^{d}	47.2^{c}	1.54^{cd}	0.00
2	1.77	2900	1638	167	407^{a}	34.3^{ab}	48.2^{c}	1.41^{f}	0.00
3	1.77	3100	1751	166	408^{a}	34.7^{a}	47.8^{c}	1.38^{f}	0.00
4	1.60	2700	1688	165	395^{ab}	33.0^{abcd}	50.1^{abc}	1.52^{cd}	0.00
5	1.60	2900	1813	168	399^{ab}	33.3^{abc}	47.9^{c}	1.44^{ef}	0.00
6	1.60	3100	1938	171	408^{a}	33.9^{abc}	46.9^{c}	1.38^{f}	0.00
7	1.40	2700	1929	169	402^{ab}	33.4^{abc}	52.2^{a}	1.56^{bc}	0.00
8	1.40	2900	2071	170	400^{ab}	33.0^{abcd}	49.7^{abc}	1.51^{cde}	0.00
9	1.40	3100	2214	166	388^{ab}	31.8^{bcd}	47.0^{c}	1.48^{de}	0.00
10	1.30	2700	2077	169	389^{ab}	31.4^{cd}	51.9^{ab}	1.65^{a}	0.00
11	1.30	2900	2231	169	394^{ab}	32.3^{abcd}	52.4^{a}	1.62^{ab}	0.00
12	1.30	3100	2385	166	387^{ab}	31.6^{cd}	48.6^{bc}	1.54^{cd}	0.00
	SEM			2.44	5.11	0.53	0.71	0.02	-
	p>F								
	Ratio Quadratic			0.78	0.0009	0.0001	0.0001	0.0001	-

a-d Means in the same column with no common superscript differ significantly ($P \leq 0.05$)

At the highest total Lys level, the 14 day weight and FCR of treatment 1 (1.77% total Lys; 2700 kcal ME/kg; 1525 ratio) were significantly lower when compared to those of treatments 2 (2900 kcal ME/kg; 1638 ratio) and 3 (3100 kcal ME/kg; 1751 ratio). However, the ADG of treatment 1 was significantly different only from that of treatment 3, but not treatment 2. The lowest FCR was achieved by treatment 6 (1.60%; total Lys 3100 kcal ME/kg; 1938 ratio).

At each one of the total Lys levels (i.e. 1.77, 1.60, 1.40 and 1.30), the 2700 kcal ME/kg treatments (1, 4, 7, and 10) FCR was significantly lower when compared to the highest ME level: total Lys level, which all had 3100 kcal/kg (3, 6, 9 and 12). However, no significant differences were observed between the lowest ME level of 2700 kcal ME/kg (1, 4, 7, and 10) and 2900 kcal/kg treatments (2, 5, 8, 11) and between the 2900 kcal ME/kg (2, 5, 8, 11) and 3100 kcal ME/kg treatments (3, 6, 9 and 12).

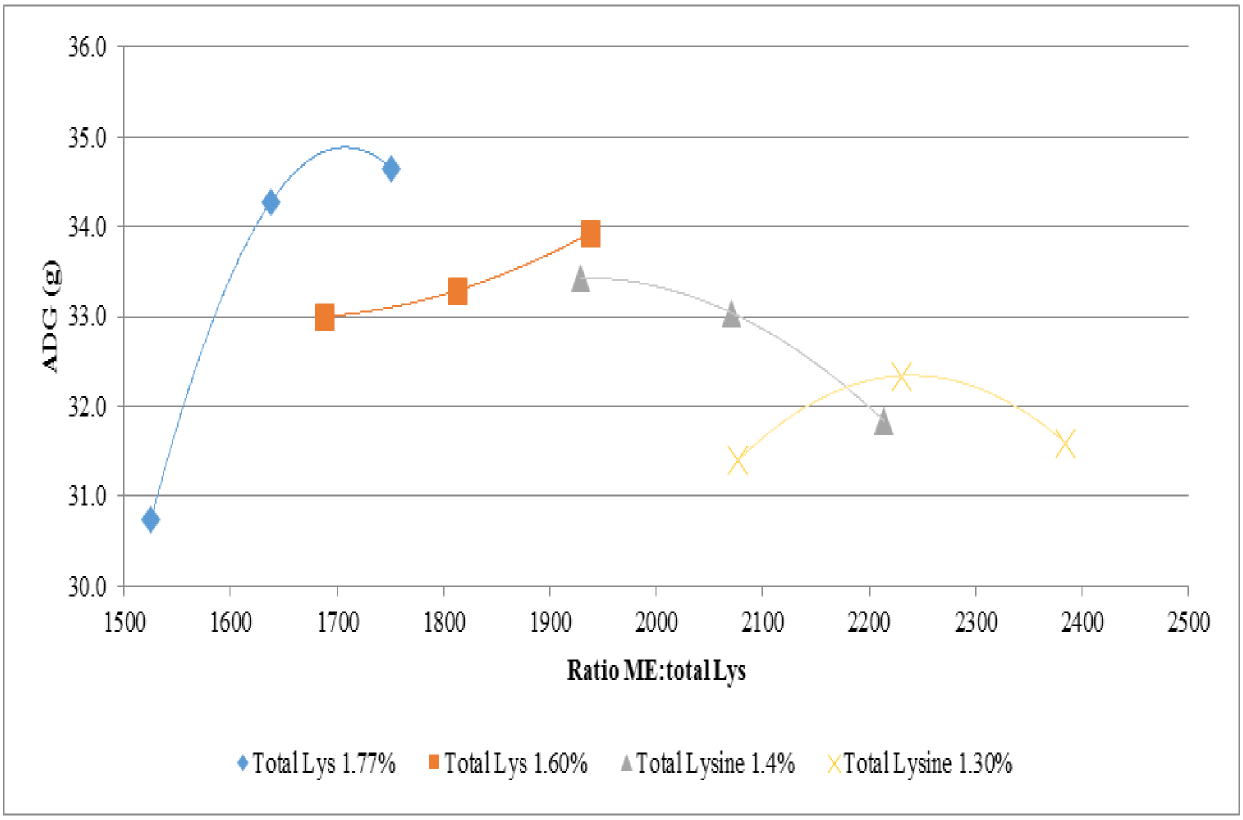

Figure 3.1: The effect of feeding 7 to 14 days of age mixed sex broilers varying amounts of dietary ME: total Lys ratios on Average Daily Gain (ADG)

Average daily feed intake tended to be less responsive with only treatment 7 (1.40% total Lys; 2700 kcal ME/kg; 1929 ratio) and treatment 11 (1.30% total Lys; 2900 kcal ME/kg; 2231 ratio) consuming significantly more feed when compared to most of the other treatments. It was observed that ADFI tended to increase with an increase in ME: total Lys and with a decrease in ME. No mortalities occurred during the trial period.

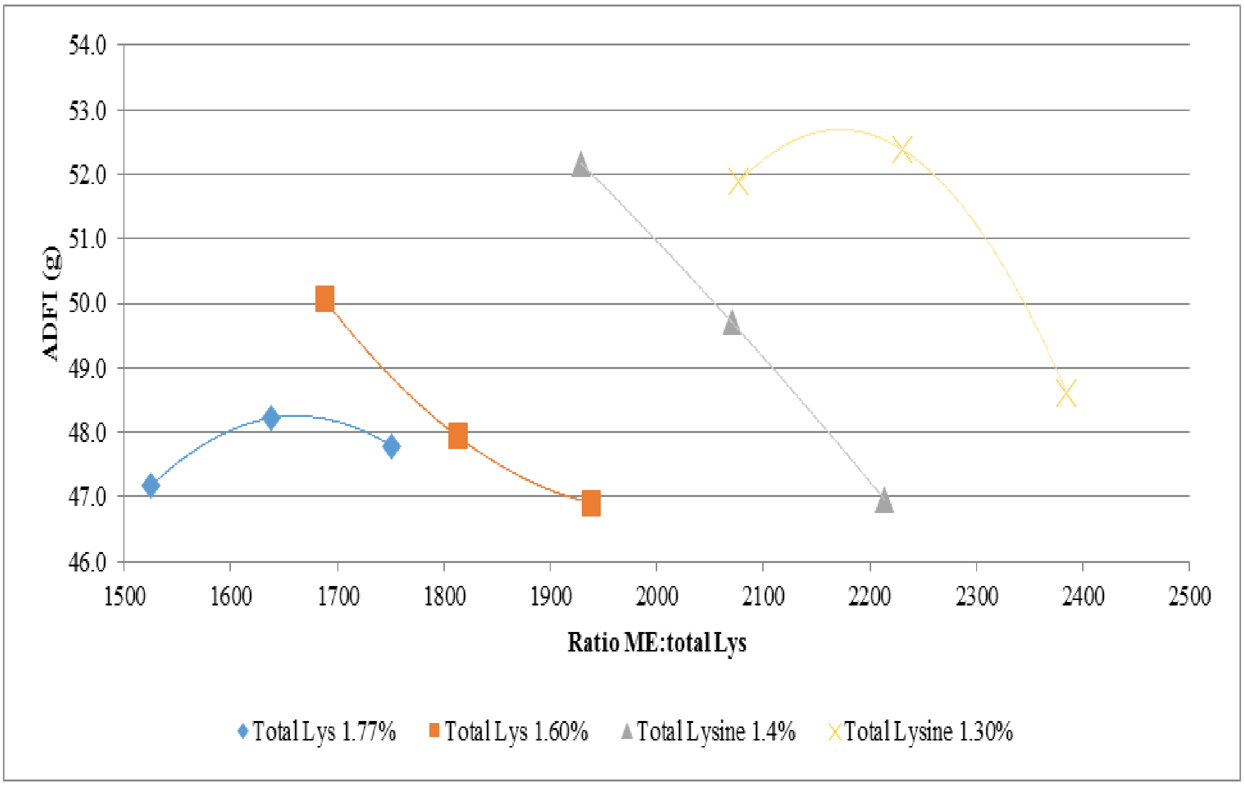

Figure 3.2: The effect of feeding 7 to 14 days of age mixed sex broilers varying amounts of dietary ME: total Lys ratios on Average Daily Feed Intake (ADFI)

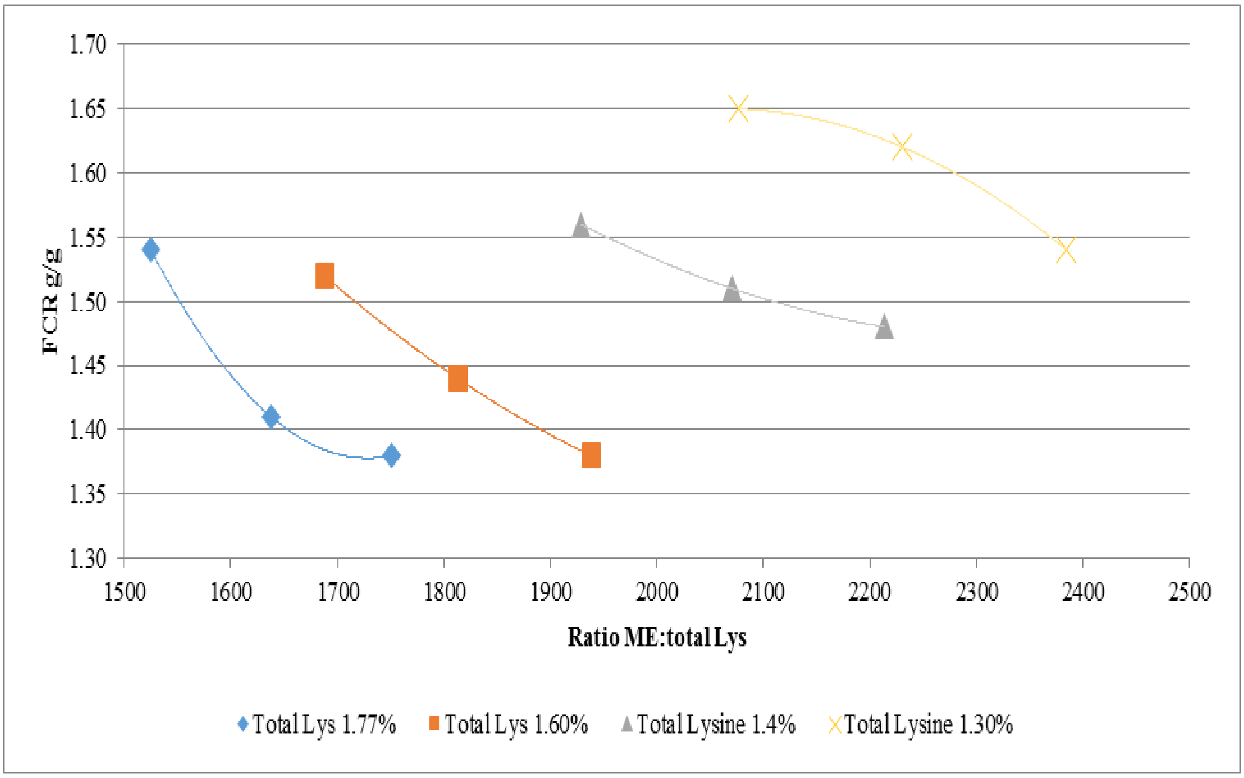

Figure 3.3: The effect of feeding 7 to 14 days of age mixed sex broilers varying amounts of dietary ME: total Lys ratios on Feed Conversion Ratio (FCR)

The statistical evaluation of the quadratic response of the broilers (7-14 days of age) to varying levels of ME: total Lys ratio yielded in the following equations for ADG, ADFI and FCR:

ADG = -3.108766 + 0.0389846 x (ME: total Lys ratio) + -0.00001038 x (ME: total Lys ratio^2)

(R^2 = 0.40) [3.3]

FCR =2.0683794+ -0.000729 x (ME: total Lys ratio) + 0.00000022155x (ME: total Lys ratio^2)

(R^2 = 0.39) [3.4]

ADFI =11.619103+0.0367668 x (ME: total Lys ratio) + -0.000008828 x (ME: total Lys ratio^2)

(R^2 = 0.17) [3.5]

A linear regression for ME: total Lys ratio indicated that the point of maximum response for ADG was 1900. It was not possible to calculate a point of maximum response in FCR as plateaus were not achieved.

3.7.2.2 The effect of feeding varying **total Lys** *levels on mixed sex broilers from 7 to 14 days of age*

The broilers' responses of 7 to 14 days of age mixed sex broilers to varying total Lys levels are presented in Table 3.7 and Figures 3.4 (ADG), 3.5 (ADFI) and 3.6 (FCR). The broilers' ADG increased and FCR decreased with increasing total Lys. The ADG of treatments 1 to 6 (total Lys of 1.77% and 1.60%) were significantly higher when compared to the lowest total Lys level of treatments 10, 11 and 12 (total Lys 1.30%). However, there were no significant differences in ADG between treatments 4 to 6 (1.60% total Lys) and treatments 7 to 9 (1.40% total Lys) and also no significant differences between in ADG between treatments 7 to 9 (1.40% total Lys) and treatments 10 to 12 (1.30% total Lys). The only significant differences in ADFI were between treatments 1-3 (total Lys 1.77%) and treatments 10 to 12 (total Lys 1.30%).

Table 3.7: The effect on the performance of 7 to 14 days of age mixed flock broilers to the feeding of varying total Lys levels (2700, 2900 and 3100 ME kcal/kg)

Total Lys (%)	7 Day weight (g)	14 Day weight (g)	ADG (g)	ADFI (g)	FCR (g/g)	Mortality (%)
1.77	166	398	33.2 [a]	47.4 [c]	1.44 [c]	0.00
1.60	167	401	33.1 [a]	48.3 [bc]	1.45 [c]	0.00
1.40	168	397	32.8 [ab]	49.6 [ab]	1.51 [b]	0.00
1.30	167	390	31.8 [b]	51.0 [a]	1.61 [a]	0.00
SEM	1.30	3.30	0.30	0.50	0.01	-
p>F						
Ratio Quadratic	0.77	0.12	0.008	0.0001	0.0001	-

[a-d] Means in the same column with no common superscript differ significantly ($P \leq 0.05$)

The lowest level of FCR was achieved by the highest total Lys level (total Lys of 1.77%) and the intermediate total Lys level (total Lys 1.60%) which were significantly lower than the lowest total Lys level (total Lys 1.40%) and treatments 10 to 12 (total Lys 1.30%). There were no significant differences between the treatments 1 to 3 (total Lys 1.77%) and treatments 4 to 6 (total Lys 1.60%). The statistical analysis of the response of broilers (7-14 days) to total Lys for ADG, ADFI and FCR is indicated in the below equations:

$$\text{ADG} = -7.14183 + 49.827886 \times \text{total Lys} + -15.27555 \times \text{total Lys}^2 \quad (R^2 = 0.99) \; [3.6]$$

$$\text{FCR} = -7.14183 + 49.827886 \times \text{total Lys} + -15.27555 \times \text{total Lys}^2 \quad (R^2 = 0.98) \; [3.7]$$

$$\text{ADFI} = 90.409345 + -47.82042 \times \text{total Lys} + 13.404034 \times \text{total Lys}^2 \quad (R^2 = 0.99) \; [3.8]$$

According to the quadratic regression for total Lys, the point of maximum response for ADG (Figure 3.4) was achieved at 1.61% while that of FCR (Figure 3.6) was achieved at 1.64%.

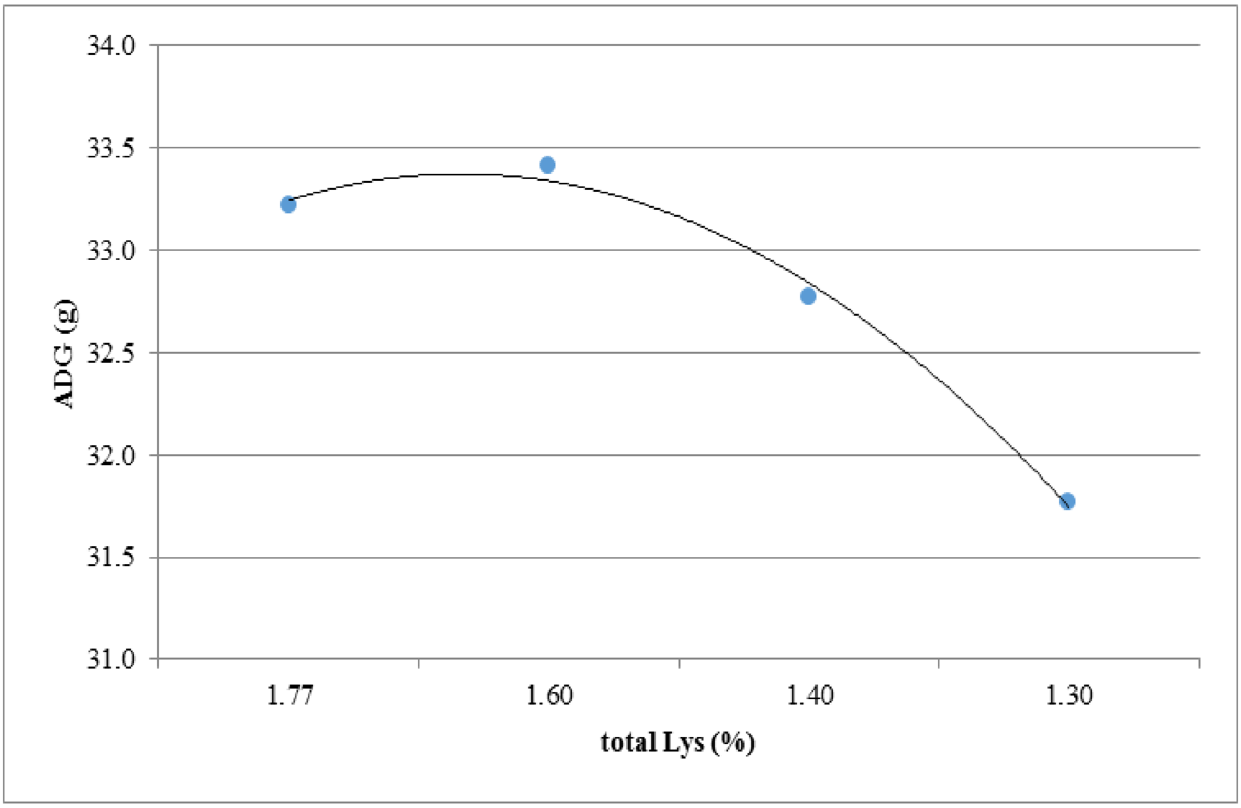

Figure 3.4: The effect of feeding 7 to 14 days of age mixed sex broilers varying amounts of dietary total Lys ratios on Average Daily Gain (ADG)

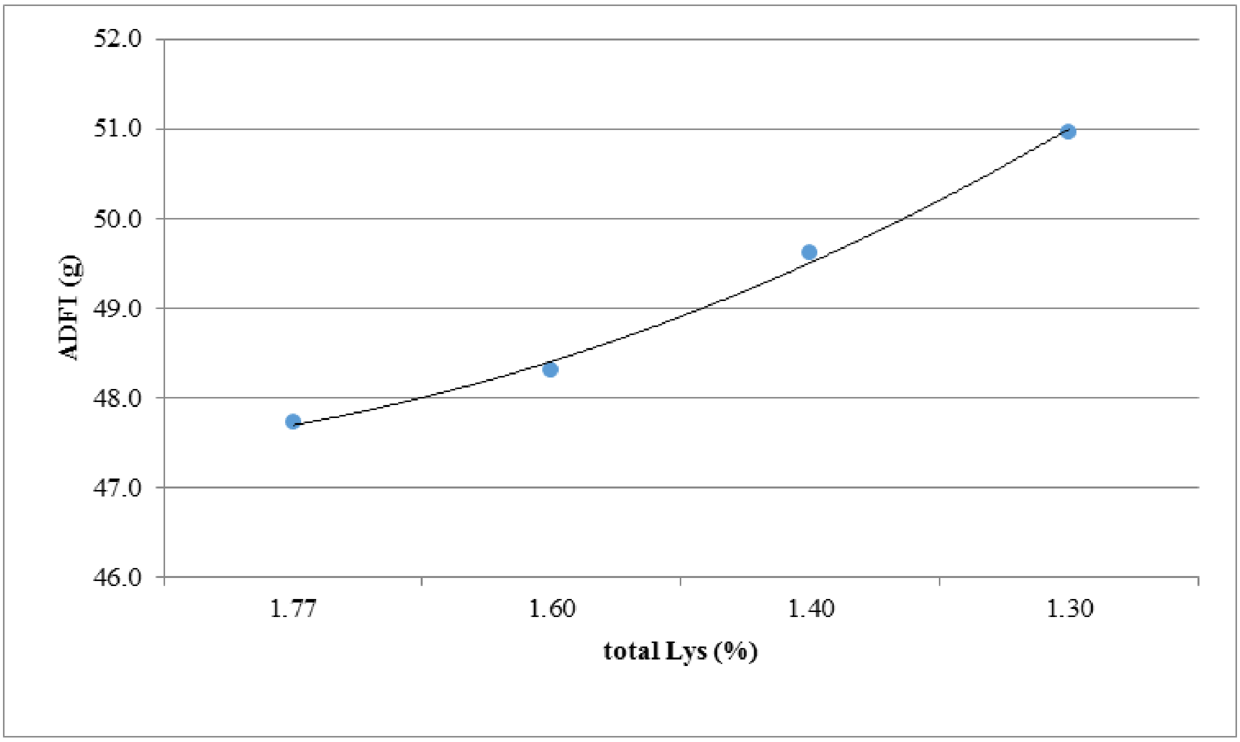

Figure 3.5: The effect of feeding 7 to 14 days of age mixed sex broilers varying amounts of dietary total Lys ratios on Average Daily Feed Intake (ADFI)

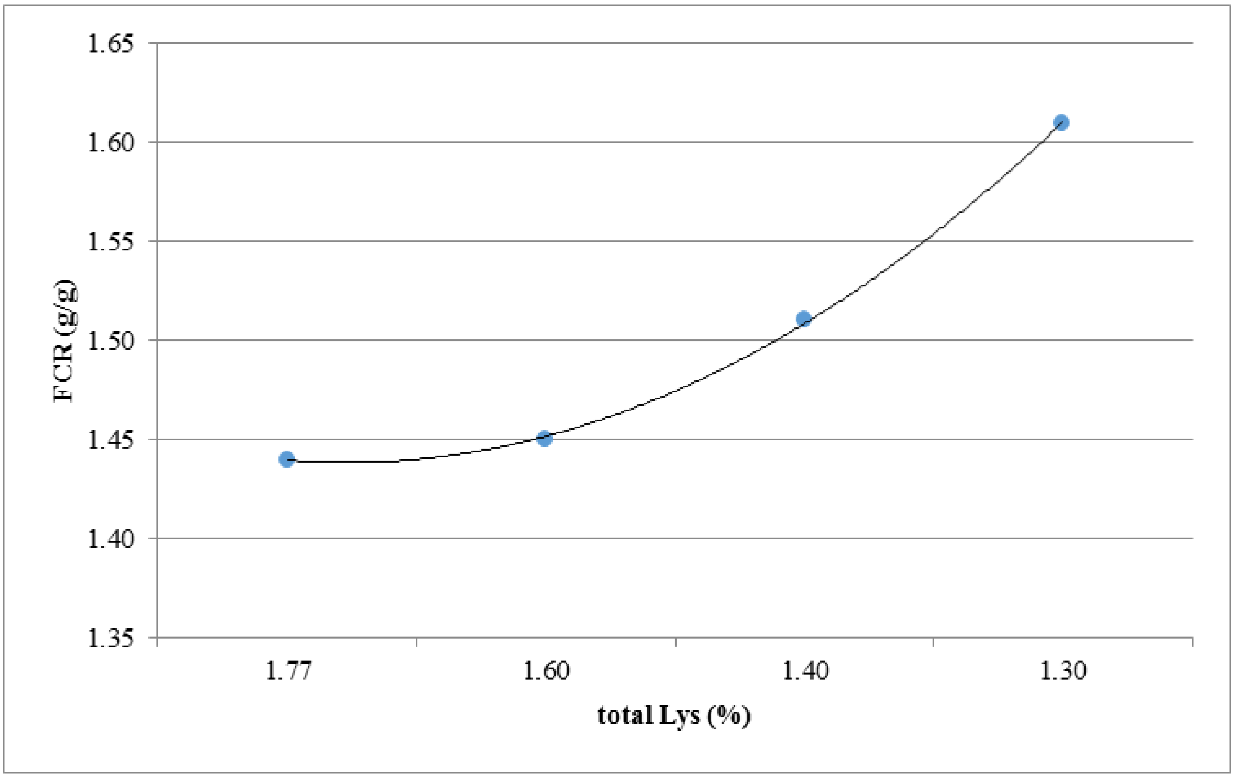

Figure 3.6: The effect of feeding 7 to 14 days of age mixed sex broilers varying amounts of dietary total Lys ratios on Feed Conversion Ratio (FCR)

3.7.2.3 The effect of feeding varying energy (ME) levels on mixed sex broilers from 7 to 14 days of age

The broilers' responses of 7 to 14 days of age mixed sex broilers to varying energy (ME) levels are presented in Table 3.8 and Figures 3.7 (ADG), 3.8 (ADFI) and 3.9 (FCR). Average daily gain were significantly higher at the highest ME treatment of 3100 kcal/kg (treatments 3, 6, 9 and 12) when compared to the lowest ME value of 2700 kcal ME/kg (treatments 1, 4, 7 and 10). However, there were no significant differences between the lowest ME of 2700 kcal/kg and 2900 kcal ME/kg (treatments 2, 5, 8 and11) and between 2900 kcal ME/kg and 3100 kcal ME/kg (treatments 3, 6, 9 and 12). For birds that were fed the highest ME, 3100 kcal ME/kg (treatments 3,6,9 and 12) ADFI was significantly lower than the 2900 and 2700 kcal ME/kg treatments. The incremental reduction in energy by 200 kcal/kg from 3200 to 2900 and 2900 to 2700 kcal ME/kg resulted each time in a significant reduction in FCR. The lowest FCR was recorded at 3100 kcal ME/kg (treatments 3, 6, 9 and 12).

The quadratic statistical analysis of the response of broilers (7-14 days) to energy (ME) for ADG, ADFI and FCR is indicated in the equations below:

$$ADG = -144.5 + 0.143475 \times ME + -0.00002437 \times ME^2 \quad (R^2 = 0.96) \quad [3.9]$$

$$FCR = 6.565 + -0.0032 \times ME + 0.0000005 \times ME^2 \quad (R^2 = 0.90) \quad [3.10]$$

$$ADFI = 468.2 + -0.2294 \times ME + 0.00003425 \times ME^2 \quad (R^2 = 0.95) \quad [3.11]$$

According to the quadratic regression for ME, the point of maximum response for ADG was achieved at 2975 kcal ME/kg while that of FCR was achieved at 3075 kcal ME/kg.

Table 3.8: The effect on the performance of 7 to 14 days of age mixed flock broilers due to the feeding of varying energy (ME) levels (1.30, 1.40, 1.60; 1.77% total Lys)

ME (kcal/kg)	7 Day weight (g)	14 Day weight (g)	ADG (g)	ADFI (g)	FCR (g/g)	Mortality (%)
2700	167	392	32.1 [b]	50.3 [a]	1.57 [a]	0.00
2900	168	400	33.2 [a]	49.6 [a]	1.49 [b]	0.00
3100	167	398	33.0 [ab]	47.6 [b]	1.45 [c]	0.00
SEM	1.2	2.8	0.5	0.6	0.01	-
p>F						
ME Quadratic	0.69	0.10	0.04	0.001	0.0001	-

[a-d] Means in the same column with no common superscript differ significantly ($P \leq 0.05$)

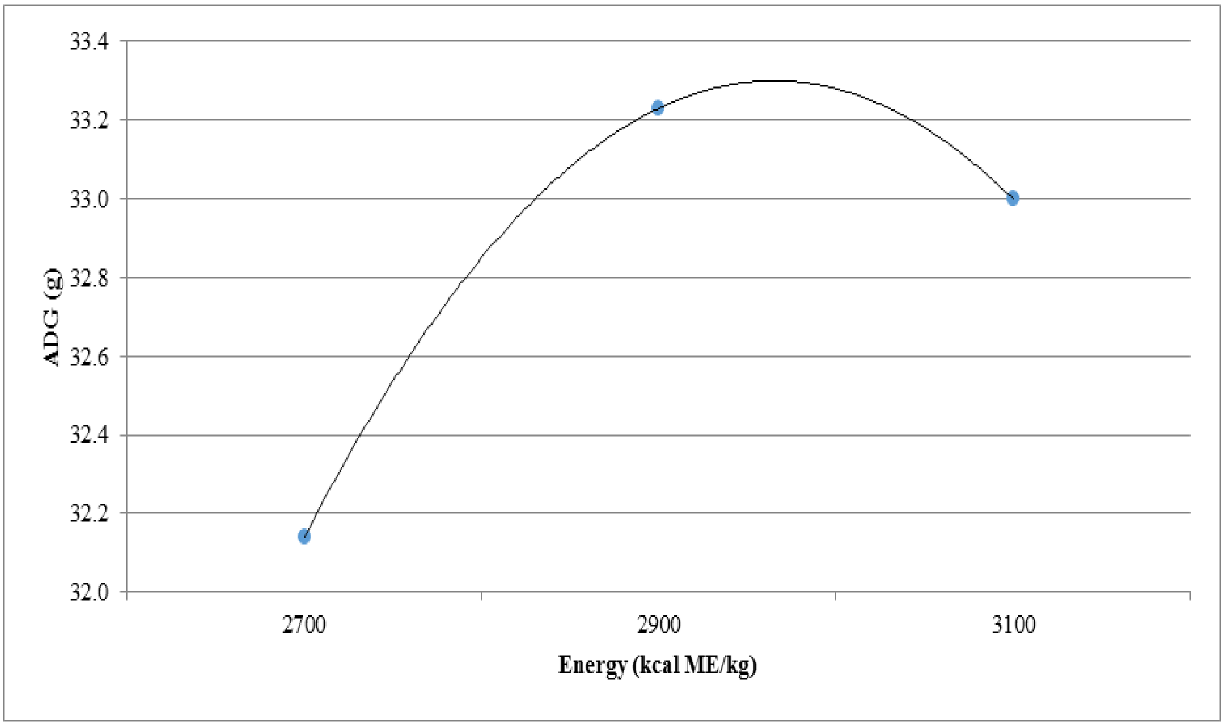

Figure 3.7: The effect of feeding 7 to 14 day of age mixed sex broiler varying amounts of energy (ME) levels on Average Daily Gain (ADG)

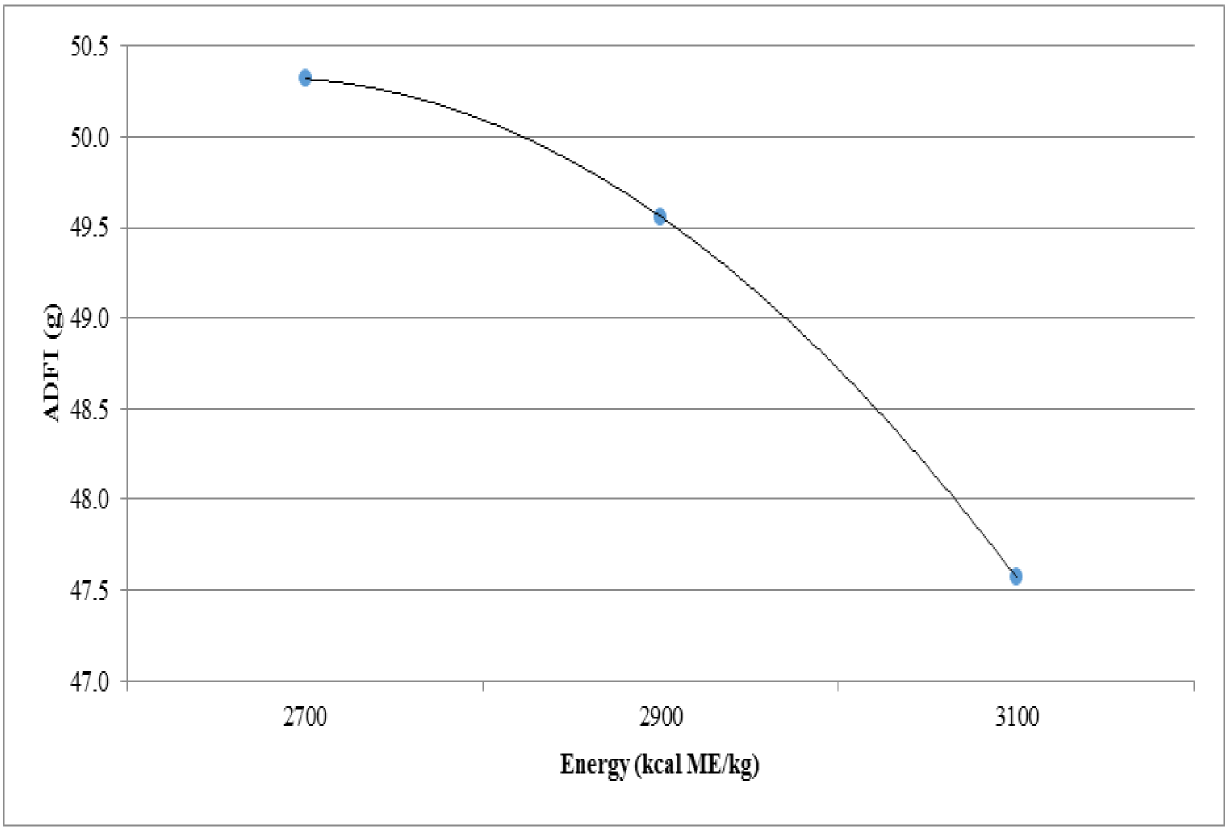

Figure 3.8: The effect of feeding 7 to 14 day of age mixed sex broiler varying amounts of energy (ME) levels on Average Daily Feed Intake (ADFI)

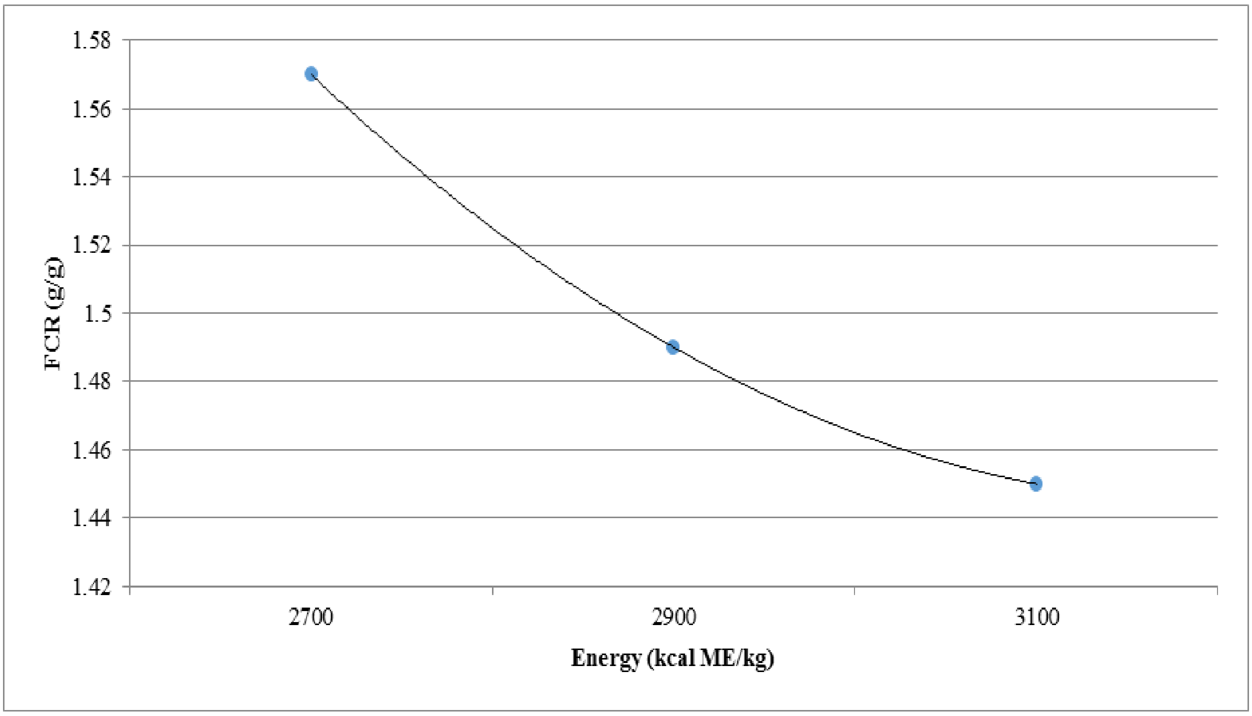

Figure 3.9: The effect of feeding 7 to 14 day of age mixed sex broiler varying amounts of energy (ME) levels on Feed Conversion Ratio (FCR)

3.8 Discussion

The majority of literature reported on broiler response to amino acid and energy requirements report only the response of broilers to Lys or energy and not the interaction between these two nutrients. For example, Dozier and Payne (2012) determined that, for Ross 708 females, the points of maximum response to total Lys for ADG were 1.55% and 1.69% for FCR while, for Hubbard × Cobb 500, it has been found that the points of maximum response to total Lys were slightly lower with ADG at 1.44% and FCR at 1.54%. Labadan *et al.* (2001) determined that mixed Ross x Avian birds required 1.28% total Lys for ADG and 1.21% for FCR. Wijtten *et al.* (2004) determined that, for Ross 708 males, the points of maximum response to total lysine were ADG at 1.50% and FCR at 1.62% while, for Ross females, Wijtten *et al.* (2004) determined that ADG was also 1.50% but slightly higher at 1.63% for FCR.

Plumstead (2005) conducted two experiments, one with Cobb 500 and another with Ross 344 x Ross 308 SF broilers from day old to 14 days. For the Cobb 500 birds maximum ADG was achieved at 1.35% total Lys and 1.50% for FCR while, for the Ross 344 x Ross 308 SF broilers birds, maximum ADG was achieved at 1.34% total Lys and 1.49% for FCR. The results of this experiment indicated that Ross 308 mixed flocks required 1.61% total Lys for maximum ADG and 1.64% for FCR. These findings are, in agreement with literature but at a higher level. It may be postulated that, compared to the trials of Wijtten *et al. (*2004); Plumstead (2005) and Dozier and Payne (2012) genetic selection over the past 5 to 10 years for higher growth rates would have resulted in a higher requirement and the associated response of higher total Lys levels.

There are only few examples in the literature where broilers received graded levels of balanced amino acid profiles at two or three dietary energy levels. For example, Plumstead (2005) conducted two experiments with a similar design to that of Bellaver *et al.* (2002). In these experiments 1 to 14 day old male Cobb 500 broilers were fed with a series of diets with increasing levels of BP (1.05% to 1.29% Dig. Lys) containing either 3000, 3100 and 3200 ME kcal/kg. It was observed that there was a significant interaction between Dig. Lys and ME at 14 days of age on BW gain and FCR. In addition, broiler 14 day BW gain increased and FCR decreased when the dietary ME was increased from 3000 to 3100 kcal/kg with no further improvement to 3200 kcal/kg ME. Increasing dietary ME or Dig. Lys had no effect on FI to 14 days. The responses were similar to those found by Bellaver *et al. (*2002) but on a higher performance level. In the first trial performed by Plumstead (2005), maximum weight gain was not achieved in the first trial and the responses were linear in nature while, at two or three energy levels. In both trials conducted by Plumstead (2005), the curves appeared to shift to the higher dig ME: total Lys ratio with decreasing energy supply.

Therefore, from the trials conducted by Plumstead (2005), it can be summarised that it appears that the dietary ME: Lys ratio increased with decreasing dietary energy level in order to achieve the same performance. In addition, the second trail also suggested that the optimum ME: total Lys ratio decreased as regards achieving maximum performance. Feed conversion improved linearly with increasing dietary BP and energy levels in both trials.

In their trial, Basurco *et al*. (2015) fed Cobb 500 broilers from day old to 7 days a range of three energy levels (3054, 3100 and 3147 kcal ME/kg) and three levels of amino acid density. The broilers' ADG and ADFI improved significantly with an increase in amino acid density but not with an increase in energy level. The FCR was significantly improved and the ADFI increased significantly with an increase in amino acid density. Although an increase in energy level did not result in a significant increase in ADG and a significant reduction in ADFI, FCR was, however, significantly reduced with an increase in energy level.

The results from the trial conducted in experiment 2 conducted by Basurco *et al.* (2015) indicated that ADG was significantly improved when the energy was increased from 2700 kcal/kg and 2900 kcal ME/kg although no further response were observed at 3000 kcal/kg. FCR was significantly improved when the energy was increased from 2700 to 2900 kcal ME/kg and also improved significantly when the energy was increased to 3100 kcal ME/kg.

In the trial conducted in Chapter 2, the ADG and FCR improve with lower ratios. This finding is in agreement with that of Bellaver *et al. (*2002), Plumstead (2005) and Basurco *et al.* (2015). In this experiment, the ADG increased significantly when the ratio was equal to or lower than 2214. The FCR improved significantly when the ME: total Lys ratio was lower than 1938. The highest response in 14 day weight and ADG were achieved with treatment 3 (1.77%; total Lys; 3100 kcal/kg; 1751 ratio) while the lowest FCR were achieved with treatment 6 (1.60%; total Lys 3100 kcal ME/kg; 1938 ratio). At each one of the total Lys levels (i.e. 1.77, 1.60, 1.40 and 1.30), the FCR of the 2700 kcal ME/kg treatments (1, 4, 7, and 10) was significantly worse when compared to the highest ME level. Average daily feed intake tended to be less responsive only with treatment 7 (1.40% total Lys; 2700 kcal ME/kg; 1929 ratio) and treatment 11 (1.30% total Lys; 2900 kcal ME/kg; 2231 ratio), consuming significantly more feed when compared to the other treatments. It was observed that ADFI tended to increase with an increase in ME: total Lys and decrease with a decrease in ME.

3.9 Conclusion

The ADG of broilers improved with an increase in total Lys content and also with an increase in energy. The point of maximum response for ADG was achieved at 1.61% total Lys and for FCR at 1.64% total Lys. Average daily intake was significantly increased when the total Lys was reduced below 1.60% while ADG was significantly improved when the energy was increased from 2700 to 2900 kcal/kg ME levels. The feed conversion ratio was significantly reduced for each increase in the level in energy. For ADG, the point of maximum response were 1.77% total Lys, 3100 kcal ME/kg, implying a ME: total Lys ratio of 1751. For FCR, the areas of maximum response were 1.60% total Lys, 3100 kcal/kg, implying a ME: total Lys ratio of 1938.

The statistical evaluation of the response of broilers (7–14 days of age) to varying levels of ME: total Lys ratio gave rise to the following equations for ADG = -3.108766 + 0.0389846 x (ME: total Lys ratio) + -0.00001038 x (ME: total Lys ratio^2) (R^2 = 0.40); FCR =2.0683794+ -0.000729 x (ME: total Lys ratio) + 0.00000022155x (ME: total Lys ratio^2) (R^2 = 0.39); ADFI =11.619103+0.0367668 x (ME: total Lys ratio) + -0.000008828 x (ME: total Lys ratio^2) (R^2 = 0.17)

Chapter 4:

The response of mixed sex broilers to varying dietary Energy: total Lys ratios fed from 14 days to 28 days of age

4.1 Abstract

This trial was conducted to study the response of broilers to varying total Lys and energy (ME) levels and the broilers' response to the interaction between these two nutrients. Seven hundred sixty-eight Ross 308 mixed male and female broilers were fed one of twelve rations. The dietary treatments consisted of four total Lys levels (1.56, 1.38, 1.23 and 1.07%) and three ME levels (2800, 3000 and 3200 kcal ME/kg). The use of the dietary summit/dilution technique, the combination of total Lys and energy levels, resulted in various ME: total Lys levels (1795, 1923, 2051, 2029, 2174, 2319, 2276, 2439, 2602, 2593, 2778 and 2963). Each one of the 12 treatments was replicated 8 times. The birds were fed the trial diets from day 14 to 28 days. The average daily gain (ADG) increased when the total Lys when increased from 1.08 to 1.38% while ADFI significantly reduced at the highest level of 1.56%. The feed conversion ratio (FCR) were reduced when the total Lys was increased from 1.08 to 1.38%. The quadratic regression revealed that the point of maximum response for ADG was achieved at 1.37% and that 1.34% total Lys was required for FCR. There was no significant difference in average daily feed intake (ADFI) between the total Lys treatments. However, ADFI was significantly lower and FCR significantly higher when the energy was reduced from 3000 to 2800 kcal ME/kg. For ADG, the point of maximum response were 1.38% total Lys, 2800 kcal ME/kg, implying a ME: total Lys ratio of 2029. For FCR, the areas of maximum response were 1.56% total Lys, 3200 kcal ME/kg, implying a ME: total Lys ratio of 2051. Breast meat and protein percentage was significantly higher and abdominal fat and carcass fat percentage significantly lower at the highest test level of total Lys. Energy levels had no significant effect on broiler carcass breast

meat, abdominal fat, protein and fat (%) parameters. It would appear that the response of broilers carcass breast meat (%), abdominal fat (%) as well as that carcass protein (%) and fat (%) due to varying ME: total Lys is influenced more by the total Lys (%) level and to a lesser extent due to the energy level in the feed.

The statistical evaluation of broilers (14-28 days of age) ADG, ADFI and FCR revealed a quadratic response to varying levels of ME: total Lys ratio: ADG = 33.606973 + 0.0302445 x ME: total Lys + -0.00000684 x ME: total Lys ^{2} (R^2 = 0.43); FCR = 4.043189 + -0.002338 x ME: total Lys + 0.00000049807 x ME: total Lys ^{2} (R^2 = 0.31); ADFI = 221.00755 + -0.112043 x ME: total Lys + 0.0000233 x ME: total Lys ^{2} (R^2 = 0.22)

4.2 Introduction

According to Dozier *et al., (*2009) genetic selection by primary breeding companies has resulted in vastly improved growth rate, feed conversion, and breast meat yield of broiler chickens as compared with the broilers of the previous decade. As a result, modern broilers require higher dietary amino acid concentrations in order to optimise both performance and breast meat yield as compared with the broilers of past years (Kidd *et al.*, 2004; Dozier *et al.*, 2008). Part of the increased requirement has been achieved by reduced feed intake (FI) per unit of growth rate. In the 1950s, Almquist (1952) clearly established that growth rate depended on the intake of the first limiting amino acid and that animal response to supplementing the diet with such amino acid depended on the supply of the second limiting amino acid. This theory has since been fully accepted by nutritionists and is used for feed formulation (Wijtten *et al.,* 2004). A method used for defining amino acid requirements is to express the requirements as a percentage of Lys and is referred to as the ideal amino acid concept. After the digestible Lys requirement has been delineated for a certain genotype and specific environmental conditions, the other non-dispensable amino acids requirements may be calculated as a ratio to Lys (Baker *et al.,* 2002).

According to Liu *et al.* (2016), despite the importance of nutrient availability in broiler diets, body weight gain in broiler chickens is determined predominantly by the amount of feed consumed. Both dietary energy density and protein concentrations have been reported to have an impact on both FI and growth performance in broilers. It is generally accepted that broilers offered high energy have relatively lower FI than would otherwise have been the case (Liu *et al.*, 2016). However, the role of energy in regulating FI in broiler chickens is not clear and research has produced contradictory results. In research comparing dietary energy contents from 2700 to 3300 kcal ME/kg in broiler chickens from 0 to 49 days of age (Leeson *et al.*, 1996), birds increased their intake of lower energy diets to maintain a similar ME intake. Older chickens (30 to 59 days of age) responded in a similar fashion to dietary energy contents ranging from 3175 to 3310 kcal ME/kg (Dozier *et al.*, 2006).

Bellaver *et al.* (2002) fed diets with graded levels of a BP and with either 3000, 3100 and 3200 kcal ME/kg to male chickens from 1 to 21 days of age. In this trial the different digestible Lys ranged from 9.2 to 12.2 g/kg. Feed intake in birds fed digestible Lys levels of 10.2 g/kg and higher on the 3200 kcal ME/kg diet was lower than that of birds with the lower energy levels. It is interesting to note that there was only a marginal difference in FI between the 3100 and 3200 kcal ME/kg treatments. Bellaver *et al.* (2002) concluded that ME: digestible Lys ratio for maximum performance decreased with an increase in the Lys content of the feed. In other words, the trial data suggested that optimum dietary BP levels should be unchanged or even decreased with increased dietary energy level in order to obtain maximum performance. The responses to increasing balanced protein (BP) levels on FCR were typical as it improved with both increasing dietary energy and BP levels. Moreover, minimum FCR was not achieved, thus suggesting a generally higher BP intake was needed than that obtained for weight gain – also documented in a literature review conducted by Pack *et al.* (2003) for various single amino acids. Plumstead *et al.* (2007) found no difference in FI for broilers (0–21 days of age) fed dietary energy levels ranging from 3000 to 3200 kcal ME/kg. They suggested that the lack of regulation in FI may be the result of intense genetic selection for growth over the years in broiler chickens and affecting satiety mechanisms. In contrast, Basurco *et al.* (2015), fed Cobb 500 broilers from day 19 to 29 days a range of three energy levels (3152, 3200 and 3250 kcal ME/kg) and three levels of amino acid density (0.96, 1.07, 1.18% Dig. Lys). Both ADG and ADFI increased significantly while FCR decreased significantly with an increase in amino acid density and energy levels.

Some reports suggest that FI regulation and, hence, response to different dietary energy is due to age (Jones and Wiseman, 1985; Brickett, *et al.*, 2007; Kamran, *et al.*, 2008). It has been found that broiler chickens younger than 24 days of age did not adjust their FI in response to dietary energy ranging from 2577 to 3533 kcal ME/kg but changed their feed intakes accordingly from 25 to 49 days of age (Jonas and Wiseman, 1985). Research conducted by Brickett *et al.* (2007) and Kamran *et al.* (2008) on broiler chickens under 14 days of age concluded that broilers were not able to regulate FI. In these cases, a limited capacity of young birds to increase FI may be the reason. Brickett *et al.* (2007) also found that, after 14 days of age, broiler chickens fed low energy diets adjusted their FI, but not to the extent expected based on dietary energy level. Reasons suggested for the age dependent response included the limited physical gastrointestinal tract capacity of young chicks and/or the influence of energy derived from yolk (Griffiths *et al.*, 1977; Kamran *et al.*, 2008). The aim of this trial was to obtain equations that related the BWG and FCR of broilers to varying total Lys and energy levels. It was anticipated that such equations would permit commercial nutritionists to determine the most economical point for broilers during the 14 to 28-day of age period. Additionally, it was important to establish if the responses in energy, total Lys and the ME: total Lys could be explained by carcass composition of the broilers.

4.3 Material and methods

A total of 384 day-old Ross 308 male and 384 Ross 308 female broilers were sexed at the hatchery and reared separately on a commercial starter feed. The commercial starter feed (2900 kcal ME/kg, 220 g CP/kg) was offered from 0-14 days old on an *ad libitum* basis to the broilers. At 14 days of age, male and female birds were randomly allocated to one of the 12 dietary treatments. The broilers were placed in a brooding facility with battery cages housing 8 birds per pen. A total of 96 cages were used. The cages were 0.8 m long x 0.5 m wide x 0.42 m height, giving a total floor area of 0.4 m^2. The stocking density for the trial was 20 birds/ m^2. A line feeder was available through the front side of the cage (0.8 m long) and water was provided by nipple drinkers (2 nipples per cage). Feed and water were available on an *ad libitum* basis. The temperatures were set to 24°C at 14 d and reduced by 1°C every day thereafter for up to 17 days at which point it was kept at 21°C to 28 days. The light intensity was set to 30 lux (14 hr light and 10 hr darkness).

4.4 Experimental diets

Dietary treatments consisted of a 3 x 4 factorial arrangement. Twelve diets were formulated to three levels of ME (2800, 3000, and 3200 kcal ME/kg) and four total Lys AA levels (1.55, 1.38, 1.22, and 1.07%) in order to achieve a range of ME: total Lys ratios (1795, 1923, 2051, 2029, 2174, 2319, 2276, 2439, 2602, 2593, 2778 and 2963). It is important to note that the same ME: total lysine ratio can be achieved with various combinations of energy and lysine. For examples a diet with 3000 kcal ME/kg and 1.50% total Lys will provide a ratio of 2000. However, the ratio of 2000 ME: total Lys can also be achieved with 2900 kcal ME/kg and 1.45% total Lys. Prior to the diet formulation, the total amino acid levels of all the ingredients was calculated as a percentage of the analysed CP content using standard procedure (CVB, 2001).

Due to different digestible co-efficient reported in literature (GfE, 1999, CVB, 2001 and Rostagno, 2005) and in order to try to make the study as applicable as possible, it was decided to rather formulate the feed on a total lysine basis and not on a digestible basis. The metabolisable energy (ME) content of the raw materials were calculated using the WPSA (1989) procedure. An inert filler (sand) was required in the diet formulation to allow the application of these principles across a wide range of ME and Lys. In order to reduce the variation among the dietary treatments that could originate as a result of the batching and mixing of ingredients, and to ensure uniform gradations of dietary total Lys within each ME level, a summit/dilution blending technique (Gous and Morris, 1983) was applied during the mixing of the diets. This technique consisted of first formulating and mixing basal diets 1, 4, 7, 10 and summit diets 3, 6, 9, 12. The four intermediate diets were then derived by blending the respective summit and dilution diets within each level of ME in the proportion presented in Table 4.1 and Table 4.2. Following the mixing and blending procedure all the diets were subsequently analysed for proximate and amino acid composition. The diets were pelleted in a pellet mill (Simon-Brown California Hyflo 67) equipped with a 3 mm die.

4.4.1 Pellet quality index hardness

A representative sample from each one of the twelve treatments was subjected to a feed texture quality test. The pellet durability index (PDI) was measured by weighing 500 grams of the diet pellets and then placing the sample in a rolling drum (300 x 300 x 125 mm) (ASAE Standards, 1987). The rolling drum was rotated at 50 revolutions per minute. After 10 minutes, the pellets and fines were removed from the drum and sieved in a 2.36 mm and 1.4 mm screen. The PDI, expressed as a percentage, was then calculated using the equation below:

$$\text{PDI (\%)} = \frac{\text{Mass of pellets after rotation}}{\text{Mass of pellets before rotation}}$$

Table 4.1 The raw material composition of the basal dilution and summit diet composition fed to mixed sex broilers from 14 days to 28 days of age.

Ingredients	Basal Diet 1	Summit Diet 3	Basal Diet 4	Summit Diet 6	Basal Diet 7	Summit Diet 9	Basal Diet 10	Summit Diet 12
	----------------------------------- Percentage (%) -------------------------------------							
Maize	44.8	43.6	52.1	51.7	58.4	58.0	63.2	63.0
Soybean oilcake	27.8	24.6	23.2	23.4	21.3	20.1	16.8	15.5
Sunflower oilcake	5.00	5.49	5.00	4.76	5.00	5.00	5.00	5.00
Corn gluten meal	6.00	6.00	5.02	5.03	3.61	3.70	3.00	3.00
Soy oil	1.99	4.00	1.18	3.59	1.00	3.15	1.00	2.74
Full fat soya	6.00	9.82	5.00	5.00	2.00	3.50	2.00	3.50
L-lysine	0.39	0.39	0.37	0.37	0.35	0.35	0.32	0.33
DL-Methionine	0.26	0.26	0.22	0.22	0.19	0.19	0.15	0.15
L-Threonine	0.06	0.06	0.06	0.06	0.05	0.05	0.05	0.05
MCP[1]	1.55	1.55	1.64	1.65	1.72	1.73	1.81	1.82
Limestone	1.57	1.57	1.55	1.55	1.53	1.53	1.52	1.52
Salt	0.43	0.43	0.44	0.43	0.42	0.42	0.42	0.42
Sodium Bicarb.	0.02	0.02	0.01	0.02	0.04	0.0	0.04	0.04
Choline (60%)	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Pre-mix[2]	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Filler (sand)	4.00	2.00	4.00	2.00	4.20	2.10	5.53	2.77

[1] MCP = Monocalcium phosphate

[2] Premix provided the following (per kg diet): Vitamin A, 13000 IA; vitamin D_3, 5000 ICU; vitamin E, 80.0 mg; vitamin B_{12}, 20.0 ug; riboflavin, 9.0 mg; niacin, 60.0 mg; d-pantothenate, 20.0 mg; menadione (K_3), 3.0 mg; folic acid, 3.2 mg; thiamine, 1.6 mg; pyridoxine, 4.0 mg; d-biotin, 250.0 ug; selenium (as Na_2SeO_3), 0.30; Manganese, 110 mg; zinc, 100 mg; iron, 70 mg; copper, 20 mg; iodine, 2.0 mg; cobalt, 0.5 g

Table 4.2 The nutrient composition of the basal dilution and summit diet fed to mixed sex broilers from 14 to 28 days of age

	Basal Diet 1	Summit Diet 3	Basal Diet 4	Summit Diet 6	Basal Diet 7	Summit Diet 9	Basal Diet 10	Summit Diet 12
				Calculated nutrients[1]				
Moisture (%)	9.40	9.11	9.57	9.43	9.71	9.55	9.63	9.69
ME [2], kcal/kg	2800	3200	2800	3200	2800	3200	2800	3200
CP, %	24.7	24.8	22.2	22.2	19.71	19.7	17.5	17.5
Lys [3], %	1.55	1.55	1.38	1.38	1.22	1.22	1.07	1.07
Met [3], %	0.68	0.68	0.60	0.60	0.53	0.53	0.46	0.46
Met & Cys [3], %	1.10	1.10	0.99	0.99	0.88	0.88	0.77	0.78
Thr [3], %	1.02	1.02	0.91	0.92	0.81	0.82	0.72	0.72
Val [3], %	1.17	1.17	1.05	1.08	0.93	0.94	0.83	0.84
Arg [3], %	1.55	1.55	1.38	1.40	1.24	1.24	1.07	1.09
SID. Lys [3], %	1.41	1.41	1.26	1.26	1.12	1.12	0.99	0.99
SID. SAA [4], %	0.99	0.99	0.88	0.88	0.78	0.78	0.69	0.69
SID. Thr [4], %	0.87	0.88	0.78	0.78	0.69	0.67	0.61	0.62
SID. Val [4], %	1.03	1.03	0.93	0.95	0.83	0.84	0.74	0.75
SID. Arg [4], %	1.44	1.47	1.27	1.27	1.12	1.13	0.98	0.98
Ca, %	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90
Avl. P %	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41
Na, %	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Cl, %	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
DEB[5], Meq/kg	171	164	152	155	143	1140	127	125
CP: ME ratio	47.3	53.8	52.8	60.4	59.4	67.8	67.0	76.4
ME: total Lys	1806	2065	2029	2319	2295	2623	2617	2991
Analysed nutrient composition								
CP, %	24.9	24.9	23.1	21.9	20.5	19.9	18.3	17.5
Lys, %	1.53	1.52	1.38	1.33	1.22	1.21	1.07	1.04

[1]Nutrient composition calculated from proximate analyses of all ingredients prior to formulation

[2]Energy content of ingredients calculated as per WPSA 3rd Ed, 1989

[3]total amino acid content of ingredients calculated as a percentage of the analysed CP content

[4]Calculated from standardised ileal digestibility coefficients applied to the calculated amino acid content (Evonik, 2014)

[5]DEB = Dietary electrolyte balance (Na + K – Cl)

Ten pellets per treatments were subject to a pellet hardness test as prescribed by Payne *et al.* (1987). During the test, a pellet is held between the jaws of a manual Kahl pellet hardness tester. Pressure is exerted by screwing down by hand onto a coil spring. The pointer indicates the load in kilogrammes at which the pellets burst. The data obtained from the pellet hardness test was used to test for statistical significance between the treatments.

4.5 Data collection

Broilers were weighed on day 14 and day 28, and FI was calculated as the difference between the feed allocated at the beginning of the trial and the amount left over at the end of the trial. Average daily gain (ADG) and feed conversion ratio (FCR) could therefore be calculated. Mortality (%) was recorded on a daily basis. However, FCR was not corrected for the mortality.

4.6 Body carcass and chemical analyses

At 28 days of age, three birds per treatment were randomly selected and slaughtered according to the technique described by Lourdes *et al.* (2010). Birds were humanely slaughtered by electrical stunning, followed by cutting of the blood vessels (carotid artery and jugular vein). After evisceration, carcasses were packed, identified, and chilled to 0 °C. Cuts were deboned 24 hours *post mortem*, packed, identified, frozen, and stored at -18 °C. Meat physical chemical analyses were performed after cuts were thawed at 4 °C for 24h. Whole carcass (without abdominal fat) and abdominal fat were weighed. Carcass, abdominal fat, and total breast meat (*pectoralis major* and *minor* muscles) yields were expressed as a percentage of 28 day carcass weight of selected broilers. Additionally, proximate analysis was conducted on minced samples of all the whole carcasses. The protein content was determined according to standard techniques of the AOAC (1997), and the lipid by means of solvent (chloroform/methanol) extraction (Lee *et al*., 1996).

4.7 Statistical analysis

JMP ® of SAS version 12.0.1 (SAS institute Inc. JMP Software, Cary, NC, 2015) was used to test for normality and the data was then analysed using Proc GLM (general linear models procedure) as a 3 (energy) x 4 (total Lys) factorial arrangement ($p \leq 0.05$). Mean differences were separated using Tukey's Honest Significant Differences Test (Tukey, 1991). For the purposes of this study, eight replications were made with 64 birds per replication. Of the 8 birds per replication, 4 birds were male and 4 birds were females.

The linear and quadratic regressions of JMP® of SAS version 12.0.1 (SAS institute, 2015) were used to observe the treatments' effect on weight, ADG, FI and FCR.

$$Y = b0 + b1 E \quad [4.1]$$

$$Y = b0 + b1E + b2 E^2 \quad [4.2]$$

Where Y predicted response, E = energy contentment of the diet, b0 = intercept and b1 and b2 = regression coefficient.

4.7 Results

4.7.1 Feed texture: pellet durability index and hardness

There was no interaction between total Lys (%) and pellet durability and pellet hardness (Table 4.4), nor was there an interaction between ME: total Lys and pellet hardness and also no interaction between ME and pellet hardness. There was however a significant interaction ($p < 0.05$) between the varying energy (ME) levels and the pellet durability index (Table 4.5). It was therefore evident from this evaluation that an increase in energy (ME) above 3000 kcal ME/kg negatively affects pellet durability.

Table 4.3: The effect of varying total Lys and energy levels (ME) in diets on the pellet durability index (PDI) and pellet hardness

Treatment	Total Lys (%)	ME (kcal/kg)	Ratio ME: total Lys	PDI (%)	Pellet hardness (kg)
1	1.56	2800	1795	95.4	1
2	1.56	3000	1923	90.5	1
3	1.56	3200	2051	84.6	1
4	1.38	2800	2029	94.1	2
5	1.38	3000	2174	95.7	1
6	1.38	3200	2319	83.3	2
7	1.23	2800	2276	94.5	1
8	1.23	3000	2439	90.9	1
9	1.23	3200	2602	84.2	1
10	1.08	2800	2593	90.9	2
11	1.08	3000	2778	87.5	1
12	1.08	3200	2963	79.1	1
SEM				5.21	
p>F					
PDI (%) quadratic				0.78	
SEM					0.50
p>F					
Pellet Hardness					0.95

[a-d] Means in the same column with no common superscript differ significantly ($p \leq 0.05$)

Table 4.4: The effect of varying total Lys (%) levels in diets on the pellet durability index (PDI) and pellet hardness

total Lys (%)	PDI (%)	Pellet hardness (kg)
1.55	90.2	1.38
1.38	91.0	0.88
1.22	89.9	0.88
1.07	85.8	0.70
SEM	3.39	-
p > F		
PDI (%) quadratic	0.71	-
SEM	-	0.27
p > F		
Hardness quadratic	-	0.37

[a-d] Means in the same column with no common superscript differ significantly ($p \leq 0.05$)

Table 4.5: The effect of varying energy (ME) levels in diets on the pellet durability index (PDI) and pellet hardness

ME (kcal/kg)	PDI (%)		Pellet hardness (kg)
2800	93.7 [a]		1.4 [a]
3000	91.1 [a]		0.9[a]
3200	82.8 [b]		0.6 [b]
SEM	1.90		-
p > F			
PDI (%) quadratic	0.007		-
SEM		-	0.19
p > F			
Hardness quadratic		-	0.05

[a-d] Means in the same column with no common superscript differ significantly ($p \leq 0.05$)

4.7.2 Animal performance

4.7.2.1 The effect of feeding varying energy (ME): total Lys ratio's to mixed sex broilers from 14 to 28 days of age

Table 4.6 and Figures 4.1 (ADG), 4.2 (ADFI) and 4.3 (FCR) present the responses of broilers (14 to 28 days of age) to varying levels of ME and total Lys for ADG, ADFI and FCR. The ADG improved with an increase in total Lys content. ADG tended to be higher with both intermediate (3000 kcal ME/kg) and lower energy values (2800 kcal ME/kg). The highest response in 28-day weight and ADG were achieved with treatment 4 (1.39%; total Lys; 2800 kcal ME/kg; 2029 ratio). The lowest FCR was achieved with treatment 3 (1.56%; total Lys 3200 kcal ME/kg; 2051 ratio). However, ADFI tended to decrease with an increase in energy. The highest ADFI was achieved with treatment 4 (total Lys 1.38%; 2800 kcal ME/kg; 2029 ratio).

Average daily gain increased with lower ME: total Lys ratios and peaked at a ratio of 2029. It tended decrease when the ratio increased further. Both ADFI and FCR tended to decrease with lower ME: total Lys ratios. There were no statistical differences observed in mortalities between the treatments. A quadratic regression for ME: total Lys ratio revealed that the area of maximum response for ADG was 2250 and that lowest FCR was achieved at 2225.

The statistical evaluation of broilers (14-28 days of age) ADG, ADFI and FCR revealed a quadratic response to varying levels of ME: total Lys ratio:

$$ADG = 33.606973 + 0.0302445 \times ME\text{: total Lys} + -0.00000684 \times ME\text{: total Lys}^2 \quad (R^2 = 0.43) \; [4.3]$$

$$FCR = 4.043189 + -0.002338 \times ME\text{: total Lys} + 0.00000049807 \times ME\text{: total Lys}^2 \quad (R^2 = 0.31) \; [4.4]$$

$$ADFI = 221.00755 + -0.112043 \times ME\text{: total Lys} + 0.0000233 \times ME\text{: total Lys}^2 \quad (R^2 = 0.22) \; [4.5]$$

Table 4.6: The effect on the performance of 14 to 28 days of age mixed flock broilers due to the feeding of varying ME: total Lys ratios

Treat-ment	total Lys (%)	ME kcal/ kg	Ratio#	Day 14 weight (g)	Day 28 weight (g)	ADG (g)	ADFI (g)	FCR (g/g)	Mortality (%)
1	1.56	2800	1795	409	1322 [ab]	64.1 [bc]	105.0 [a]	1.63 [a]	3.13
2	1.56	3000	1923	397	1349 [ab]	67.5 [abc]	85.5 [cde]	1.27 [de]	1.56
3	1.56	3200	2051	402	1308 [b]	64.7 [abc]	79.3 [e]	1.23 [e]	0.00
4	1.38	2800	2029	414	1389 [a]	69.1 [a]	89.3 [bcd]	1.30 [cde]	1.56
5	1.38	3000	2174	412	1372 [ab]	68.5 [ab]	86.3 [bcde]	1.26 [de]	0.00
6	1.38	3200	2319	407	1354 [ab]	65.8 [abc]	84.4 [de]	1.29 [de]	1.56
7	1.23	2800	2276	391	1334 [ab]	66.8 [abc]	89.5 [bcd]	1.34 [bcd]	1.56
8	1.23	3000	2439	404	1328 [ab]	66.0 [abc]	89.2 [bcd]	1.35 [bcd]	0.00
9	1.23	3200	2602	409	1343 [ab]	66.2 [abc]	86.9 [bcd]	1.32 [cde]	1.56
10	1.08	2800	2593	397	1306 [b]	64.5 [abc]	93.5 [b]	1.45 [b]	1.56
11	1.08	3000	2778	406	1312 [ab]	64.3 [bc]	92.0 [bc]	1.43 [b]	1.56
12	1.08	3200	2963	404	1294 [b]	63.4 [c]	88.7 [bcd]	1.40 [bc]	1.56
SEM				5.20	16.7	1.00	1.53	0.02	0.23
$p > F$									
Ratio quadratic				0.69	0.004	0.001	0.0001	0.0001	0.78

[a-e] Means in the same column with no common superscript differ significantly ($p \leq 0.05$)

Ratio = ME: total Lys

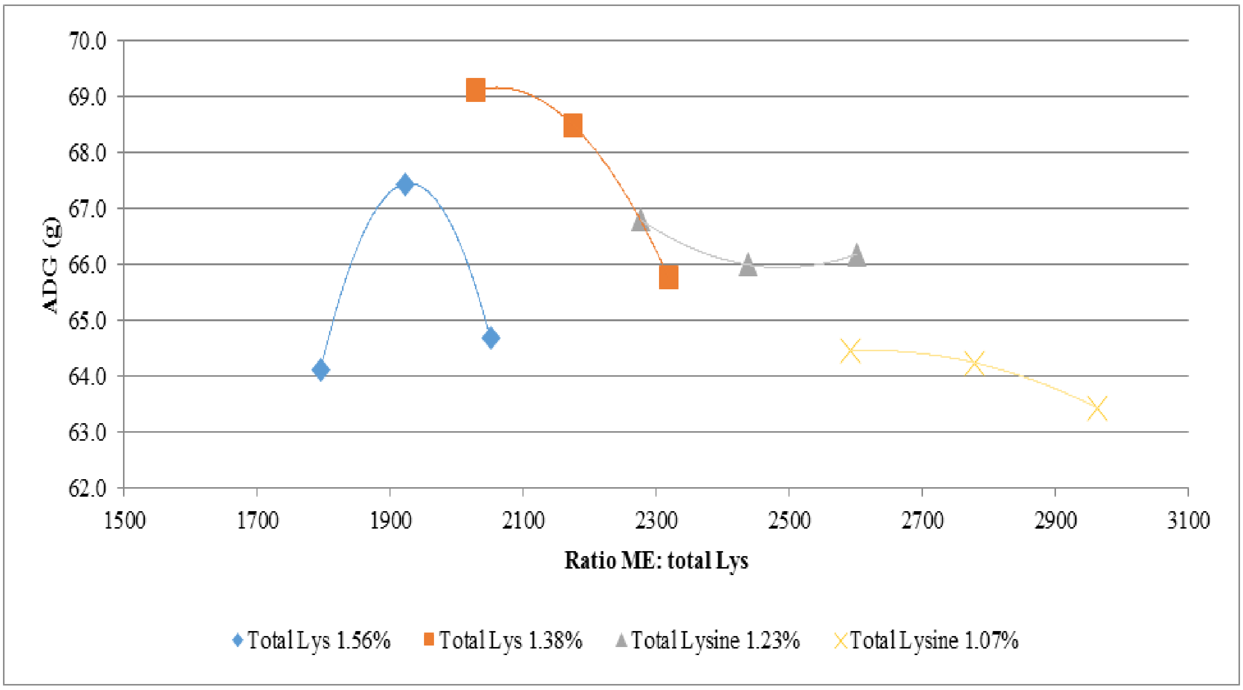

Figure 4.1: The effect of feeding 14 to 28 days of age mixed sex broilers varying amounts of dietary ME: total Lys ratios on Average Daily Gain (ADG)

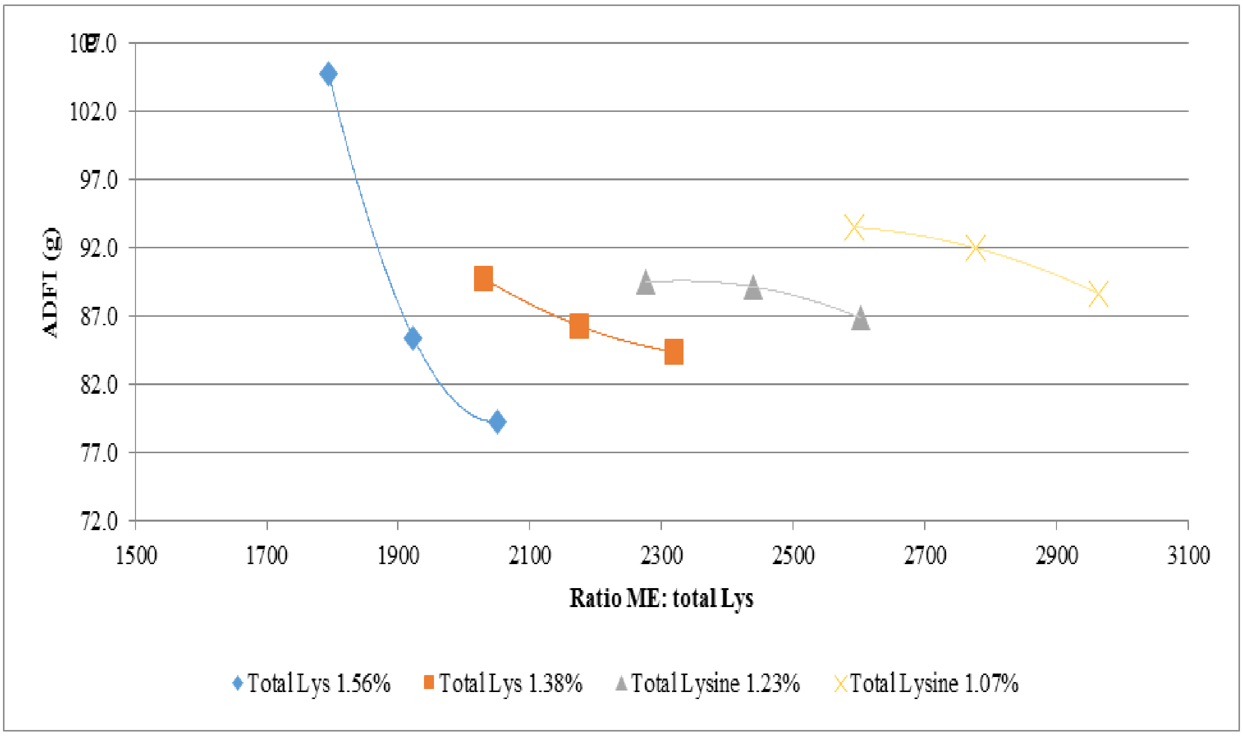

Figure 4.2: The effect of feeding 14 to 28 days of age mixed sex broilers varying amounts of dietary ME: total Lys ratios on Average Daily Feed Intake (ADFI)

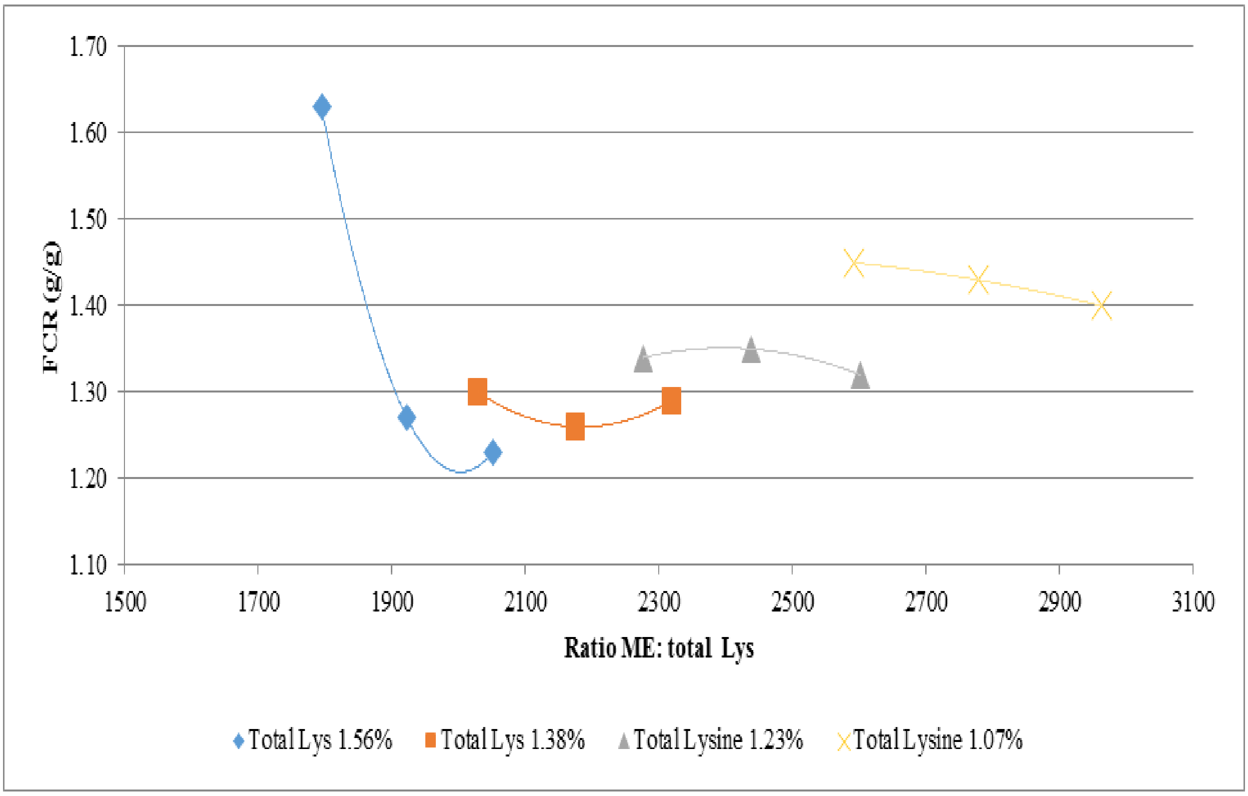

Figure 4.3: The effect of feeding 14 to 28 days of age mixed sex broilers varying amounts of dietary ME: total Lys ratios on Feed Conversion Ratio (FCR)

4.7.2.2 The effect of feeding varying **total Lys** *levels on mixed sex broilers from 14 to 28 days of age*

The broilers' responses (14-28 days) to varying total Lys are depicted in Table 4.7 and Figures 4.4 (ADG), 4.5 (ADFI) and 4.6 (FCR). The broilers' ADG increased with increasing total Lys and the FCR decreased with an increase in total Lys. The ADG for treatments 4, 5 and 6 (total Lys 1.38%) was significantly higher than that of the highest level of Lys treatments 1, 2 and 3 (total Lys 1.56%) as well as the lowest Lys treatments of 10, 11 and 12 (total Lys 1.08%). There was no significant difference in ADG between the total Lys 1.38% (4, 5 and 6) and total Lys 1.23% (7, 8 and 9). Total Lys level had no significant effect on ADFI. The FCR for treatments 4, 5 and 6 (total Lys 1.38%) was significantly lower than that of the highest Lys treatment 1, 2 and 3 (total Lys 1.56%) as well as the lowest Lys treatments of 10, 11 and 12 (total Lys 1.08%). There was no significant difference in FCR between the total Lys 1.38% (4, 5 and 6) and total Lys 1.23% (7, 8 and 9).

The quadratic statistical analysis of the response of broilers (14–28 days of age) to total Lys for ADG, ADFI and FCR is indicated in the equations below:

ADG = -18.27109 + 126.45999 x total Lys + -46.63198 x total Lys^2 (R^2 = 0.95) [4.6]

FCR = 4.6352704 + -4.913784x total Lys + 1.8064332 x total Lys^2 (R^2 = 0.96) [4.7]

ADFI = 419.890 + -642.97 x total Lys + 241.745 x total Lys^2 (R^2 = 0.97) [4.8]

According to the quadratic regression for total Lys, the point of maximum response for ADG was achieved at 1.37% while that of FCR was achieved at 1.34%.

Table 4.7: The effect on the performance of 14 to 28 days of age mixed flock broilers to the feeding of varying total Lys levels (2800, 3000 and 3200 ME kcal/kg)

total Lys (%)	14-day weight (g)	28-day weight (g)	ADG (g)	ADFI (g)	FCR (g/g)	Mortality (%)
1.56	403	1325[b]	65.4 [bc]	89.8	1.37 [ab]	1.60
1.38	411	1365 [a]	67.8 [a]	86.9	1.28[c]	1.00
1.23	402	1335 [ab]	66.3 [ab]	88.5	1.34 [bc]	1.00
1.08	402	1304 [b]	64.1 [c]	91.4	1.43 [a]	1.60
SEM	5.18	23.7	1.43	1.50	0.03	0.00
$p > F$						
tot. Lys quadratic	0.09	0.004	0.001	0.001	0.0001	0.36

[a-d] Means in the same column with no common superscript differ significantly ($p \leq 0.05$)

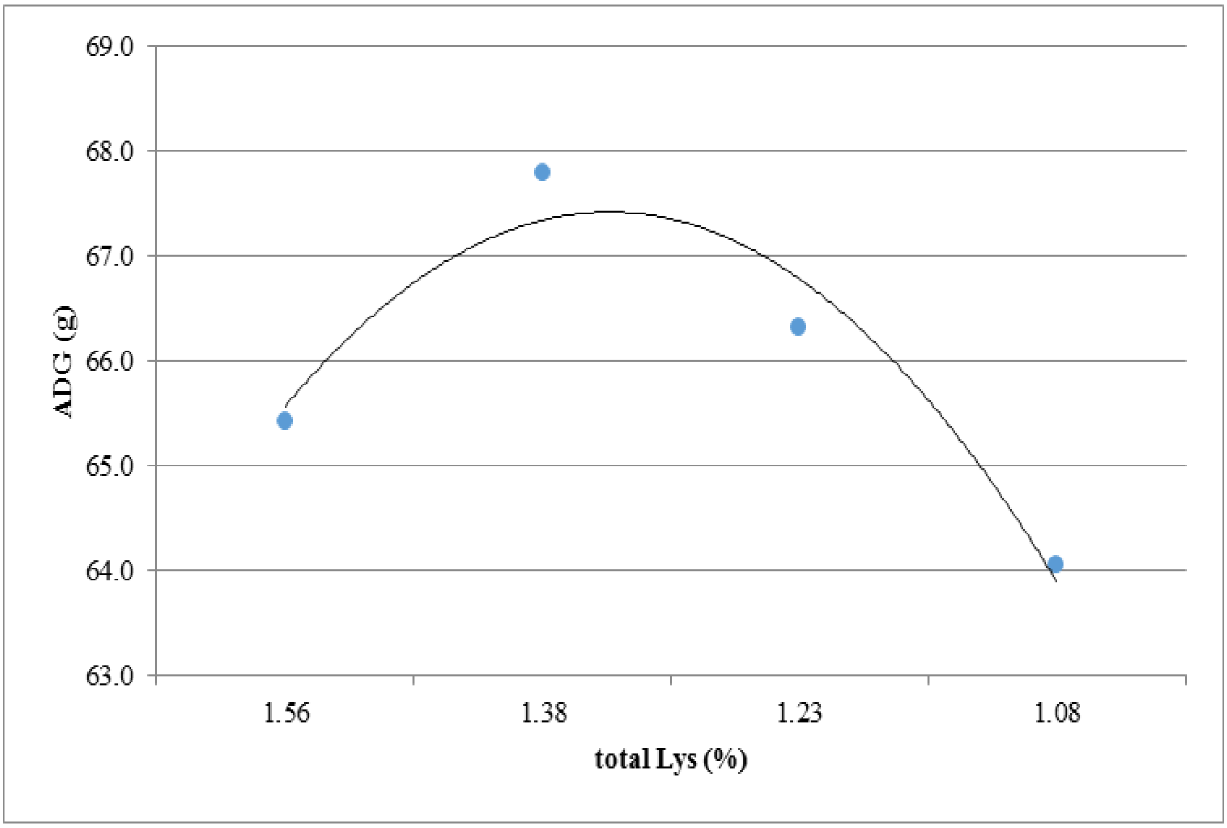

Figure 4.4: The effect of feeding 14 to 28 days of age mixed sex broilers varying amounts of dietary total Lys ratios on Average Daily Gain (ADG)

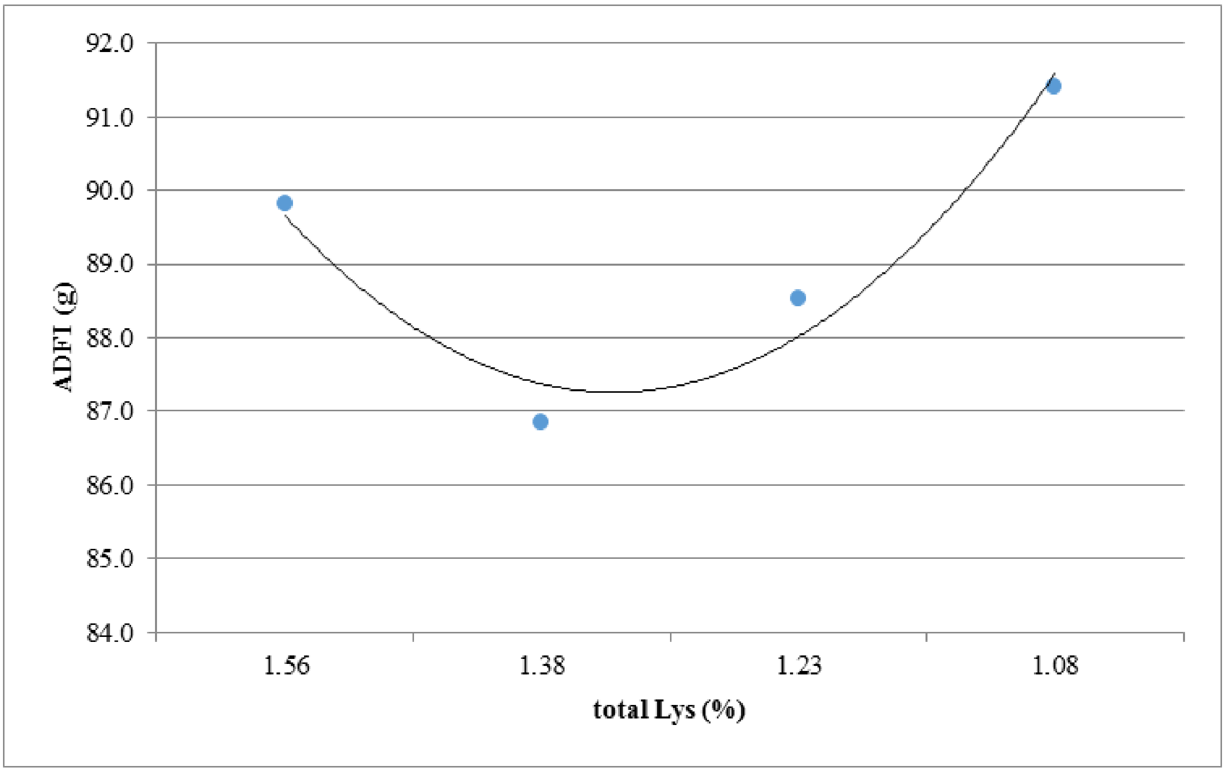

Figure 4.5: The effect of feeding 14 to 28 days of age mixed sex broilers varying amounts of dietary total Lys ratios on Average Daily Feed Intake (ADFI)

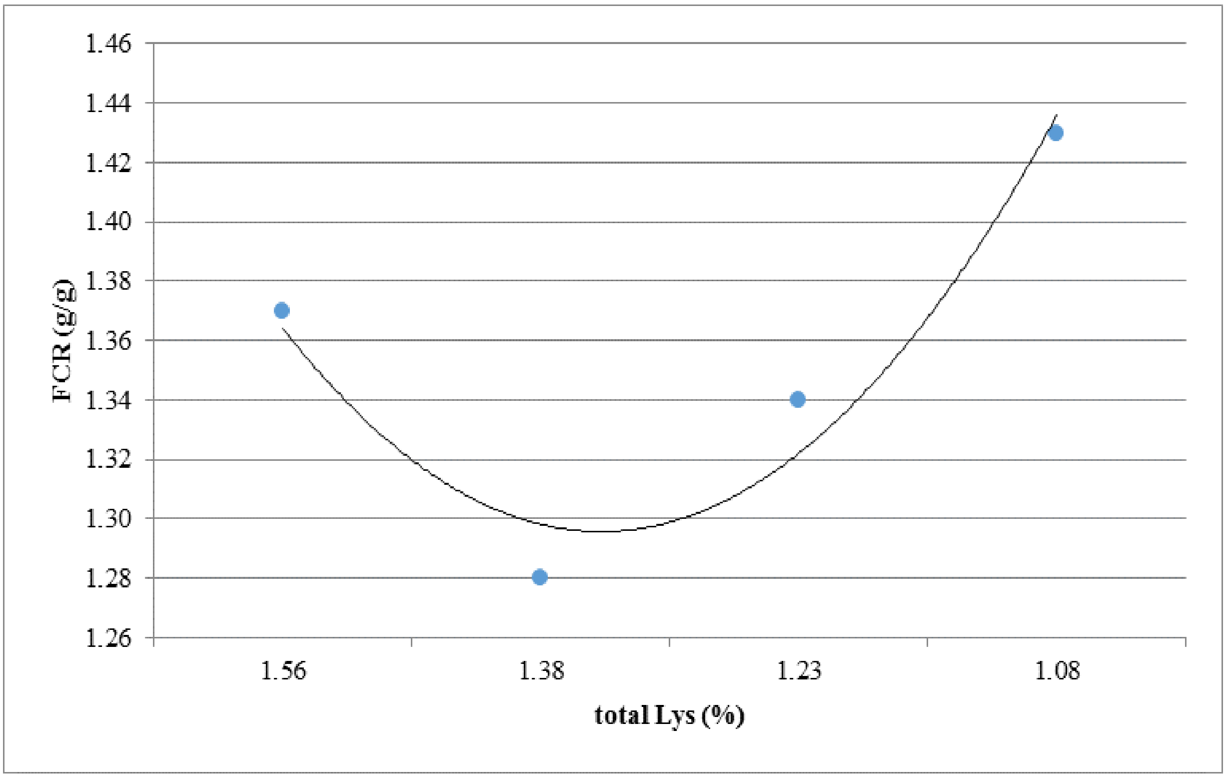

Figure 4.6: The effect of feeding 14 to 28 days of age mixed sex broilers varying amounts of dietary total Lys ratios on Feed Conversion Ratio (FCR)

4.7.2.3 The effect of feeding varying energy (ME) levels on mixed sex broilers from 14 to 28 days of age

The broilers' responses (14-28 days) to varying energy (ME) are depicted in Table 4.8 and Figures 4.7 (ADG), 4.8 (ADFI) and 4.9 (FCR). Neither an increase in the energy level by 200 kcal ME/kg or even by 400 kcal ME/kg from 2800 ME kcal/kg to 3200 ME kcal/kg had a significant effect on ADG. However, ADFI and FCR were significantly decreased when the energy was increased by 200 kcal ME/kg from 2800 ME kcal/kg to 3000 ME kcal/kg. There was no further significant improvement in ADFI and FCR when the energy was increased by another 200 kcal ME/kg from 3000 kcal ME/kg to 3200 kcal ME/kg.

Table 4.8: The effect on the performance of 14 to 28 days of age mixed flock broilers due to the feeding of varying energy (ME) levels (1.08, 1.23, 1.38; 1.56% total Lys)

ME (kcal/kg)	Day 14 weight (g)	Day 28 weight (g)	ADG (g)	ADFI (g)	FCR (g)	Mortality (%)
2800	403	787	66.1	94.4^{a}	1.43^{a}	2.00
3000	405	786	66.6	88.3^{b}	1.32^{b}	0.80
3200	406	768	65.0	84.8^{b}	1.31^{b}	1.20
SEM	2.71	9.15	0.80	1.50	0.02	1.30
p > F						
ME Quadratic	0.75	0.23	0.0001	0.0001	0.0001	0.29

$^{a-d}$ Means in the same column with no common superscript differ significantly ($P \leq 0.05$)

The quadratic statistical analysis of the response of broilers (14-28 days of age) to energy (ME) for ADG, ADFI and FCR is indicated in the equations below:

$$ADG = -144.5 + 0.143475 \times ME + -0.00002437 \times ME^2 \quad (R^2 = 0.89) \; [4.9]$$

$$FCR = 4.6352704 + -4.913784 \times ME + 1.8064332 \times ME^2 \quad (R^2 = 0.96) \; [4.10]$$

$$ADFI = 468.2 + -0.2294 \times ME + 0.00003425 \times ME^2 \quad (R^2 = 0.93) \; [4.11]$$

Since there were no significant differences between the 2800 kcal ME/kg, 3000 kcal ME/kg and 3200 kcal ME/kg treatments for ADG, an optimum ME value could not be predicted. However, for FCR the point of maximum response was achieved at 3075 kcal ME/kg.

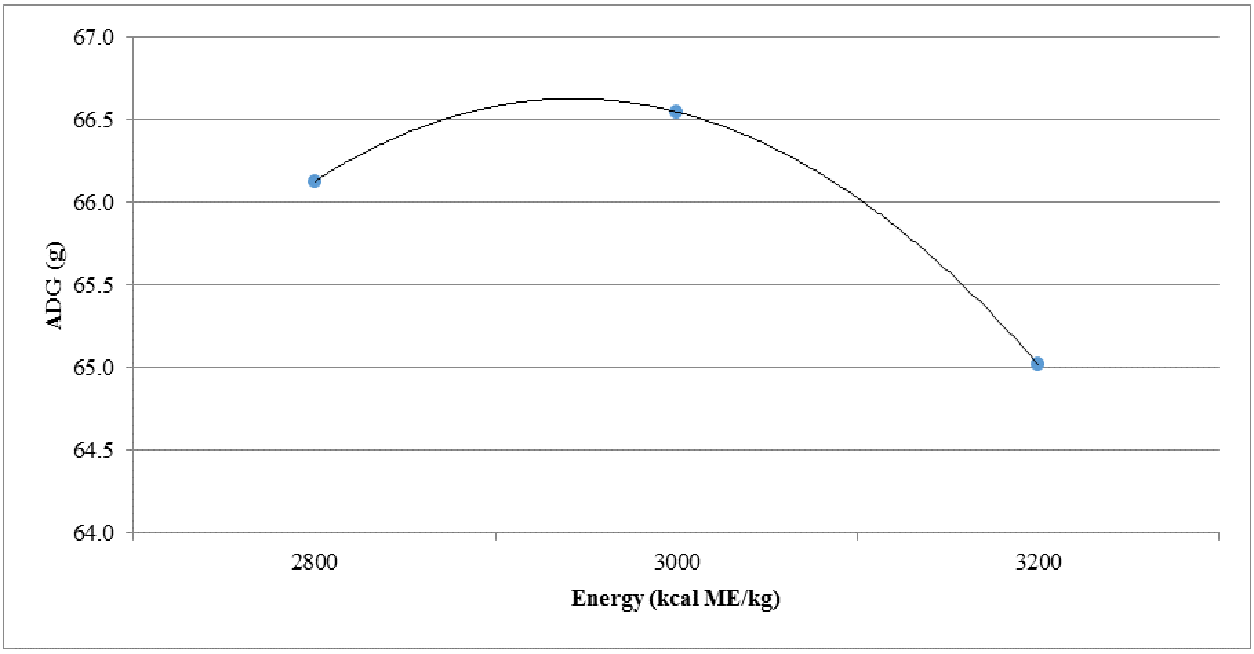

Figure 4.7: The effect of feeding 14 to 28 day of age mixed sex broiler varying amounts of energy (ME) levels on Average Daily Gain (ADG)

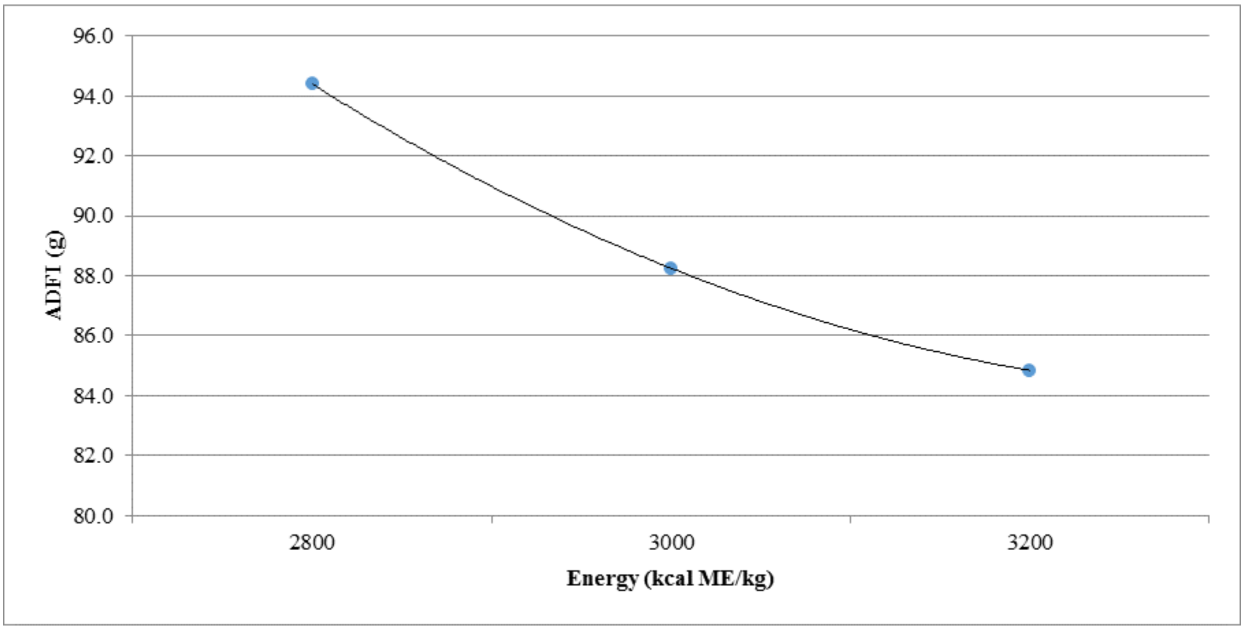

Figure 4.8: The effect of feeding 14 to 28 day of age mixed sex broiler varying amounts of energy (ME) levels on Average Daily Intake (ADI)

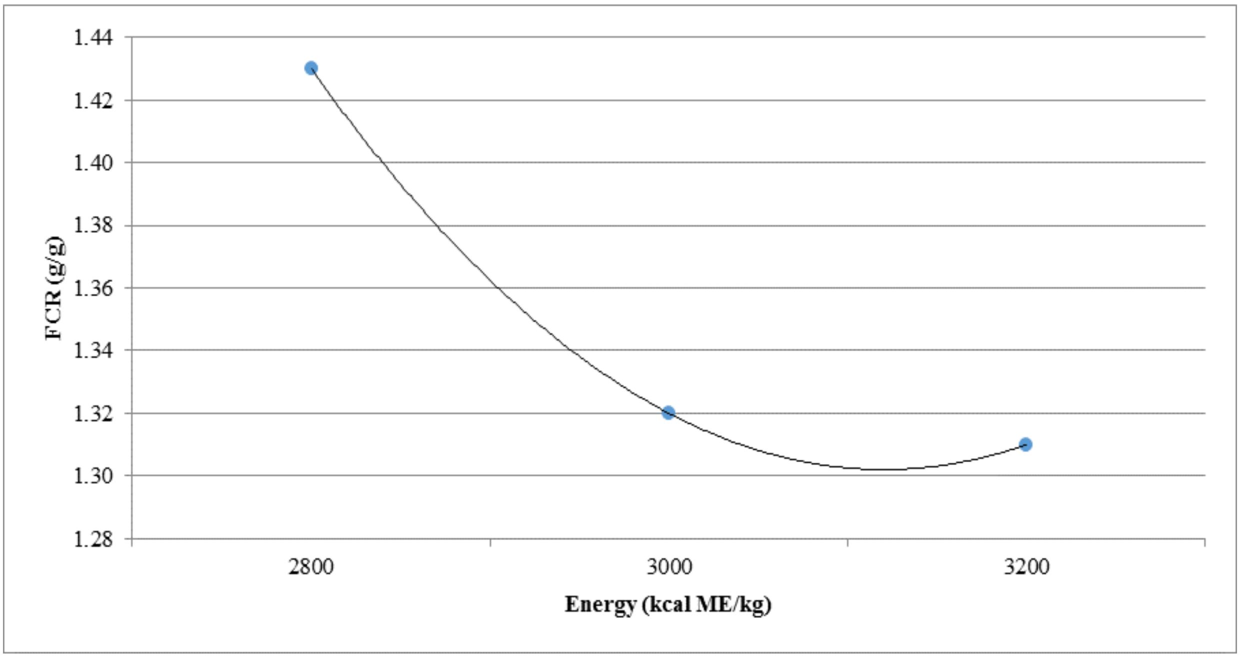

Figure 4.9: The effect of feeding 14 to 28 day of age mixed sex broiler varying amounts of energy (ME) levels on Feed Conversion Ratio (FCR)

4.7.2.4 Carcass composition

Table 4.9 presents the effect of varying ME: total Lys ratios on carcass composition of broilers (14–28 days of age). Only treatment 2 (ME: total Lys 1923, total Lys % 1.56 3000 kcal ME/kg) was significantly different from treatment 12 (ME: total Lys 2963, total Lys % 1.08, 3200 kcal ME/kg). The statistical analysis of the quadratic response of broilers to varying ME: total Lys for whole carcass (%), whole breast (%), breast meat (%), abdominal fat (%) is described by the equations below:

Whole carcass (%) = 107.69172+ -0.010045 x total Lys + 0.000001825 x total Lys ^{2}

(R^2 = 0.03) [4.15]

Abdominal fat (%) = -2.040821 + 0.0025054 x total Lys + -0.0000004043 x total Lys ^{2}

(R^2 = 0.19) [4.16]

Whole breast (%) = 41.200308 + -0.011911 x total Lys + 0.0000019342 total Lys ^{2}

(R^2 = 0.35) [4.17]

Breast meat (%) = 41.869439+ -0.017194 x total Lys + 0.0000029777 x total Lys ^{2}

(R^2 = 0.34) [4.18]

Table 4.9: The effects of feeding 14 to 28 days' mix sex broilers varying ME: total Lys ratio on carcass protein and fat composition (% of carcass weight at processing)

Treatment	total Lys (%)	ME kcal/kg	Ratio ME/total Lys	Carcass (%)	Abdominal fat (%)	Whole breast (%)	Breast meat (%)
1	1.56	2800	1795	94.2	1.90 ^{ab}	25.2 ^{ab}	19.9 ^{ab}
2	1.56	3000	1923	97.8	0.90 ^{b}	26.8 ^{a}	21.2 ^{a}
3	1.56	3200	2051	92.5	1.30 ^{ab}	24.9 ^{ab}	18.3 ^{ab}
4	1.38	2800	2029	94.7	1.60 ^{ab}	25.7 ^{ab}	20.3 ^{ab}
5	1.38	3000	2174	95.5	1.10 ^{ab}	24.4 ^{ab}	18.3 ^{ab}
6	1.38	3200	2319	96.2	1.30 ^{ab}	22.6 ^{b}	16.6 ^{ab}
7	1.23	2800	2276	93.8	2.10 ^{ab}	23.5 ^{ab}	17.7 ^{ab}
8	1.23	3000	2439	94.1	2.00 ^{ab}	23.6 ^{ab}	17.9 ^{ab}
9	1.23	3200	2602	93.5	1.70 ^{ab}	23.7 ^{ab}	17.7 ^{ab}
10	1.08	2800	2593	92.4	2.10 ^{a}	23.8 ^{ab}	17.6 ^{ab}
11	1.08	3000	2778	93.7	1.90 ^{ab}	23.8 ^{ab}	18.3 ^{ab}
12	1.08	3200	2963	94.6	1.60 ^{ab}	22.3 ^{b}	16.2 ^{b}
SEM				1.70	0.30	0.70	0.90
$p > F$							
Ratio Quadratic				0.67	0.02	0.03	0.03

$^{a-d}$ Means in the same column with no common superscript differ significantly ($p \leq 0.05$)

Table 4.10 presents the effect of varying total Lys (%) on broilers' (14-28 days of age) body composition. A general trend was observed that a decrease in the total Lys ratio resulted in higher abdominal fat and a lower whole breast percentage as well as lower breast meat percentage. There was a significant increase in abdominal fat (%), a decrease in whole breast (%) as well a decrease in breast meat (%) when the total Lys was reduced from 1.56% (treatments 1, 2 and 3) to 1.23% (treatments 7, 8 and 9).

Table 4.10: The effects of feeding 14 to 28-day mix sex broilers varying total Lys (%) on broiler carcass protein and fat composition (% of carcass weight at processing)

total Lys (%)	Carcass (%)	Abdominal fat (%)	Whole breast (%)	Breast meat (%)
1.56	94.8	1.20 [c]	25.6 [a]	19.8 [a]
1.38	95.5	1.30 [bc]	24.2 [ab]	18.4 [ab]
1.23	93.8	1.80 [ab]	23.6 [b]	17.8 [ab]
1.08	93.6	1.90 [b]	23.3 [b]	17.4 [b]
SEM	0.97	0.14	0.50	0.60
p > F				
total Lys quadratic	0.48	0.002	0.01	0.03

[a-d] Means in the same column with no common superscript differ significantly ($p \leq 0.05$)

The statistical analysis of the quadratic response of broilers to varying total Lys for whole carcass (%), whole breast (%), breast meat (%) and abdominal fat (%) is described in the equations below:

Whole carcass (%) = 72.783536 + 29.95829 x total Lys + -10.0828 x total Lys ^{2}

(R^2 = 0.05) [4.19]

Abdominal fat (%) = -2.040821 + 0.0025054 x total Lys + -0.0000004037 x total Lys ^{2}

(R^2 = 0.18) [4.20]

Whole breast (%) = 41.869 + -0.017194 x total Lys + 0.00000297773 x total Lys ^{2}

(R^2 = 0.35) [4.21]

Breast meat (%) = 41.869339 + -0.017238 x total Lys + 0.0000029773 x total Lys ^{2}

(R^2 = 0.34) [4.22]

Table 4.11 illustrates the effect of varying energy (ME) ratios on broiler (14–28 days of age) body composition. No significant differences were observed between the treatments.

Table 4.11: The effects of feeding 14 to 28-day mix sex broilers varying ME on broiler carcass protein and fat composition (% of carcass weight at processing)

ME (kcal/kg)	Carcass (%)	Abdominal fat (%)	Whole breast (%)	Breast meat (%)
2800	93.8	1.80	24.5	19.0
3000	95.3	1.40	24.6	19.0
3200	94.2	1.50	23.4	17.0
SEM	0.84	0.10	0.50	0.52
p > F				
ME Quadratic	0.45	0.06	0.15	0.05

[a-d] Means in the same column with no common superscript differ significantly ($p \leq 0.05$)

The statistical analysis of the quadratic response of broilers to varying energy (ME) for whole carcass (%), whole breast (%), breast meat (%) and abdominal fat (%) is described in the equations below:

Whole carcass (%) = -195.8858 + 0.19305 x ME + -0.000032 x ME ^{2}

($R^2 = 0.05$) [4.23]

Whole breast (%) = -112.8125 + 0.0944958 x ME + -0.00001623 x ME ^{2}

($R^2 = 0.12$) [4.24]

Breast meat (%) = -158.5617 + 0.1225563 x ME + -0.00002114 x ME ^{2}

($R^2 = 0.18$) [4.25]

Abdominal fat (%) = 68.013333 + -0.043592 x ME + 0.000007125 x ME ^{2}

($R^2 = 0.08$) [4.26]

4.7.2.5 Body carcass protein and fat composition of 14 to 28 day old mixed sex broilers fed varying ME: total Lys ratio's

Table 4.12 and Figure 4.10 and Figure 4.11 presents the effect of varying ME: total Lys ratios on the carcass protein and fat composition of broilers from 14 to 28 days of age. There appeared to be a general trend that a decrease in the ME: total Lys ratio resulted in a lower carcass protein (%) content and a higher fat (%) content.

Table 4.12: The effects of feeding 14 to 28 day old mix sex broilers varying ME: total Lys ratios and the composition of broiler carcass protein and fat

Treatment	total Lys (%)	ME kcal/kg	Ratio ME/total Lys	Protein (%)	Fat (%)
1	1.56	2800	1795	60.0 ^{a}	17.8 ^{c}
2	1.56	3000	1923	56.8 ^{abcd}	21.6 ^{abc}
3	1.56	3200	2051	56.9 ^{abcd}	20.0 ^{bc}
4	1.38	2800	2029	57.3 ^{abc}	22.3 ^{abc}
5	1.38	3000	2174	59.3 ^{ab}	20.1 ^{bc}
6	1.38	3200	2319	56.7 ^{abcd}	22.5 ^{abc}
7	1.23	2800	2276	54.6 ^{abcd}	25.0 ^{abc}
8	1.23	3000	2439	50.6 ^{cd}	27.2 ^{ab}
9	1.23	3200	2602	49.3 ^{cd}	26.7 ^{ab}
10	1.08	2800	2593	51.3 ^{bcd}	27.9 ^{ab}
11	1.08	3000	2778	48.5 ^{d}	28.3 ^{a}
12	1.08	3200	2963	50.2 ^{cd}	26.2 ^{abc}
SEM				2.38	1.62
p > F					
Ratio Quadratic				0.0001	0.0007

[a-d] Means in the same column with no common superscript differ significantly ($p \leq 0.05$)

The statistical analysis of the quadratic response of broilers to varying ME: total Lys for carcass protein (%) and carcass fat (%) is described in the equations below:

Carcass protein (%) = 89.520824+ -0.019946 x ME: total Lys + 0.0000020243 x ME: total Lys^2

(R^2 = 0.59) [4.27]

Carcass fat (%) = -37.91186 + 0.0448105 x ME: total Lys + -0.000000077 x ME: total Lys^2

(R^2 = 0.54) [4.28]

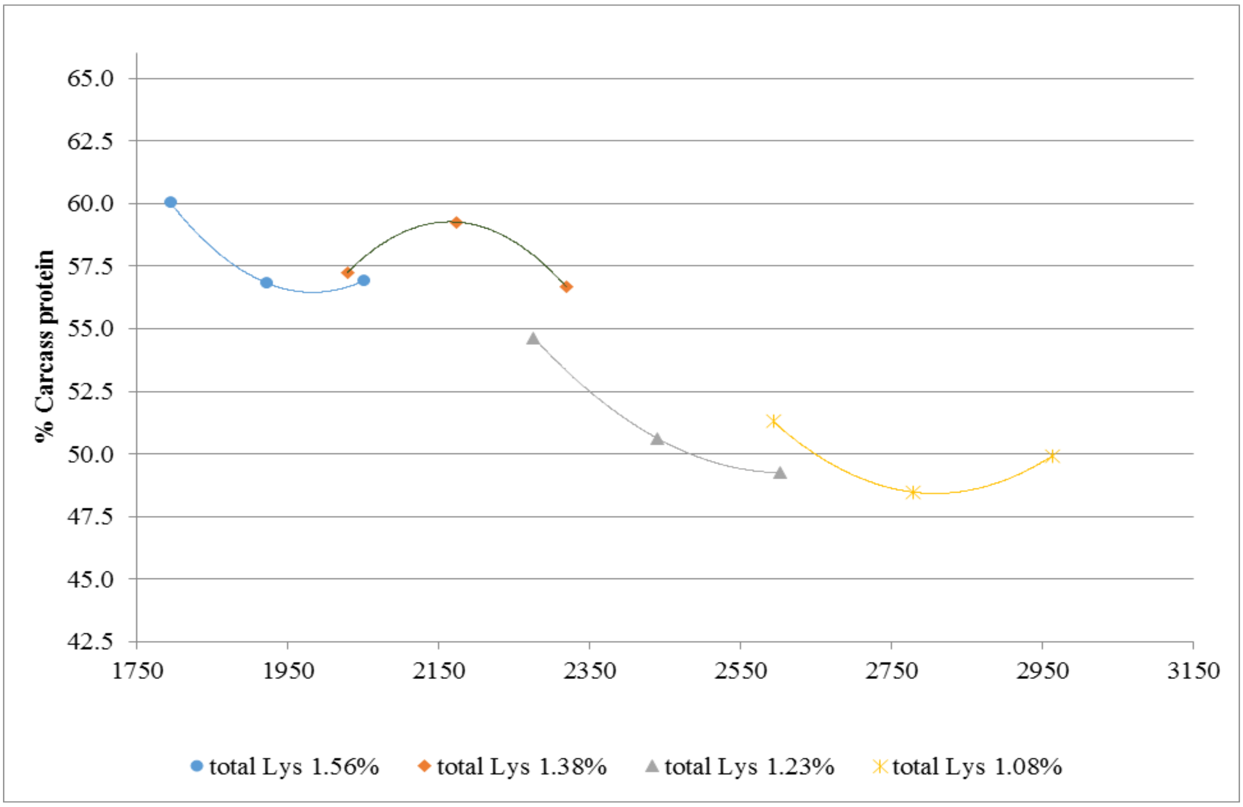

Figure 4.10: The effect of feeding 14 to 28 days of age mixed sex broilers varying amounts of dietary energy: total Lys ratios on carcass protein (%)

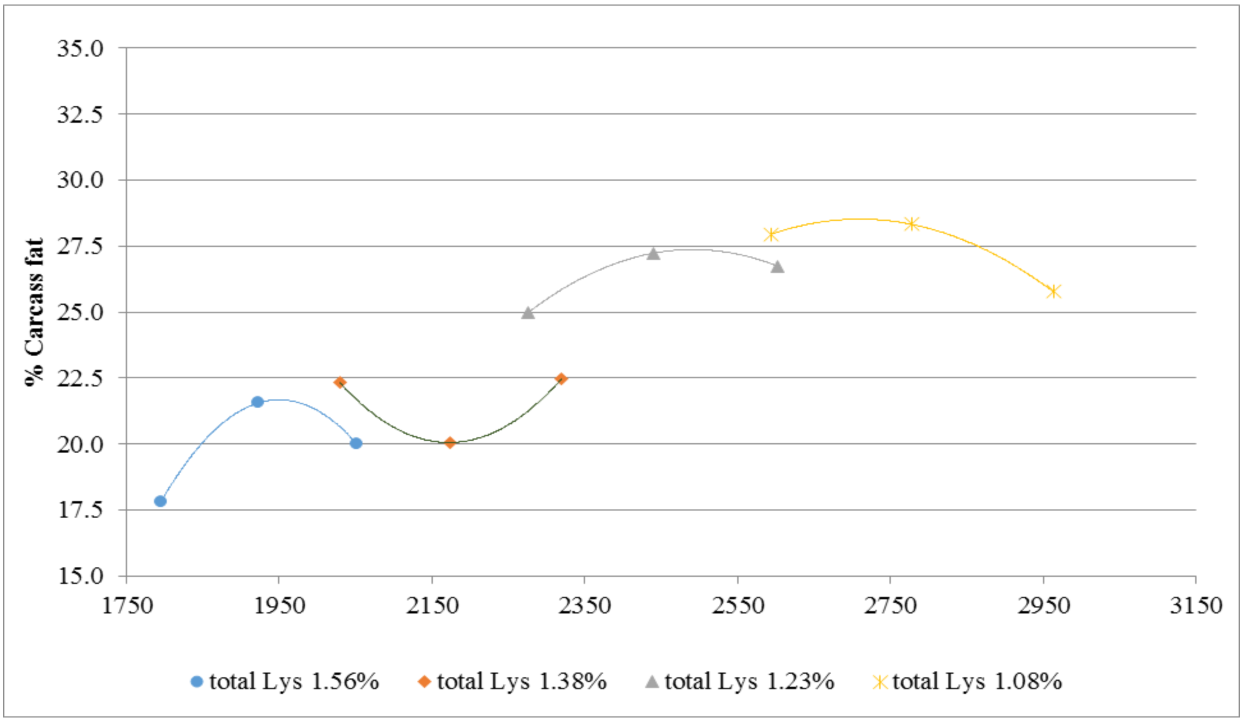

Figure 4.11: The effect of feeding 14 to 28 days of age mixed sex broilers varying amounts of dietary energy: total Lys ratios on carcass fat (%)

Table 4.13 presents the effect of varying total Lys (%) on broilers from 14 to 28 days of age carcass protein (%) and fat (%) composition. A general trend was observed that a decrease in the total Lys ratio resulted in lower carcass protein (%) and a higher carcass fat (%). There was a significant difference in carcass (%) and fat (%) between the two highest total Lys levels (1.56 and 1.38%) and the two lowest Lys levels (1.23 and 1.08%).

Table 4.13: The effects of feeding 14 to 28-day old mixed sex broilers varying total Lys (%) on the protein and fat composition of broiler carcass

total Lys (%)	Protein (%)	Fat (%)
1.56	58.0 ^{a}	19.9 ^{b}
1.38	57.8 ^{a}	21.6 ^{b}
1.23	51.5 ^{b}	26.3 ^{a}
1.08	49.9 ^{b}	27.4 ^{a}
SEM	1.01	1.29
$p > F$ total Lys quadratic	0.0001	0.0001

$^{a\text{-}d}$ Means in the same column with no common superscript differ significantly ($p \leq 0.05$)

The statistical analysis of the quadratic response of broilers to varying total Lys for carcass protein (%) and fat (%) is described in the equations below:

$$\text{Carcass protein (\%)} = -7.843872 + 76.457735 \times \text{total Lys} + -21.78503 \times \text{total Lys}^2 \quad (R^2 = 0.54) \; [4.29]$$

$$\text{Carcass fat (\%)} = 44.950369 + -15.11784 \times \text{total Lys} + -0.758366 \times \text{total Lys}^2 \quad (R^2 = 0.56) \; [4.30]$$

Table 4.14 illustrates the effect of varying energy (ME) levels on broiler (14–28 days of age) body carcass protein (%) and fat (%). The data indicated that varying energy levels did not have a significant effect of broiler carcass protein and fat (%) content.

Table 4.14: The effects of feeding 14 to 28-day old mixed sex broilers varying ME levels and the composition of broiler carcass protein and fat

ME (kcal/kg)	Protein (%)	Fat (%)
2800	55.9 [a]	23.3 [a]
3000	53.8 [a]	24.3 [a]
3200	53.2 [a]	23.8 [a]
SEM	1.35	1.22
p > F		
ME Quadratic	0.36	0.84

[a-d] Means in the same column with no common superscript differ significantly ($p \leq 0.05$)

The statistical analysis of the quadratic response of broilers to varying energy (ME) for carcass protein (%) and carcass fat (%) is illustrated in the equations below:

Carcass protein (%) = 233.06667 + -0.112875 x ME + 0.0000177 x ME ^{2} ($R^2 = 0.06$) [4.31]

Carcass fat (%) = -158.3167 + 0.1205625 x ME + -0.0000199 x ME ^{2} ($R^2 = 0.01$) [4.32]

4.8 Discussion

There is considerable literature describing the response of 14 to 28 days' broilers to varying amounts of Lys. However, majority of literature reported on broiler response to amino acid and energy requirements report only the response of broilers to energy or Lys and not the interaction between these two nutrients (Han *et al.*, 1991; Dozier *et al.*, 2009). Moreover, the authors did not report what the effect was of the treatment with amino acids or energy on broiler carcass composition. Dozier *et al.* (2009) determined that, for Ross x Ross TP 16 females fed a diet containing 3126 kcal/kg, the areas of maximum response to total Lys for ADG were 1.23% and 1.24% for FCR (ME: total Lys ratio for ADG, 2541; for FCR, 2520). They concluded that Ross x Ross TP 16 males had a higher requirement and that the point of maximum response to total Lys for ADG were 1.36% and 1.44% for FCR (ME: total Lys ratio for ADG, 2298; for FCR, 2170). A trial conducted by Lemme *et al.* (2003) observed that, for Ross male 208 fed a diet from 14 to 35 days containing 2850 kcal ME/kg, the point of maximum response to total Lys for

ADG were 1.34% and 1.41% for FCR (ME: total Lys ratio for ADG, 2126; for FCR, 2021). For a diet containing 3000 kcal ME/kg, the point of maximum response to total Lys for ADG were 1.48% and 1.48% for FCR (ME: total Lys ratio for ADG an FCR, 2027), while for a diet containing 3150 kcal ME/kg the point of maximum response to total Lys for ADG were 1.41% and 1.41% for FCR (ME: total Lys ratio for ADG an FCR, 2234). Interestingly for the birds fed diets containing the 3150 kcal ME/kg, the ADG response was, on average, lower than that of birds fed the 2850 kcal ME/kg diet. The level of response on the 3000 kcal ME/kg diet was, on average, the highest.

Both the ADFI and FCR on the 3150 kcal ME/kg diet were the lowest with the 3000 and 2850 kcal ME/kg diet consuming, as expected, more feed and demonstrating a higher FCR at each level of dietary increase in energy. A follow up trial conducted by Lemme *et al.* (2006) established that, for Ross male 308 from 14 to 28 days of age fed a diet containing 3000 kcal ME/kg, the point of maximum response to total Lys for ADG were 1.30% and 1.41% for FCR (ME: total Lys ratio for ADG, 2307; for FCR, 2112). In addition, for Ross male 308 from 14 to 35 days fed a diet containing 3000 kcal ME/kg, the point of maximum response to total Lys for ADG were 1.30% and 1.46% for FCR (ME: total Lys ratio for ADG, 2307; for FCR, 2054).

The results from this Experiment are both in agreement with the findings of the trials conducted by Lemme *et al.* (2003, 2006) and Dozier *et al.* (2009) and according to the quadratic regression for total Lys, the point of maximum response for ADG was achieved at 1.37%, while that of FCR was achieved at 1.34%. However, the highest ADG was achieved with the lowest test energy level, 2800 kcal ME/kg (ME: total Lys 2029) while the lowest FCR was achieved by the broilers fed the highest total Lys level and highest test energy level (total Lys 1.56%; 3200 kcal ME/kg; ME: total Lys 2051). Average daily feed intake was influenced significantly by the energy level only and did not respond to a change in total Lys treatment. Despite the fact that the pelleted feed produced at the highest energy levels (3200 kcal ME/kg) had significantly lower pellet durability, no interaction with ADG was observed. Breast meat (%) was significantly higher and abdominal fat percentage significantly lower at the highest test level of total Lys of 1.56%. Energy levels had no significant effect on broiler carcass parameters. It could also be observed that a decrease in the total Lys ratio resulted in lower carcass protein (%) and a higher carcass fat (%). The results indicated that varying energy levels had a non-significant effect of broiler carcass protein and fat (%) composition. It would thus appear that the response of broilers carcass breast meat (%), abdominal fat (%) as well as that carcass protein (%) and fat (%) due to varying ME: total Lys is influenced more by the total Lys (%) level and to a lesser extent due to the energy level in the feed.

Research has indicated that mortality increases when the energy content of the diet is increased (Dale and Villacres, 1988). However, as has been the case in certain other studies (Hidalgo *et al.,* 2004; Brickett *et al.,* 2007), this finding was also not observed in this study.

4.9 Conclusion

The ADG of broilers increased as total Lys increased from 1.08% to 1.38%, but was significantly reduced at the highest level of 1.56%. The quadratic regression revealed that the point of maximum response for ADG was achieved at 1.37%, while 1.34% was required for FCR. There was no significant difference in ADFI between total Lys treatments nor was there any significant difference in ADG between energy treatments. Average daily feed intake was significantly lower and FCR significantly higher when the energy was reduced from 3000 to 2800 kcal ME/kg. For ADG, the point of maximum response were 1.38% total Lys, 2800 kcal

ME/kg, implying a ME: total Lys ratio of 2029. For FCR, the point of maximum response were 1.56% total Lys, 3200 kcal ME/kg, implying a ME: total Lys ratio of 2051.

It could also be observed that a decrease in the total Lys ratio resulted in lower carcass protein (%) and a higher carcass fat (%). In contrast to total Lys, energy levels had no significant effect of broiler carcass parameters. Additionally, there appeared to be a general trend that a decrease in the ME: total Lys ratio resulted in a higher carcass protein (%) composition and a lower fat (%) composition. It would thus appear that the response of broilers carcass breast meat (%), abdominal fat (%) as well as that carcass protein (%) and fat (%) due to varying ME: total Lys is influenced more by the total Lys (%) level and to a lesser extent due to the energy level in the feed.

The statistical evaluation of broilers (14-28 days of age) ADG, ADFI and FCR revealed a quadratic response to varying levels of ME: total Lys ratio: ADG = 33.606973 + 0.0302445 x ME: total Lys + -0.00000684 x ME: total Lys ^{2} (R^2 = 0.43); FCR = 4.043189 + -0.002338 x ME: total Lys + 0.00000049807 x ME: total Lys ^{2} (R^2 = 0.31); ADFI = 221.00755 + -0.112043 x ME: total Lys + 0.0000233 x ME: total Lys ^{2} (R^2 = 0.22)

Chapter 5:

The response of mixed sex broilers to varying dietary energy: total Lys ratios fed from 28 days to 35 days of age

5.1 Abstract

This experiment was conducted to study the response of broilers to varying energy (ME) and total Lys levels and also the broilers' response to the interaction between these two nutrients. Five hundred seventy-six Ross 308 mixed male and female broilers were fed one of twelve rations. The dietary treatments consisted of four total Lys levels (1.42, 1.25, 1.11 and 0.96%) and three ME levels (2800, 3050 and 3300 kcal ME/kg). The use of the dietary summit/dilution technique, the combination of total Lys and energy levels, resulted in various ME: total Lys levels (1972, 2148, 2324, 2240, 2440, 2640, 2523, 2748, 2973, 2917, 3177 and 3438). Each one of the 12 treatments was replicated eight times. The birds were fed the trial diets from day 28 to 35 days. The ADG was not significantly different between the four total Lys levels. The quadratic regression revealed that the point of maximum response for FCR was 3250 kcal ME/kg while ADFI was significantly lower with higher total Lys treatments. FCR were significantly improved when the total Lys was increased from 1.11 to 1.25% and significantly improved further when the total Lys was increased to 1.42%. Increasing the energy from 2800 to 3300 kcal ME/kg resulted in significantly lower ADG, ADFI and FCR. The pellet durability was significantly reduced when the energy was increased from 2800 to 3300 kcal ME/kg. This was probably responsible for the significantly lower ADG, ADFI and higher FCR at 3300 kcal ME/kg compared to the 2800 kcal ME/kg treatments. For ADG, the point of maximum response were 1.42% total Lys, 2800 kcal/kg, implying a ME: total Lys ratio of 1972. For FCR, the point of maximum response were 1.42% total Lys, 3050 kcal ME/kg, implying a ME: total Lys ratio of 2148.

Lower ratios of ME: total Lys tended to increase the breast percentage of broilers and decrease the abdominal fat percentage. Breast meat percentage was significantly higher at the highest test level of total Lys of 1.42% compared to the lowest total Lys of 0.96%. Energy level did not have a significant effect on broiler carcass parameters. Considering all the carcass data observations in the response of broilers fed varying ME: total Lys levels, it would appear that the total Lys levels have a more pronounced effect on the body carcass composition compared to the energy level of the diet. The statistical analysis of the response of the broilers (28–35 days) to total Lys for ADG, ADFI and FCR revealed a quadratic response to varying levels of ME: total Lys ratio: ADG = 136.87248 + -0.045396 x ME: total Lys ratio + 0.0000077407 x ME: total Lys ratio^2 (R^2 = 0.30); FCR = 0.1641301 + 0.0011762 x ME: total Lys ratio + -0.0000001848 x ME: total Lys ratio^2 (R^2 = 0.37); ADFI = 127.57202 + 0.003209 x ME: total Lys ratio + 0.00000059861 x ME: total Lys ratio^2 (R^2 = 0.07)

5.2 Introduction

There has been a notable increase in the growth rate and feed efficiency in commercial broiler chickens in the last 20 years. It is widely recognised that feed constitutes the most significant cost in broiler production. Most production cost estimates range from a feed cost of 60 to 80% (Durunna *et al.*, 2005). Based on the research conducted by Classen (2013), it would appear there are two schools of thought on the energy to protein or energy: Lys ratio. The research into these two opposing views of thought is based on either the view that the modern broiler eats to meet its energy requirement (Leeson *et al.,* 1996; Lemme *et al.,* 2005 and Dozier *et al.*, 2006), or the opposite view, namely, that the modern broiler is unable to adjust its FI owing to different energy levels in its diet (Plumstead *et al.,* 2007; Latshaw, 2008; Delezie *et al.,* 2010; Li, *et al.,* 2011, Gous, 2013).

The challenge for commercial nutritionists lies in the latter school of thought, as precise knowledge of the FI of a given animal of a given food when housed in a given environment makes it possible to define the optimum economic levels of energy and essential nutrients in the feed for broilers (Gous, 2013). In this context, the decision regarding the optimum dietary level of ME appears to be merely a function of the price of the energy sources and the premium achieved for the improved FCR (Aftab, 2009). In order to ensure the maximum utilisation of energy, Lys and every nutrient in the diet, the correct proportions of these nutrients are necessary to ensure the optimum growth of the birds and to minimise a surplus of vital dietary components.

According to Gous (2013), chickens attempt to control their FI to enable them to achieve a particular level of fatness. This fatness level is generally lower than that which is observed in many commercial operations while it also differs between strains, sexes and degrees of maturity (Gous *et al.*, 1990). The fact that this goal is not often achieved provides adequate proof that birds do not 'eat to satisfy an energy requirement', and that FI regulation is more complex than this statement suggests. The complexity of the process of FI regulation is evidenced by the fact that birds that are fat will always attempt to reduce the excessive amounts of lipid. They accomplish this by making use of these excessive amounts as an energy source when this is possible (Gous *et al.*, 2010). They do this because they always attempt to maintain an inherent ratio between body lipid and body protein. They fail to maintain this ratio when imbalanced feeds are offered but are able to correct the ratio if given the opportunity to do so. It appears that strain differences exist in the extent to which broilers utilise their body lipid reserves as an energy source, although this may be accounted for when simulating FI and growth by assuming a higher desired lipid to protein ratio in genetically fat strains (Gous *et al.*, 2010).

Further evidence that birds and animals attempt to maintain a genetically determined level of fatness, and that they do this by utilising body lipid reserves as an energy source when appropriate, was provided by Kyriazakis and Emmans (1992). The researchers showed that when pigs are made fat by feeding them a low protein food and the pigs are then given a choice between foods of low and high protein contents, they will select a diet of the composition of which will correct the effects of previous misfeeding.

The aim of this experiment was to obtain equations that could relate the body weight gain and feed conversion of broilers to varying total Lys and energy levels. It was anticipated that these equations would enable commercial nutritionists to determine the most economical point for the production of broilers during the 28 to 35-day period.

5.3 Material and methods

A total of 288 Ross 308 male and 288 Ross 308 female broilers were sexed at the hatchery and reared separately on a commercial starter and grower feed. The commercial starter feed (2900 kcal ME/kg, 220 g CP/kg) was offered from 0 to 14 days old and the grower feed (3150 kcal ME/kg, 210 g CP/kg) from 14 to 28 days. Both were fed on an *ad libitum* basis to the broilers. At 28 days of age, male and female birds were randomly allocated to one of the 12 dietary treatments. The broilers were placed in a brooding facility with battery cages of 6 birds per pen, 3 males and 3 females. A total of 96 cages were used. The cages were 0.8 m long x 0.5 m wide x 0.42 m height, giving a total floor area of 0.4 m^2. The stocking density for the trial was 15 birds/ m^2. A line feeder was available through the front side of the cage (0.8 m long) and water provided by nipple drinkers (2 nipples per cage). Feed and water were available on an *ad-libitum* basis. The temperatures were set to 21°C from 21 days to 28 days. The light intensity was set to 30 lux (14 hr light and 10 hr darkness).

5.4 Experimental diets

Dietary treatments consisted of a 3 x 4 factorial arrangement. Twelve diets were formulated to three levels of metabolisable energy (2800, 3050 and 3300 kcal ME/kg) and four total Lys AA (1.42, 1.25, 1.11 and 0.96 %) in order to achieve a combination of energy: Lys ratios (1972, 2148, 2324, 2240, 2440, 2640, 2523, 2748, 2973, 2917, 3177 and 3438). It is important to note that the same ME: total lysine ratio can be achieved with various combinations of energy and lysine. For examples a diet with 3000 kcal ME/kg and 1.50% total Lys will provide a ratio of 2000. However, the ratio of 2000 ME: total Lys can also be achieved with 2900 kcal ME/kg and 1.45% total Lys. Prior to the diet formulation, the total AA of all the ingredients was calculated as a percentage of the analysed CP content using standard procedure (CVB, 2001). Due to different digestible co-efficient reported in literature (GfE, 1999, CVB, 2001 and Rostagno, 2005) and in order to try to make the study as applicable as possible, it was decided to rather formulate the feed on a total lysine basis and not on a digestible basis. The metabolisable energy (ME) content of the raw materials was calculated using the WPSA (1989) procedure. An inert filler (sand) was required in the diet formulation to allow the application of these principles across a wide range of ME and Lys. In order to reduce the variation between the dietary treatments that could originate as a result of the batching and mixing of ingredients, and to ensure uniform gradations of dietary total Lys within each ME level, a summit/dilution blending technique (Gous and Morris, 1983) was applied during the mixing of the diets. This technique consisted of first formulating and mixing basal diets 1, 4, 7, 10 and summit diets 3, 6, 9, 12. The four intermediate diets were then derived by blending the respective summit and dilution diets within each level of ME in the proportions depicted in Tables 5.1 and 5.2. Following the mixing and blending procedure all the diets were analysed for proximate and amino acid composition. The diets were pelleted in a pellet mill (Simon-Brown California Hyflo 67) equipped with a 3 mm die.

5.4.1 Experimental diets

Table 5.1 The raw material composition of the basal dilution and summit diet composition fed to mixed sex broilers from 28 days to 35 days of age

	Basal Diet 1	Summit Diet 3	Basal Diet 4	Summit Diet 6	Basal Diet 7	Summit Diet 9	Basal Diet 10	Summit Diet 12
Ingredients	----------------------------------- Percentage (%) ---							
Maize	44.7	46.4	49.5	52.2	53.4	56.7	57.5	61.9
Soy bean oilcake	26.3	24.9	21.4	19.0	17.5	13.9	13.3	9.5
Sunflower oilcake	8.00	5.63	8.00	6.76	8.00	8.00	8.00	8.00
Corn gluten meal	5.95	5.97	4.62	4.45	3.55	3.48	2.40	2.49
Soy oil	4.00	5.59	4.00	5.29	4.00	5.05	4.0	4.65
Full fat soya	0.00	4.00	0.00	4.00	0.00	4.00	0.00	4.00
L-lysine	0.42	0.39	0.39	0.38	0.37	0.37	0.34	0.35
DL-methionine	0.22	0.22	0.19	0.19	0.16	0.16	0.13	0.12
L-threonine	0.06	0.06	0.06	0.05	0.05	0.05	0.05	0.05
MCP[1]	1.36	1.36	1.46	1.46	1.54	1.54	1.63	1.63
Limestone	1.39	1.38	1.37	1.37	1.35	1.35	1.34	1.34
Salt	0.43	0.43	0.43	0.42	0.42	0.42	0.42	0.42
Sodium Bicarb.	0.03	0.03	0.03	0.03	0.04	0.04	0.04	0.05
Choline (60%)	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Pre-mix[2]	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Filler (sand)	7.05	3.53	8.44	4.22	9.55	4.77	10.8	5.40

[1]MCP = Monocalcium phosphate

[2] Premix provided the following (per kg diet): Vitamin A, 12000 IA; vitamin D_3, 4000 ICU; vitamin E, 55.0 mg; vitamin B_{12}, 20.0 ug; riboflavin, 9.0 mg; niacin, 60.0 mg; d-pantothenate, 15.0 mg; menadione (K_3), 2.1 mg; folic acid, 2.3 mg; thiamine, 2.3 mg; pyridoxine, 6.0 mg; d-biotin, 180.0 ug; selenium (as Na_2SeO_3), 0.30; Manganese, 110 mg; zinc, 100 mg; iron, 50 mg; copper, 15 mg; iodine, 2.0 mg; cobalt, 0.5 g

5.4.2 Pellet hardness and quality index

A representative sample from each one of the twelve treatments was subjected to a feed texture quality test. The pellet durability index (PDI) was measured by weighing 500 grams of the diet pellets and then placing the sample in a rolling drum (300 x 300 x 125 mm) (ASAE Standards, 1987). The rolling drum was rotated at 50 revolutions per minute. After 10 minutes, the pellets and fines were removed from the drum and sieved in a 2.36 mm and 1.4 mm screen.

The PDI, expressed as a percentage, was then calculated by using the equation below:

$$\text{PDI (\%)} = \frac{\text{Mass of pellets after rotation}}{\text{Mass of pellets before rotation}}$$

Table 5.2 The nutrient composition of the basal dilution and summit diet fed to mixed sex broilers from 28 to 35 days of age

	Basal Diet 1	Summit Diet 3	Basal Diet 4	Summit Diet 6	Basal Diet 7	Summit Diet 9	Basal Diet 10	Summit Diet 12
Calculated nutrients[1]								
Moisture (%)	9.06	9.27	9.00	9.35	8.95	9.39	8.89	9.52
ME [2], kcal/g	2800	3300	2800	3300	2800	3300	2800	3300
CP, %	22.8	23.1	20.0	20.2	17.8	18.0	15.4	15.7
Lys [3], %	1.42	1.42	1.25	1.25	1.11	1.11	0.96	0.96
Met [3], %	0.63	0.63	0.55	0.55	0.48	0.48	0.41	0.40
Met & Cys [3], %	1.02	1.03	0.90	0.90	0.80	0.80	0.69	0.70
Thr [3], %	0.94	0.9	0.820	0.84	0.73	0.75	0.63	0.64
Val [3], %	1.08	1.10	0.95	0.97	0.84	0.86	0.73	0.75
Arg [3], %	0.91	0.91	0.80	0.80	0.71	0.71	0.61	0.62
SID. Lys [4], %	1.28	1.30	1.13	1.15	1.01	1.03	0.87	0.89
SID. SAA [4], %	0.91	0.91	0.81	0.81	0.71	0.71	0.62	0.62
SID. Thr [4], %	0.79	0.81	0.70	0.72	0.62	0.64	0.54	0.56
SID. Val [4], %	0.95	0.97	0.84	0.86	0.75	0.77	0.65	0.68
SID. Arg [4], %	1.30	1.32	1.14	1.16	1.01	1.03	0.87	0.89
Ca, %	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Avl. P %	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37
Na, %	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Cl, %	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
DEB[5], Meq/kg	152	158	134	137	121	118	106	103
CP:ME	51.3	59.7	58.4	68.4	65.8	76.7	76.1	87.7
ME: total Lys	1972	2324	2240	2640	2523	2946	2917	3438
Analysed nutrient composition								
CP, %	22.9	23.1	20.4	20.3	17.8	17.9	15.3	15.8
Lys, %	1.34	1.37	1.22	1.21	1.06	1.09	0.94	0.92

[1]Nutrient composition calculated from proximate analyses of all ingredients prior to formulation

[2]Energy content of ingredients calculated as per WPSA 3rd Ed, 1989

[3]Total amino acid content of ingredients calculated as a percentage of the analysed CP content

[4]Calculated from standardized ileal digestibility coefficients applied to the calculated amino acid content (Evonik, 2014)

[5]DEB = Dietary electrolyte balance (Na + K – Cl)

Ten pellets per treatments were subjected to a pellet hardness test, as prescribed by Payne *et al.* (1987). During the test, a pellet is held between the jaws of manual Kahl pellet hardness tester. Pressure is exerted by screwing down by hand onto a coil spring. The pointer indicates the load in kilogrammes at which the pellets bursts. The data obtained from the pellet hardness test was used to test for statistical significance between the treatments.

5.5 Data collection

Broilers were weighed on day 28 and on day 35. The FI was calculated as the difference between the feed allocated at the beginning of the trial and the amount left over at the end of the trial. The average daily gain (ADG) and feed conversion ratio (FCR) could, therefore, be calculated. Mortality (%) was recorded on a daily basis. However, FCR was not corrected for the mortality.

5.6 Carcass evaluation

At 35 days, three birds per treatment were randomly selected and slaughtered according to the technique described by Lourdes *et al.* (2010). Birds were humanely slaughtered by electrical stunning, followed by cutting of the blood vessels (carotid artery and jugular vein). After evisceration, carcasses were packed, identified, and chilled to 0 °C. Cuts were deboned 24 hours *post mortem*, packed, identified, frozen, and stored at -18 °C. Meat physical chemical analyses were performed after cuts were thawed at 4 °C for 24h. Whole carcass (without abdominal fat) and abdominal fat were weighed. Carcass, abdominal fat, and total breast meat (*pectoralis major* and *minor* muscles) yields were expressed as a percentage of 35 day carcass weight of selected broilers. Additionally, proximate analysis was conducted on minced samples of all the whole carcasses. The protein content was determined according to standard techniques of the AOAC (1997), and the lipid by means of solvent (chloroform/methanol) extraction (Lee *et al*., 1996).

5.7 Statistical analysis

JMP® of SAS version 12.0.1 (SAS institute Inc. JMP Software, Cary, NC, 2015) was used to test for normality and the data was then analysed using Proc GLM (General Linear Models Procedure) as a 3 energy x 4 lysine factorial arrangement ($p \leq 0.05$). The mean differences were separated using Tukey's honest significant differences test (Tukey, 1991). For the purposes of this study, eight replications were made with 48 birds per replication. Of the 6 birds per replication, 3 birds were male and 3 birds were females The linear and quadratic regressions of JMP ® of SAS version 12.0.1 (SAS institute, 2015) were used to observe the treatment effect on weight, ADG, FI and FCR.

$$Y = b0 + b1 E \quad [5.1]$$

$$Y = b0 + b1E + b2 E^2 \quad [5.2]$$

Where Y predicted response, E = energy contentment of the diet, b0 = intercept and b1 and b2 = regression coefficient.

5.8 Results

5.8.1 Feed texture: pellet hardness and pellet quality index

The effect of varying ME: total Lys, total Lys and ME on pellet hardness and pellet quality is indicated in table 5.3, 5.4 and 5.5. In this experiment a significant interaction ($p < 0.05$) was observed between varying energy (ME) levels and the pellet durability index. This was evident from the varying energy levels (Table 5.5). It was, therefore, evident from this evaluation that an increase in energy (ME) above 3050 kcal ME/kg negatively affected pellet durability. There was no interaction (Table 5.4) between total Lys (%) and pellet durability and pellet hardness, no interaction between ME: total Lys and pellet hardness and also not between ME and pellet hardness.

Table 5.3: The effect of varying total Lys and energy levels (ME) in diets and the pellet durability index (PDI) and pellet hardness

Treatment	Total Lys (%)	ME (kcal/kg)	ME: total Lys	PDI (%)	Pellet hardness (kg)
1	1.42	2800	1972	96.8	1
2	1.42	3050	2148	92.3	1
3	1.42	3300	2324	76.5	1
4	1.25	2800	2240	91.7	1
5	1.25	3050	2440	93.9	1
6	1.25	3300	2640	78.9	1
7	1.11	2800	2523	97.3	1
8	1.11	3050	2748	88.4	1
9	1.11	3300	2973	72.9	1
10	0.96	2800	2917	97.1	2
11	0.96	3050	3177	85.7	2
12	0.96	3300	3438	72.3	1
SEM				9.11	
$p > F$					
PDI (%) quadratic				0.34	
SEM					0.37
$p > F$					
Pellet Hardness					0.89

[a-d] Means in the same column with no common superscript differ significantly ($p \leq 0.05$)

Table 5.4: Varying total Lys (%) levels in diets and the effect on the pellet durability index (PDI) and pellet hardness

	Total Lys (%)	PDI (%)	Pellet hardness (kg)
	1.42	88.5	1.40
	1.25	88.2	0.70
	1.11	86.2	0.70
	0.96	85.0	0.60
SEM		6.41	-
$p > F$			
PDI (%) quadratic		0.97	-
SEM		-	0.21
$p > F$			
Hardness quadratic		-	0.07

[a-d] Means in the same column with no common superscript differ significantly ($p \leq 0.05$)

Table 5.5: Varying energy (ME) levels in diets and the effect on the pellet durability index (PDI) and pellet hardness

ME (kcal/kg)	PDI (%)		Pellet hardness (kg)
2800	95.7 [a]		1.20
3050	90.1 [a]		0.80
3300	75.2 [b]		0.50
SEM	1.609		-
$p > F$			
PDI (%) quadratic	0.0001		-
SEM		-	0.22
$p > F$			
Hardness quadratic		-	0.16

[a-d] Means in the same column with no common superscript differ significantly ($p \leq 0.05$)

5.8.2 Animal performance

5.8.2.1 The effect of feeding varying energy (ME): total Lys ratios to mixed sex broilers from 28 to 35 days of age

Table 5.6 and Figures 5.1 (ADG), 5.2 (ADFI) and 5.3 (FCR) present the responses of broilers (28–35 days of age) to varying levels of ME and total Lys for ADG, ADFI and FCR. The ADG tended to decrease with an increase in the ME: total Lys ratio at each level of amino acid as well as from the lowest (1972) to the highest ME: total Lys ratio (3438).

However, apart from the difference between treatment 1 lowest ratio (ME: total Lys ratio, 1972) consisting of the highest total Lys (1.42%) and lowest energy level (2800 kcal/kg), and treatment 7 (total Lys 1.11%; 2800 kcal ME/kg; ME: total Lys ratio 2523) and treatment 9 (total Lys 1.11%; 3300 kcal/kg; ME: total Lys ratio 2973), no other significant differences in ADG were observed.

Average daily feed intake also tended to decrease at each one of the amino acid levels although there was no consistent increase or decrease in ADFI as the ratio increased from the lowest level of 1972 to the highest level of 3438. The ADFI in treatment 10 with a ME: total Lys ratio of 2917 (total Lys 0.96%; ME 2800 kcal ME/kg) was significantly higher than that in all other treatments with the exception of treatment 11 with a ME: total Lys ratio of 3177 (total Lys 0.96%; ME 3050 kcal ME/kg). The ADFI of treatment 3 with a ME: total Lys ratio of 2324 (total Lys 1.42%; 3300 kcal ME/kg) was significantly less than that of treatment 10 with a ME: total Lys ratio of 2917 (total Lys 0.96%; ME 2800 kcal ME/kg) and treatment 11 with a ME: total Lys ratio of 3177 (total Lys 0.96; ME 3050 kcal ME/kg). Treatment 10 with a ME: total Lys ratio of 2917 (total Lys 0.96%; ME 2800 kcal ME/kg) differed significantly from all treatments from treatment 1 to treatment 9.

Feed conversion ratio were significantly decreased with an increase in the ME: total Lys. Treatment 7 with a ME: total Lys ratio of 2523 (total Lys 1.11%; ME 2800 kcal/kg) differed significantly from all the other treatments with the exception of treatment 10 (ME: total Lys ratio of 2917; total Lys 0.96%; ME 2800 kcal/kg) and treatment 11 (ME: total Lys ratio of 3177, total Lys 0.96%; ME 3050 kcal/kg). The lowest FCR was achieved by treatment 6 with a ME: total Lys ration of 2640 (total Lys 1.25%; ME 3300 kcal/kg). The statistical analysis revealed that there was not a significant interaction between treatment and mortality

Table 5.6: The effect on the performance of 28 to 35 days of age mixed flock broilers due to the feeding of varying ME: total Lys ratio's

Treat-ment	total Lys (%)	ME (kcal/kg)	Ratio (ME: tot Lys)	28 Day weight (g)	35 Day weight (g)	ADG (g)	ADFI (g)	FCR (g/g)	Mortality (%)
1	1.42	2800	1972	1411	1968	79.6 [a]	144.7 [bcd]	1.82 [d]	0.00
2	1.42	3050	2148	1383	1910	75.3 [ab]	132.9 [cde]	1.78 [d]	0.00
3	1.42	3300	2324	1429	1894	66.5 [b]	119.9 [e]	1.81 [d]	1.61
4	1.25	2800	2240	1409	1936	75.4 [ab]	143.4 [bcd]	1.91 [cd]	0.00
5	1.25	3050	2440	1396	1922	75.2 [ab]	137.8 [bcd]	1.84 [d]	1.61
6	1.25	3300	2640	1396	1899	71.9 [ab]	131.3 [de]	1.83 [d]	1.61
7	1.11	2800	2523	1417	1882	66.5 [b]	146.1 [bc]	2.21 [a]	0.00
8	1.11	3050	2748	1386	1894	73.3 [ab]	144.3 [bcd]	1.97 [bcd]	1.61
9	1.11	3300	2973	1372	1859	68.1 [b]	132.8 [cde]	1.95 [bcd]	0.00
10	0.96	2800	2917	1421	1945	74.9 [ab]	161.3 [a]	2.15 [ab]	0.00
11	0.96	3050	3177	1408	1904	71.1 [ab]	148.8 [ab]	2.10 [abc]	0.00
12	0.96	3300	3438	1422	1919	71.0 [ab]	139.8 [bcd]	1.98 [bcd]	0.00
SEM				0.80	26.6	2.30	2.90	0.47	0.13
$p > F$									
Ratio Quadratic				21.3	0.32	0.0019	0.0001	0.0001	0.23

[a-d] Means in the same column with no common superscript differ significantly ($P \leq 0.05$)

The statistical analysis of the response of the broilers (28–35 days) to total Lys for ADG, ADFI and FCR revealed a quadratic response to varying levels of ME: total Lys ratio:

ADG = 136.87248 + -0.045396 x ME: total Lys ratio + 0.0000077407 x ME: total Lys ratio^2

(R^2 = 0.30) [5.3]

FCR = 0.1641301 + 0.0011762 x ME: total Lys ratio + -0.0000001848 x ME: total Lys ratio^2

(R^2 = 0.37) [5.4]

ADFI = 127.57202 + 0.003209 x ME: total Lys ratio + 0.00000059861 x ME: total Lys ratio^2

(R^2 = 0.07) [5.5]

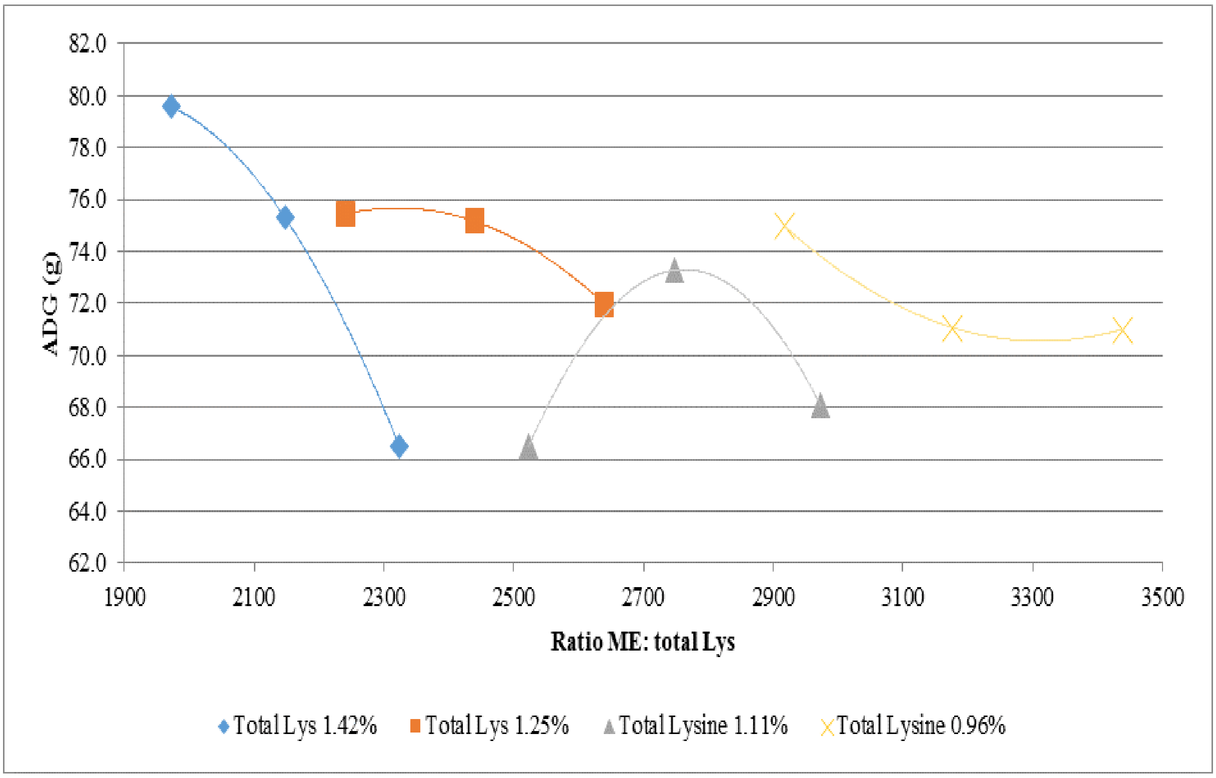

Figure 5.1: The effect of feeding 28 to 35 days of age mixed sex broilers varying amounts of dietary ME: total Lys ratios on Average Daily Gain (ADG)

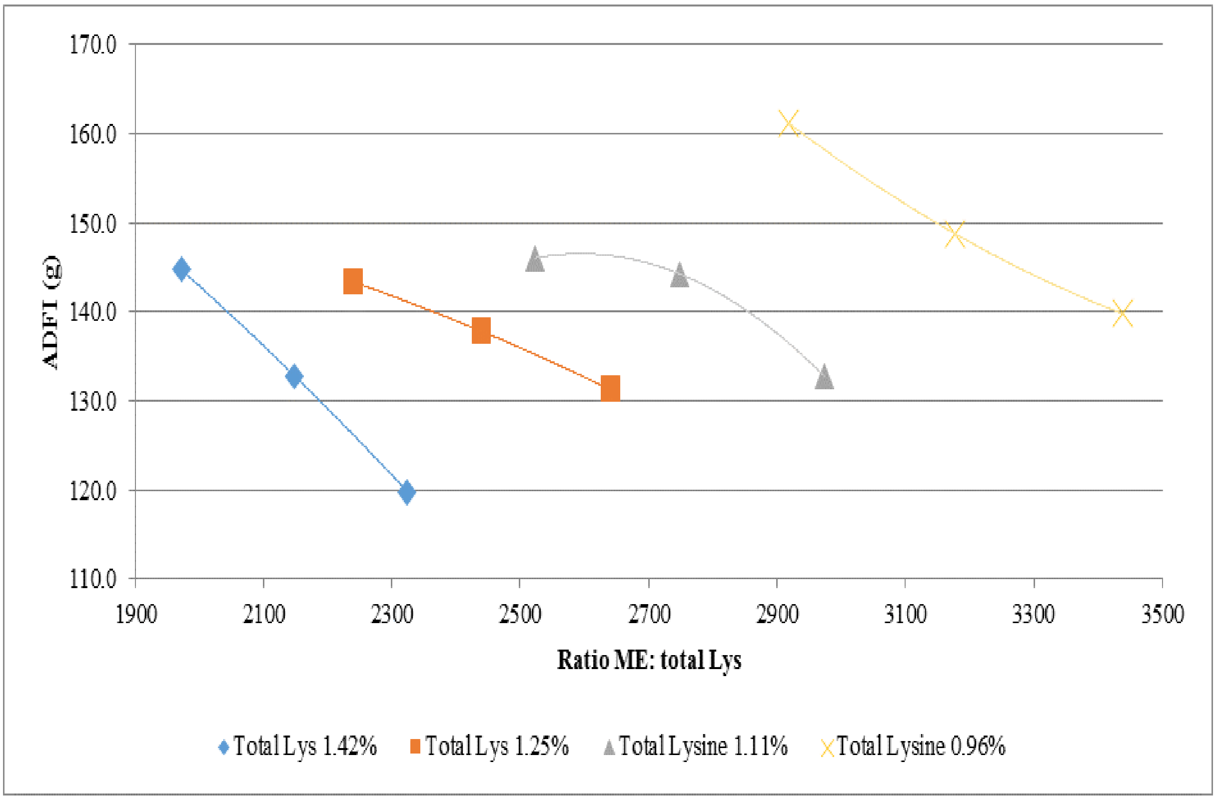

Figure 5.2: The effect of feeding 28 to 35 days of age mixed sex broilers varying amounts of dietary ME: total Lys ratios on Average Daily Feed Intake (ADFI)

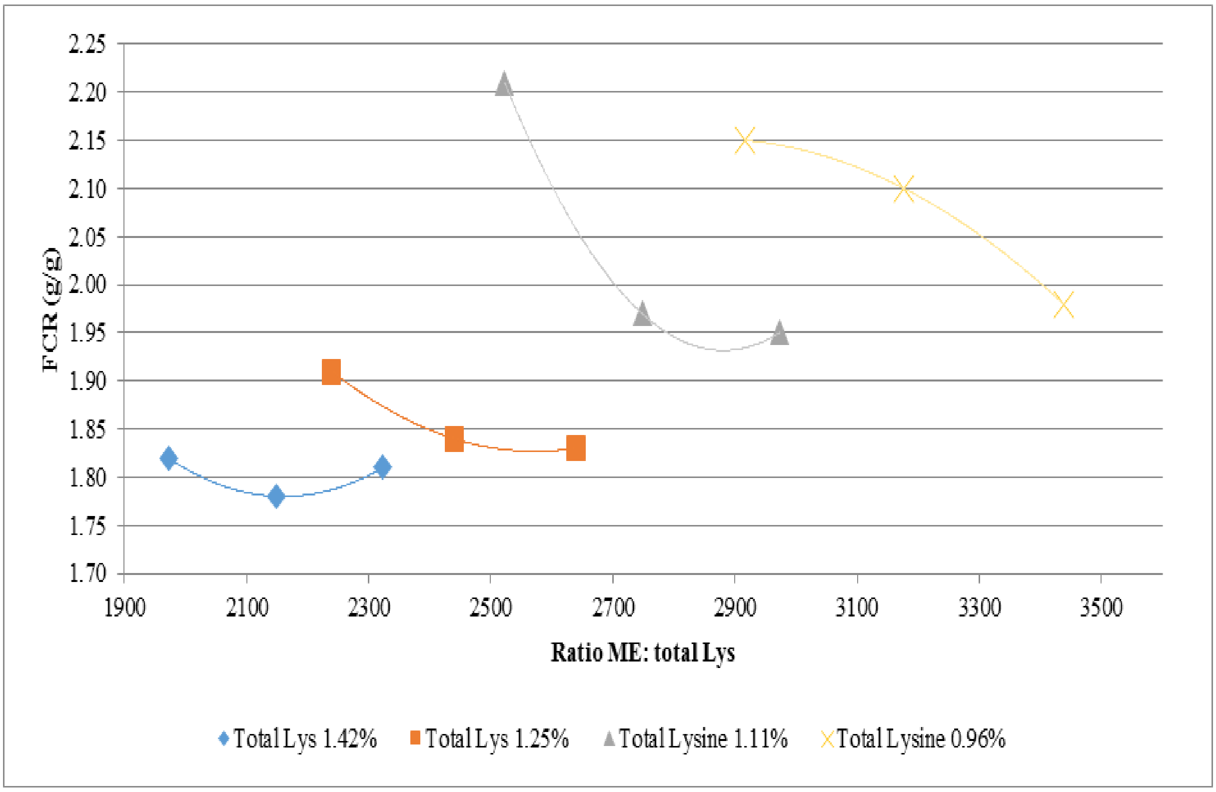

Figure 5.3: The effect of feeding 28 to 35 days of age mixed sex broilers varying amounts of dietary ME: total Lys ratios on Feed Conversion Ratio (FCR)

5.8.2.2 The effect of feeding varying total Lys *levels on mixed sex broilers from 28 to 35 days of age*

The broilers' responses (28–35 days) to varying total Lys levels are depicted in Table 5.7 and Figures 5.4 (ADG), 5.5 (ADFI) and 5.6 (FCR). There was not a significant difference in ADG between treatments. On the other hand, the FCR and ADFI were significantly increased when the total Lys was reduced from 1.42% to 1.11% and 1.49%. An increase in the Lys level did not have a significant effect on mortality (%).

The quadratic statistical analysis of the response of broilers (28-35 days) to total Lys for ADG, ADFI and FCR is indicated in the equations below:

ADG = 137.32145 + -0.045764 x total Lys + 0.0000078137 x total Lys ^{2} (R^2 = 0.09) [5.6]

$$FCR = 0.0946415 + 0.0012273 \times \text{total Lys} + -0.0000001946 \times \text{total Lys}^2 \quad (R^2 = 0.21) \quad [5.7]$$

$$ADFI = 126.46071 + 0.0039613 \times \text{total Lys} + 0.00000048712 \times \text{total Lys}^2 \quad (R^2 = 0.05) \quad [5.8]$$

According to the quadratic regression for total Lys, the point of maximum response for ADG was achieved at 1.27%. It was not possible to reach a point of maximum response for FCR, not even at the highest level of total Lys 1.42%.

Table 5.7: The effect on the performance of 28 to 35 days of age mixed flock broilers to the feeding of varying total Lys levels (2800, 3050 and 3300 ME kcal/kg)

total Lys (%)	28 Day weight (g)	35 Day weight (g)	ADG (g)	ADFI (g)	FCR (g/g)	Mortality (%)
1.42	1408	1924	73.8	132^{c}	1.80^{b}	3.10
1.25	1400	1919	74.2	138^{bc}	1.86^{b}	1.61
1.11	1392	1879	69.2	141^{b}	2.05^{a}	1.61
0.96	1417	1924	72.4	150^{a}	2.08^{a}	0.00
SEM	12.1	15.2	1.40	3.30	0.04	0.00
$p > F$						
tot. Lys quadratic	0.50	0.13	0.07	0.0001	0.0001	0.36

$^{a-d}$ Means in the same column with no common superscript differ significantly ($p \leq 0.05$)

5.8.2.3 The effect of feeding varying energy (ME) levels on mixed sex broilers from 28 to 35 days of age

The broilers' responses (28–35 days) to varying energy (ME) are depicted Table 5.8 and Figures 5.7 (ADG), 5.8 (ADI) and 5.9 (FCR). It was observed that an increase in the energy level by 250 kcal ME/kg from 2800 kcal ME/kg to 3050 kcal ME/kg did not have a significant effect on ADG. On the other hand, ADG, ADI and FCR were significantly reduced when the energy was further increased by another 250 kcal ME/kg to 3300 kcal ME/kg.

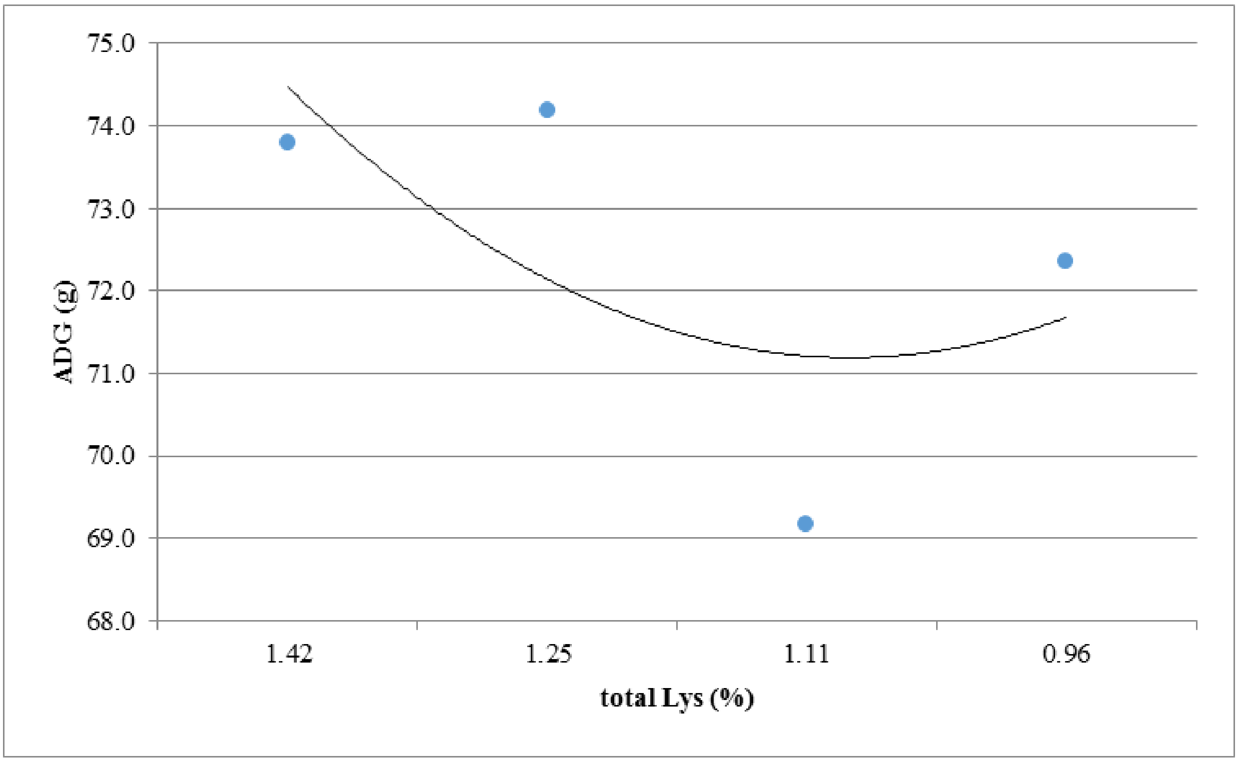

Figure 5.4 The effect of varying total Lys levels on Average Daily Gain (ADG) of broilers from 28 days to 35 days

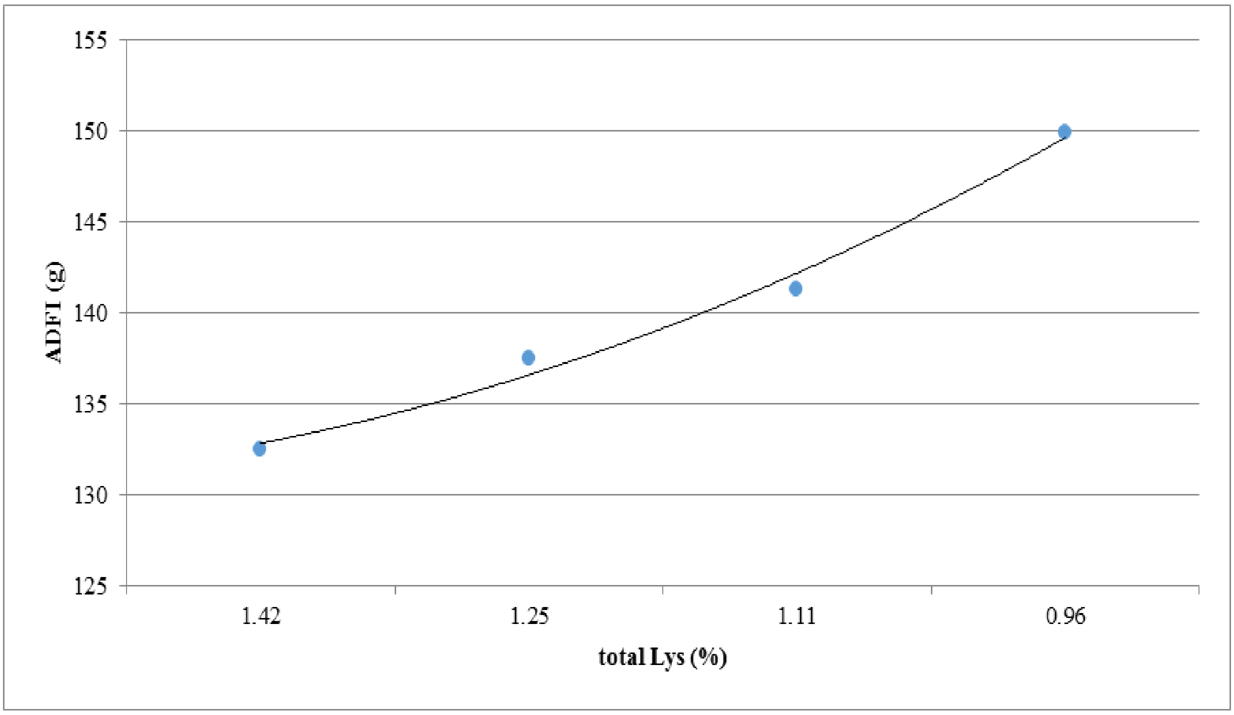

Figure 5.5 The effect of varying total Lys levels on Average Daily Feed Intake (ADFI) of broilers from 28 days to 35 days

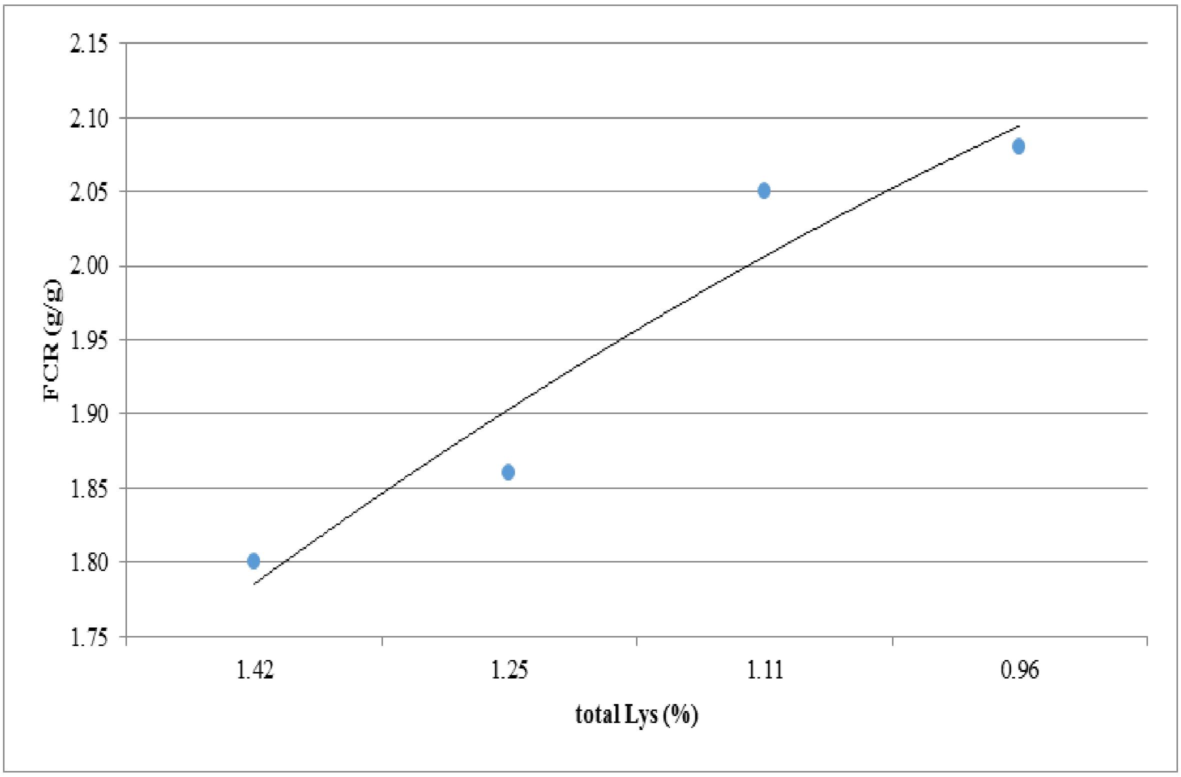

Figure 5.6: The effect of varying total Lys levels on Feed Conversion Ratio) FCR of broilers from 28 days to 35 days

According to the quadratic regression for energy (ME), the point of maximum response for FCR was achieved at 3250 kcal ME/kg. There was not significant interaction between ME level and mortality (%).

The quadratic statistical analysis of the response of broilers (28–35 days) to energy (ME) for ADG, ADI and FCR is indicated in the equations below:

ADG = -176.806 + 0.17362 x ME + -0.00003 x ME ^{2} (R^2 = 0.92) [5.9]

FCR = 6.444+ -0.0027 x ME + -0.0000004 x ME ^{2} (R^2 = 0.93) [5.10]

ADFI = 64.626 + 0.08608 x ME + -0.00002 x ME ^{2} (R^2 = 0.89) [5.11]

Table 5.8: The effect on the performance of 14 to 28 days of age mixed flock broilers due to the feeding of varying energy (ME) levels (0.96, 1.11, 1.25; 1.42% total Lys)

ME (kcal/kg)	28 Day weight (g)	35 Day weight (g)	ADG (g)	ADFI (g)	FCR (g/g)	Mortality (%)
2800	1414	1933	74.1[a]	149[a]	2.02 [a]	3.10
3050	1392	1908	73.7[ab]	141[ab]	1.93 [ab]	1.61
3300	1404	1894	69.4[b]	131[b]	1.89 [b]	0.00
SEM	14.80	18.9	1.80	2.60	0.05	0.30
$p > F$	0.37	0.12				
ME quadratic			0.03	0.0001	0.02	0.23

[a-d] Means in the same column with no common superscript differ significantly ($p \leq 0.05$)

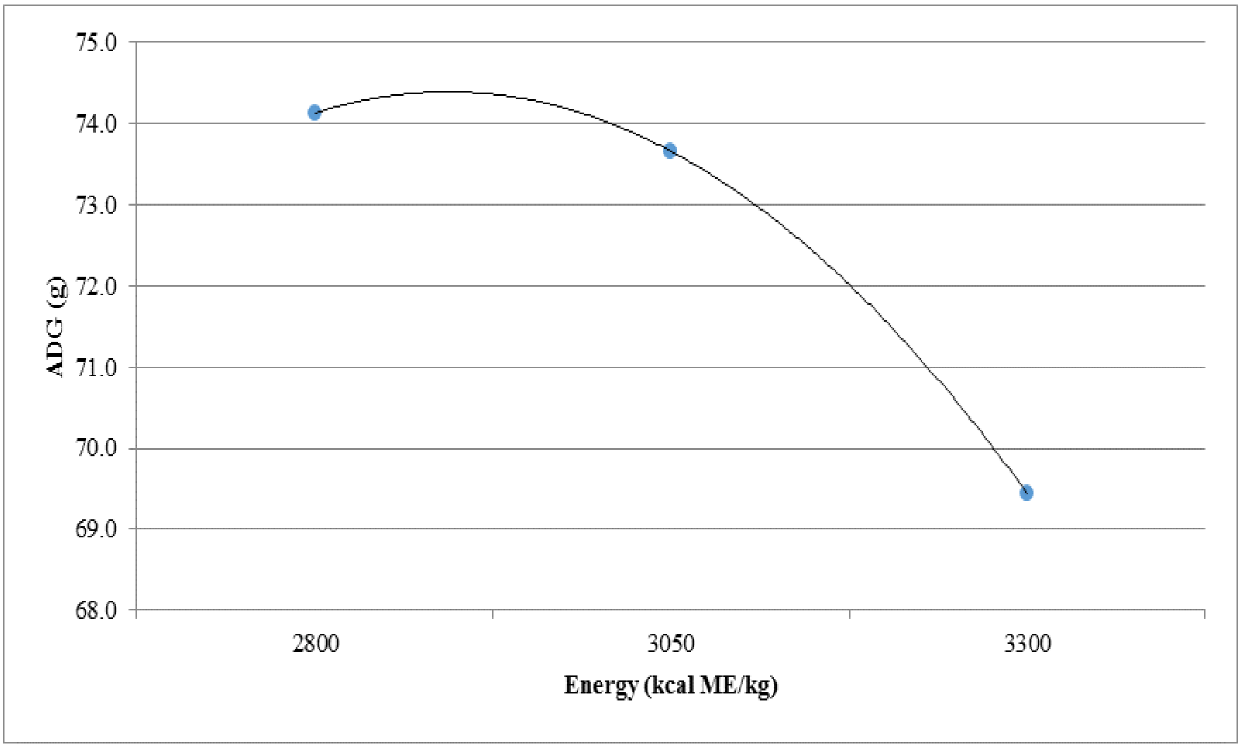

Figure 5.7: The effect of feeding 28 to 35 day of age mixed sex broiler varying amounts of energy (ME) levels on Average Daily Gain (ADG)

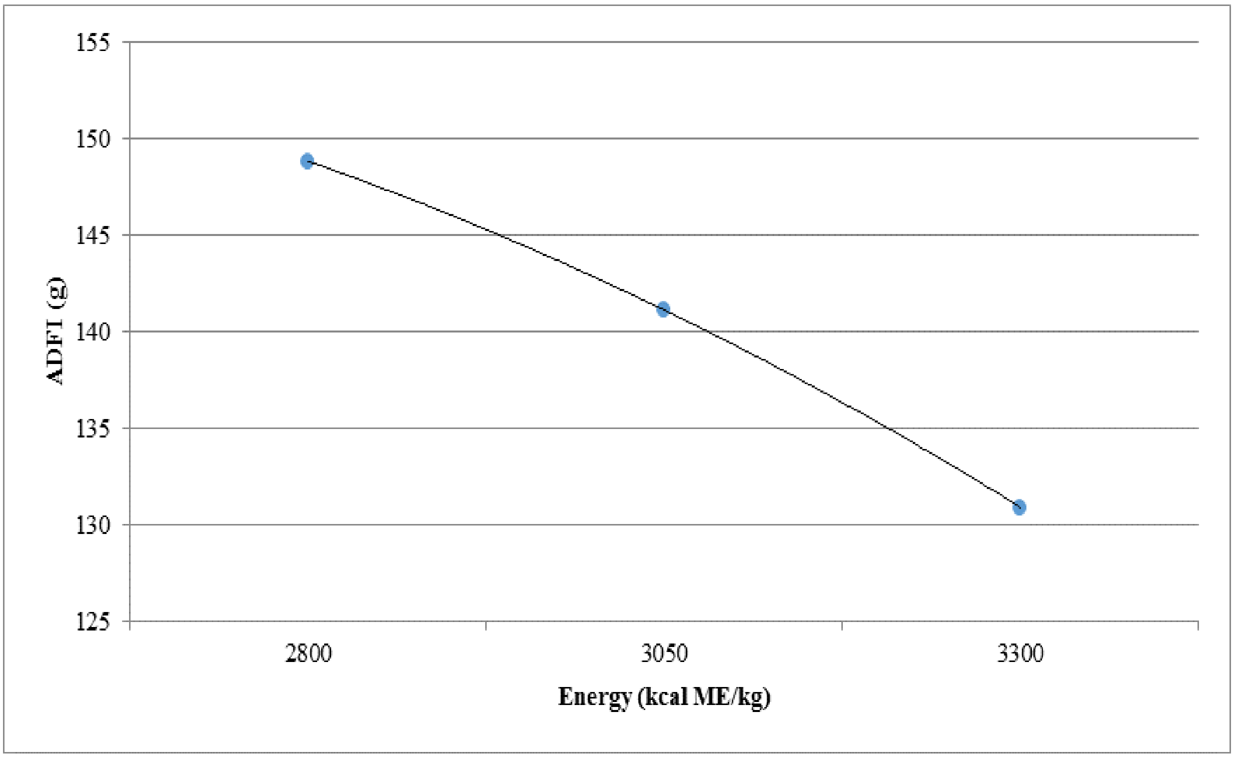

Figure 5.8: The effect of feeding 28 to 35 day of age mixed sex broiler varying amounts of energy (ME) levels on Average Daily Feed Intake (ADFI)

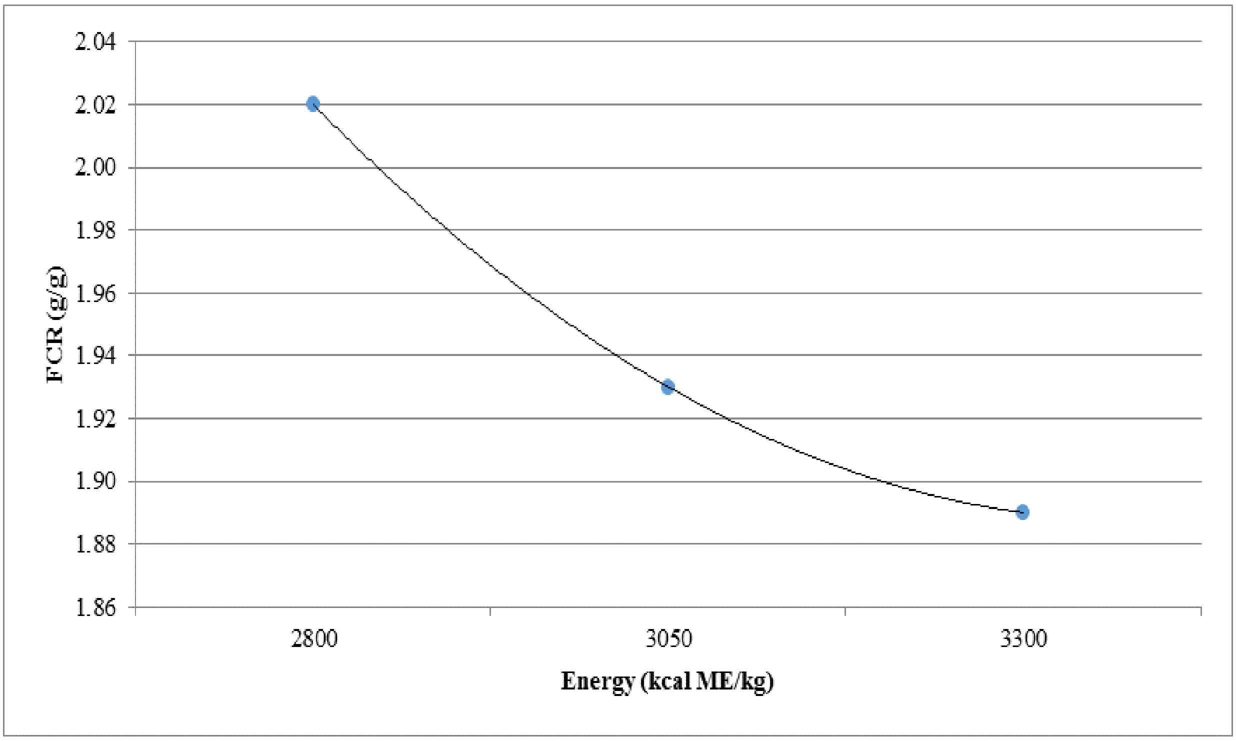

Figure 5.9: The effect of feeding 28 to 35 day of age mixed sex broiler varying amounts of energy (ME) levels on Feed Conversion Ratio (FCR)

5.8.2.4 Carcass composition

Table 5.9 presents the effect of varying ME: total Lys ratios on the carcass portions of broilers (28-35 days). An increase in the ME: total Lys ratio resulted in a significant increase in abdominal fat (%), lower whole breast (%) as well as a lower breast meat (%). However, in terms of whole breast (%), as well lower breast meat (%), only treatment 1 (ME: total Lys 1972, total Lys % 1.42 2800 kcal ME/kg) and treatment 4 (ME: total Lys 2240, total Lys % 1.25 3050 kcal ME/kg) were significantly different from treatment 12 (ME: total Lys 3438, total Lys % 0.96, 3300 kcal ME/kg).

Table 5.9: The effects of feeding 28 to 35 days' mix sex broilers varying ME: total lysine ratio on carcass protein and fat composition (% of carcass weight at processing)

Treatment	total Lys (%)	ME (kcal/ kg)	Ratio ME: tot. Lys	Carcass (%)	Abdominal fat (%)	Whole breast (%)	Breast meat (%)
1	1.42	2800	1972	85.3	1.20	26.5 ^{a}	21.2 ^{a}
2	1.42	3050	2148	86.1	1.30	25.2 ^{ab}	20.7 ^{a}
3	1.42	3300	2324	86.9	1.00	24.4 ^{ab}	19.6 ^{ab}
4	1.25	2800	2240	86.7	1.10	25.8 ^{a}	20.5 ^{ab}
5	1.25	3050	2440	86.9	0.90	24.9 ^{ab}	19.5 ^{ab}
6	1.25	3300	2640	88.6	1.70	24.2 ^{ab}	18.3 ^{ab}
7	1.11	2800	2523	87.6	1.40	22.5 ^{ab}	17.5 ^{ab}
8	1.11	3050	2748	88.1	1.10	22.5 ^{ab}	17.5 ^{ab}
9	1.11	3300	2973	83.8	1.80	24.7 ^{ab}	19.4 ^{ab}
10	0.96	2800	2917	88.0	1.50	23.2 ^{ab}	18.5 ^{ab}
11	0.96	3050	3177	83.3	1.60	23.0 ^{ab}	19.0 ^{ab}
12	0.96	3300	3438	86.3	1.30	20.6 ^{b}	16.01 ^{b}
SEM				1.90	0.22	0.92	0.90
$p > F$							
Ratio quadratic				0.72	0.05	0.007	0.015

[1] Data presented of random selected birds

[a-d] Means in the same column with no common superscript differ significantly ($p \leq 0.05$)

The statistical analysis of the quadratic response of broilers to varying ME: total Lys for whole carcass (%), whole breast (%), breast meat (%), abdominal fat (%) is illustrated in the equations below:

Whole carcass (%) = 62.08519 + 0.019284 x ME: total Lys + -0.000003713 x ME: total Lys ^{2}

(R^2 = 0.06) [5.15]

Abdominal fat (%) = -1.223625 + 0.0016398 x ME: total Lys+ -0.0000002464 x ME: total Lys ^{2}

(R^2 = 0.14) [5.16]

Whole breast (%) = 33.271886+ -0.004045 x ME: total Lys+ 0.00000018727 x ME: total Lys ^{2}

(R^2 = 0.40) [5.17]

Breast meat (%) = 33.681659 + -0.008554 x ME: total Lys + 0.000001094 x ME: total Lys ^{2}

(R^2 = 0.35) [5.18]

Table 5.10 presents the effect of varying total Lys (%) on the carcass composition of broilers (28–35 days). There was a significant decrease in whole breast (%) as well as a decrease in breast meat (%) when the total Lys was reduced from 1.42% to total Lys 0.96%.

Table 5.10: The effects of feeding 28 to 35 days mixed sex broilers varying total Lys (%) on broiler carcass protein and fat composition (% of carcass weight at processing)

Total Lys (%)	Carcass (%)	Abdominal fat (%)	Whole breast (%)	Breast meat (%)
1.42	86.1	1.20	25.4 ^{a}	20.5 ^{a}
1.25	87.4	1.20	25.0 ^{a}	19.4 ^{ab}
1.11	86.5	1.40	23.3 ^{ab}	18.1 ^{b}
0.96	85.9	1.50	22.3 ^{b}	17.8 ^{b}
SEM	1.10	0.10	0.60	0.60
$p > F$				
total Lys quadratic	0.77	0.29	0.0015	0.006

$^{a\text{-}d}$ Means in the same column with no common superscript differ significantly ($p \leq 0.05$)

The statistical analysis of the quadratic response of broilers to varying total Lys for whole carcass (%), whole breast (%), breast meat (%), abdominal fat (%) is described in the equations below:

Whole carcass (%) = 50.502864 + 50.454248 x total Lys + -2081432 x total Lys ^{2}

(R^2 = 0.03) [5.19]

Abdominal fat (%) = 1.767248 + -0.038749 x total Lys + -0.270735 x total Lys ^{2}

(R^2 = 0.1) [5.20]

Whole breast (%) = 4.5447896 + 25.974748 x total Lys + -7.925339 x total Lys ^{2}

(R^2 = 0.36) [5.21]

Breast meat (%) = 21.29707 + -10.4654 x total Lys + 7.0156113 x total Lys ^{2}

(R^2 = 0.31) [5.22]

Table 5.11 illustrates the effect of varying energy (ME) ratios on broilers' (14–28 days) body composition. There were no significant differences observed between the treatments.

Table 5.11: The effects of feeding 28 to 35 days' mix sex broilers varying ME on broiler carcass protein and fat composition (% of carcass weight at processing)

ME (kcal/kg)	Carcass (%)	Abdominal fat (%)	Whole breast (%)	Breast meat (%)
2800	86.9	1.29	24.5	19.4
3050	86.1	1.23	23.9	19.1
3300	86.4	1.45	23.5	18.3
SEM	1.02	0.10	0.62	0.65
p > F				
ME quadratic	0.83	0.34	0.48	0.38

$^{a\text{-}d}$ Means in the same column with no common superscript differ significantly ($p \leq 0.05$)

The statistical analysis of the quadratic response of broilers to varying energy (ME) for whole carcass (%), whole breast (%), breast meat (%), abdominal fat (%) is illustrated in the equations below:

Whole carcass (%) = 176.87533 + -0.058645 x ME + 0.0000094667 x ME ^{2} (R^2 = 0.01) [5.23]

Abdominal fat (%) = 20.741333 + -0.013103 x ME + 0.0000022 x ME ^{2} (R^2 = 0.06) [5.24]

Whole breast (%) = 37.678667 + -0.006947 x ME + 0.0000008 x ME ^{2} (R^2 = 0.04) [5.25]

Breast meat (%) = -17.09233 + 0.02591 x ME + -0.0000046 x ME ^{2} (R^2 = 0.06) [5.26]

5.8.2.5 Body carcass protein and fat composition of 28 to 35 day old mixed sex broilers fed varying ME: total Lys ratio's

Table 5.12 and Figures 5.10 and 5.11 presents the effect of varying ME: total Lys ratios on the body carcass protein (%) and fat (%) composition of broilers (28-35 days). An increase in the ME: total Lys ratio resulted in a significant lower protein (%).

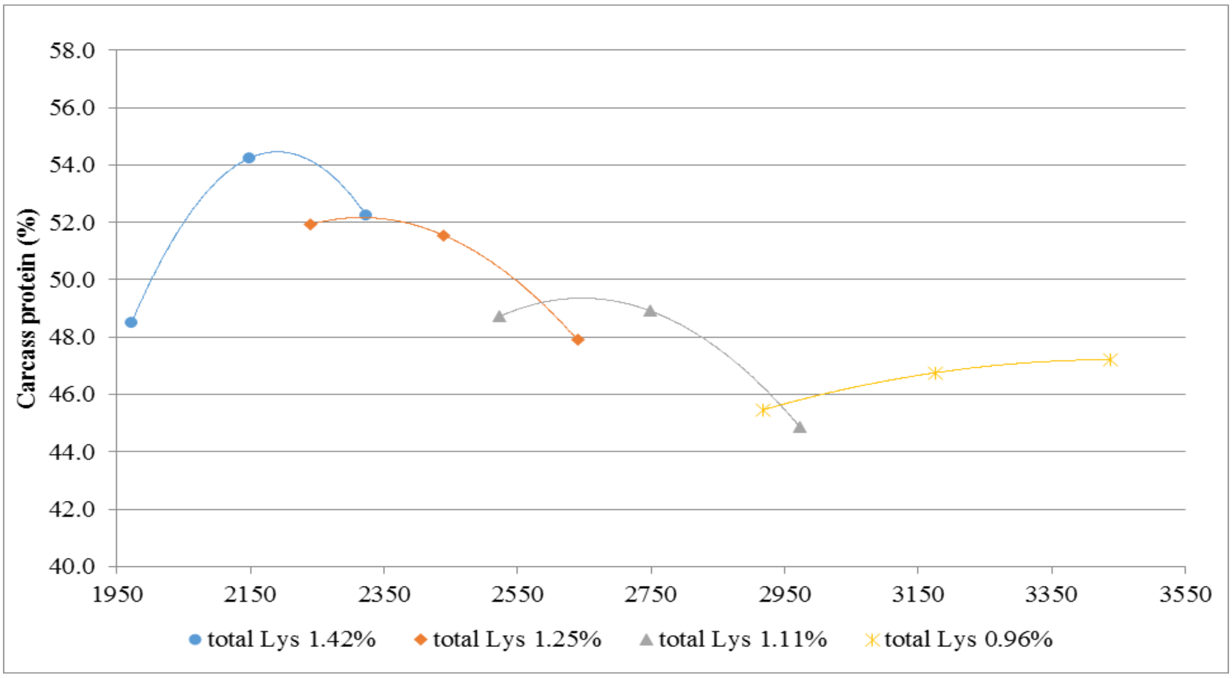

Figure 5.10: The effect of feeding 28 to 35 days of age mixed sex broilers varying amounts of dietary total Lys ratios on carcass protein (%)

Table 5.12: The effects of feeding 28 to 35 day old mix sex broilers varying ME: total Lys ratios and the composition of broiler carcass protein and fat

total Lys (%)	ME (kcal/kg)	Ratio ME: total Lys	Protein (%)	Fat (%)
1.42	2800	1972	48.5 [abcd]	30.7 [a]
1.42	3050	2148	54.2 [a]	28.5 [a]
1.42	3300	2324	51.9 [ab]	28.0 [a]
1.25	2800	2240	52.2 [abc]	26.5 [a]
1.25	3050	2440	51.5 [abc]	27.1 [a]
1.25	3300	2640	48.7 [abcd]	31.6 [a]
1.11	2800	2523	47.9 [abcd]	30.5 [a]
1.11	3050	2748	48.9 [abcd]	29.6 [a]
1.11	3300	2973	45.4 [d]	31.2 [a]
0.96	2800	2917	44.9 [cd]	33.3 [a]
0.96	3050	3177	46.7 [bcd]	31.5 [a]
0.96	3300	3438	47.4 [bcd]	31.5 [a]
SEM			1.81	1.77
p > F				
Ratio quadratic			0.0004	0.25

[a-d] Means in the same column with no common superscript differ significantly ($p \leq 0.05$)

The statistical analysis of the quadratic response of broilers to varying ME: total Lys for carcass protein (%) and carcass fat (%) is illustrated in the equations below:

Carcass protein (%) = 69.384712 + -0.010892 x ME: total Lys + 0.00000 x to11678 ME: total Lys ^{2}

(R^2 = 0.35) [5.27]

Carcass fat (%)= 29.594328+ -0.002491 x ME: total Lys + -0.00000098087 x ME: total Lys ^{2}

(R^2 = 0.14) [5.28]

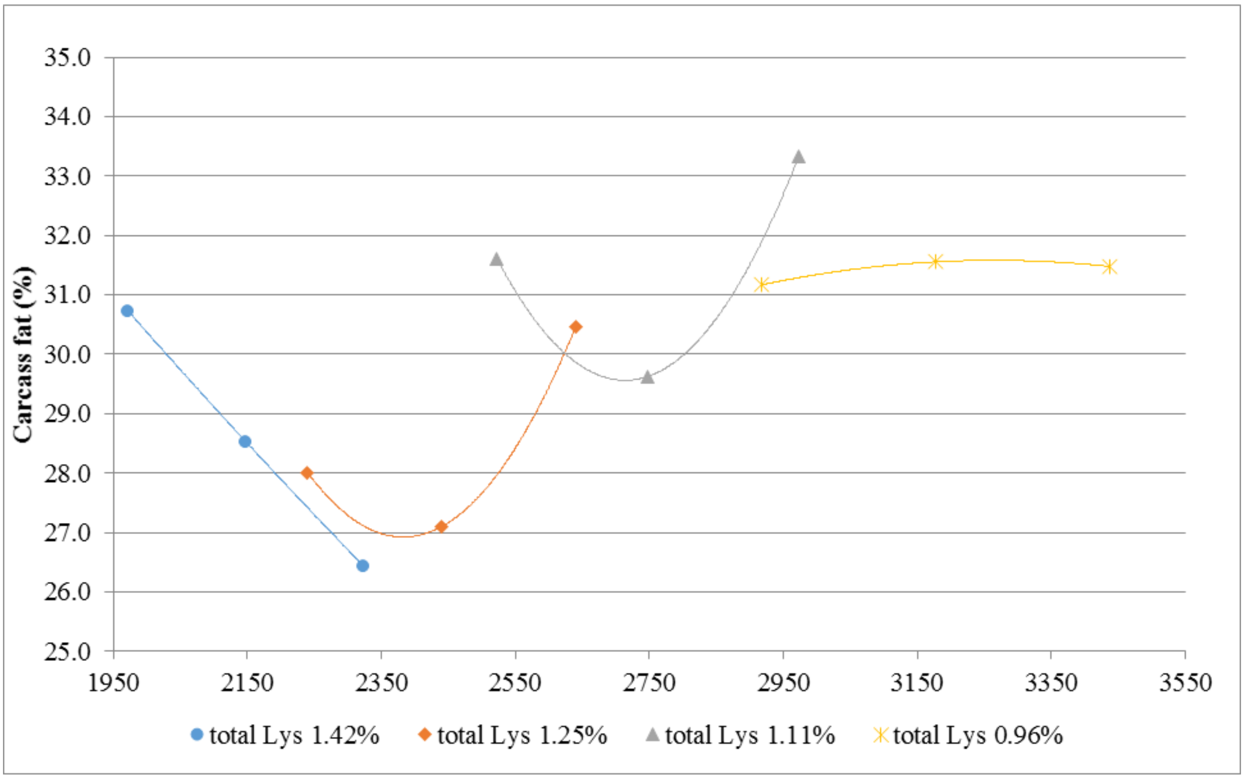

Figure 5.11: The effect of feeding 28 to 35 days of age mixed sex broilers varying amounts of dietary total Lys ratios on carcass fat (%)

Table 5.13 presents the effect of varying total Lys (%) on the carcass protein (%) and fat (%) composition of broilers (28–35 days). A general trend could be observed that a decrease in the total Lys ratio resulted in a significant lower carcass protein (%).

Table 5.13: The effects of feeding 28 to 35 day old mixed sex broilers varying total Lys (%) and the composition of broiler carcass protein and fat

total Lys (%)	Protein (%)	Fat (%)
1.42	51.6 [a]	28.5 [a]
1.25	50.5 [ab]	28.5 [a]
1.11	47.5 [bc]	31.5 [a]
0.96	46.5 [c]	31.4 [a]
SEM	0.90	1.01
p > F		
total Lys quadratic	0.0008	0.06

[1] Data presented of random selected birds

[a-d] Means in the same column with no common superscript differ significantly ($p \leq 0.05$)

The statistical analysis of the quadratic response of broilers to varying total Lys for carcass protein (%) and carcass fat (%) is described in the equations below:

Carcass protein (%) = 32.969902 + 15.14693 x total Lys + -1.317697 x total Lys ^{2}

(R^2 = 0.38) [5.29]

Carcass fat (%) = 41.121263 + -11.41075 x total Lys + 1.6730276 x total Lys ^{2}

(R^2 = 0.16) [5.30]

Table 5.14 illustrates the effect of varying energy (ME) ratios on broilers' (28–35 days) body carcass fat (%) composition. There were no significant differences observed between the treatments.

Table 5.14: The effects of feeding 28 to 35 day old mixed sex broilers varying ME levels and the composition of broiler carcass protein and fat

ME (kcal ME/kg)	Protein (%)	Fat (%)
2800	48.7	30.4
3050	50.4	29.2
3300	48.1	30.4
SEM	0.95	0.95
p > F		
ME quadratic	0.23	0.58

a-d Means in the same column with no common superscript differ significantly ($p \leq 0.05$)

The statistical analysis of the quadratic response of broilers to varying energy (ME) for carcass protein (%) and carcass fat (%) is described in the equations below:

Carcass protein (%) = -240.925 + 0.1920833 x ME + 0.00001920833 x ME ^{2} (R^2 = 0.004) [5.31]

Carcass fat (%) = 208.735 + - 0.117833 x ME + 0.000019333 x ME ^{2} (R^2 = 0.03) [5.32]

5.9 Discussion

The results of Experiment 4 showed an interesting relationship between total Lys, energy levels, the ME: total Lys ratio and pellet hardness and FI. Increasing the total Lys did not significantly affect ADG. It may be postulated that the lack in response in performance results might have been due to the levels of total Lys identified for the trial were already beyond the area of maximum response and, thus, if lower total Lys levels had been selected, that is, 0.75, 0.8, 0.85 and 0.90%, a response may have been observed.

Both ADFI and FCR were significantly reduced with higher total Lys levels. In addition, higher energy levels (3300 kcal ME/kg) resulted in significant reductions in ADG, ADFI and FCR. This response may be explained in terms of the poorer pellet produced with the highest energy level treatments. Accordingly, ADG was not significantly affected by either lower or higher ME: total Lys levels although both ADFI and FCR were low at the lowest ME: total Lys ratios. The variation in the FI response in relationship to dietary energy as noted in scientific literature is considerable and may be due to a number of factors, including dietary fat content, feed form, the pellet quality, amino acid content of the diet and the genetic strain of broilers (Gous *et al.*, 1990). Support for the argument is evident in a trial conducted by Wijtten *et al.* (2001). Male and female Cobb 500 broilers from 14 to 35 days of age were fed corn-soybean meal-based diets with increasing BP levels at either 3035 or 3240 kcal ME/kg. The Dig. Lys ranged between 10.7 and 13.8 g/kg. The FI increased linearly with increasing BP but was, on average, 150 g lower in the high energy treatments as compared with the corresponding lower energy treatments. Although the response to increasing BP was relatively small, the growth responses always differed between the dietary energy levels at identical ME: Dig. Lys ratios. Feed conversion improved by approximately ten points (g/g) from the lowest to the highest BP levels but was always, on average, six points (g/g) lower in the birds fed 3240 kcal ME/kg as compared with those fed 3035 kcal ME/kg. In a follow-up trial, 14 to 35 days old male Ross

308 broilers were fed graded levels of BP in diets containing 3100, 3240 or 3380 kcal ME/kg (Lemme *et al.,* 2003). Balanced protein levels ranged between 10.2 and 12.9 g Dig. Lys /kg. Feed intake decreased significantly with increasing ME content while increasing dietary BP had little effect on FI. Lemme *et al.* (2003) were not able to conclusively explain the decline in weight gain at Dig. Lys to ME ratios higher than 3500 in the birds which received the 3380 kcal ME/kg diets. It may, however, be postulated that there may be two possible explanations for this observation. One such argument may be that very high energy diets (3200 kcal ME/kg and more) tend to be associated with poor pellet quality and the other may be that the BP levels were too high.

It has been found that broiler chickens decreased their FI with increasing dietary energy and fat content of the diet when dietary carbohydrate was held constant (Leeson *et al.*, 1996). A similar response was observed when the dietary energy and sunflower oil content increased from 2985 to 3200 kcal ME/kg and 3% to 7%, respectively, with varying carbohydrate content in the diet (Sanz *et al.*, 2000). Furthermore, the high fat content that is often found in high energy diets may decrease pellet quality (Briggs *et al.*, 1999) and this may, in turn, affect FI. In addition, the feed form and pellet quality of the diet could affect the FI in broiler chickens. Pelleted diets are widely known both to increase FI and to improve performance in broilers as compared to mash diets (Svihus *et al.,* 2004; Amerah *et al.,* 2007; Mirghelenj and Golian, 2009). This could be due to increased nutrient density of the diet promoting more rapid and efficient eating. In addition, pelleted diets have been shown to decrease feed wastage, which could be interpreted as reduced FI (Hamilton and Proudfoot, 1995; Ahmerah *et al.*, 2007). Furthermore, pellet quality may affect FI in broilers with poor pellet quality reducing FI (Lemme *et al.*, 2006).

Another possible explanation of the poorer performance observed in the trial of Lemme *et al.* (2003) with higher energy levels may be that the nutrient density of the diet was too high with

BP levels (12.3 and 12.9 g Dig. Lys/kg; 3380 kcal ME/kg) for the birds. However, at 3100 and 3240 kcal ME/kg, weight gain decreased with a lower ME: digestible Lys ratio. In a review, Lemme (2007) concluded that lower energy to BP ratios are required either to maintain weight gain or to achieve maximum performance when dietary energy is reduced. Lemme (2007), therefore, postulated that there is no optimum fixed ME: total Lys ratio as is sometimes indicated when recommendations are in gram amino acid per unit dietary energy.

Lemme (2007) further commented that it appeared that expressing the amino acid recommendations as g per kcal (or ME) cannot be justified although the one or other question remained unresolved. It would seem that, if dietary energy is reduced, dietary amino acids should be reduced to a lesser extent, if at all. It is possible that birds may adjust their FI to their net energy requirement rather than to their ME intake. However, this may become obvious only in case of drastic changes in the diet formulation. Nevertheless, while a part of the ingested ME is retained in the body as protein or fat another part is lost as heat. Subtracting this heat energy from the ME then reveals that the amount of energy which is available for maintenance and production. A typical consequence of or reason for energy reduction is the reduction of ME from fat and the increase of the ME from carbohydrates. The effects of such changes have been controversial. For example, Wappechowski *et al.* (2004) reported that the utilisation of the ME for NE was minimal but still significantly higher in broilers fed high fat diets as compared with high starch diets. In contrast, MacLeod (1997) was not able to find any differences in the utilisation of ME in his experiments, including in high and low fat as well as high and low protein diets. Noblet *et al.* (2003) investigated the energy utilisation of broilers fed either adequate or low protein diets and could also not find a difference between the dietary treatments as regards the utilisation of ME. In order to assess the effects of the energy system Lemme (2007) transformed the diets used in his experiments conducted in 2005 to NE according to the

NE system as proposed by Beyer *et al.* (2003). Weight gain data was then plotted against the ratio of Met and Cys and NE. However, it was found that curves did not shift together, thus indicating that NE, as such, may not be the main reason for the non-linear relationship between optimum BP levels at changing dietary ME levels in the broiler diets evaluated. It is possible that other metabolic regulation mechanisms may be responsible for the observed non-proportional optimum ratio between BP and ME. This, in turn, may refer back to the work of Gonzales and Pesti (1993) who could also not find a correlation between dietary amino acid to energy ratio and FI and weight gain but who were able to predict these performance parameters by the dietary protein and energy levels. This suggests that birds regulate their FI not only according to their energy needs but also to their protein or, more likely, to their amino acid requirements. Lemme (2007) postulated that an alternative view could be that physiological feed quality may alter with changing dietary energy because high energy diets contain a significant amount of fat which sometimes makes it difficult to produce a good pellet. This effect is linked to the dietary energy of the diet.

From the carcass composition it could be observed that an increase in the ME: total Lys ratio resulted in a statistical significant lower carcass protein (%). Additionally, it could be observed that a decrease in the total Lys levels resulted in a significant lower carcass protein (%). It was also observed from the data that varying energy levels did not have a significant effect on body carcass fat (%) nor carcass protein (%). Considering all the carcass data observations in the response of broiler fed varying ME: total Lys levels, it would appear that the total Lys levels have a more pronounced effect on the carcass composition compared to the energy level of the diet.

5.10 Conclusion

The results of this Experiment showed that the ADG was not significantly different for the four total Lys levels. The quadratic regression for energy revealed that the point of maximum response for FCR was 3250 kcal ME/kg while ADFI was significantly lower with higher total lysine treatments. On the other hand, FCR was significantly improved when the total Lys was increased from 1.11 to 1.25% and further significantly improved when the total Lys was increased to 1.42%. Increasing the energy from 2800 to 3300 kcal ME/kg resulted in significantly lower ADG, ADFI and FCR. The pellet durability was significantly reduced when the energy was increased from 2800 to 3300 kcal ME/kg. This was probably responsible for the significantly lower ADG, ADI and FCR at 3300 kcal ME/kg versus the 2800 kcal ME/kg treatments. For ADG, the point of maximum response were 1.42% total Lys, 2800 kcal ME/kg, implying a ME: total Lys ratio of 1972. For FCR, the point of maximum response were 1.42% total Lys, 3050 kcal ME/kg, implying a ME: total Lys ratio of 2148.

Lower ratios of ME: total Lys tended to increase the breast (%) and protein (%) of broilers and also decrease the abdominal fat (%). The broilers' breast meat (%) was significantly more at the highest test level of total lysine of 1.42% as compared to the lowest total Lys of 0.96%. It was also concluded from the data that varying energy levels did not have a significant effect on body carcass fat (%) content. Considering all the carcass data observations in the response of broiler fed varying ME: total Lys levels, it would appear that the total Lys levels have a more pronounced effect on the carcass composition compared to the energy level of the diet.

The statistical analysis of the response of the broilers (28–35 days) to total Lys for ADG, ADFI and FCR revealed a quadratic response to varying levels of ME: total Lys ratio: ADG = 136.87248 + -0.045396 x ME: total Lys ratio + 0.0000077407 x ME: total Lys ratio^2 (R^2 =

0.30); FCR = 0.1641301 + 0.0011762 x ME: total Lys ratio + -0.0000001848 x ME: total Lys ratio^2 (R^2 = 0.37); ADFI = 127.57202 + 0.003209 x ME: total Lys ratio + 0.00000059861 x ME: total Lys ratio^2 (R^2 = 0.07)

General discussion

Dietary energy and amino acids constitute the largest components of poultry diets and also the most expensive. Accordingly, an understanding of the relationship between these dietary components is of major significance. Genetic selection by primary breeding companies has resulted in the vastly improved growth rate, feed conversion, and breast meat yield of broiler chickens as compared with the broilers of the previous decade (Dozier *et al.*, 2009). As a result, modern broilers require higher dietary amino acid concentrations in order to optimise performance and breast meat yield as compared with the broilers of past years (Kidd *et al.*, 2004; Dozier *et al.*, 2008). This increased dietary amino acid requirement has been partly achieved by a reduced FI per unit of growth rate. Despite the importance of nutrient availability in broiler diets, the body weight gain in broiler chickens is predominantly determined by the amount of feed consumed. It has been reported that both dietary energy density and protein concentrations have an impact on the FI and growth performance of broilers. It is generally accepted that broilers which are offered high energy would demonstrate relatively lower FI (Liu *et al.*, 2016). Nevertheless, the role of energy in regulating FI in broiler chickens is not clear and research has produced contradictory results.

During the trial period 0 to 7 days (Experiment 1), the FI of broilers was not significantly affected by either the Lys content nor by the energy level. Some reports suggest that FI regulation and, hence, response to different dietary energy is due to age (Jones and Wiseman, 1985; Brickett *et al.*, 2007; Kamran *et al.*, 2008). It was found that broiler chickens that were younger than 24 days of age did not adjust their FI in response to dietary energy ranging from 2577 to 3533 kcal ME/kg but changed their FI accordingly from 25 to 49 days of age (Jonas and Wiseman, 1985). Research conducted by Brickett *et al.* (2007) and Kamran *et al.* (2008) with broiler chickens under 14 days of age concluded that broilers were not able to regulate their FI.

In such cases, the limited capacity of the young birds to increase their FI may be the reason. Brickett *et al.* (2007) also found that, after 14 days of age, broiler chickens fed low energy diets adjusted their FI but not to the extent expected based on dietary energy level. Reasons suggested for the age dependent response included limited physical gastrointestinal tract capacity of young chicks and/or the influence of the energy derived from yolk (Griffiths *et al.*, 1977; Kamran *et al.*, 2008).

In Experiments 1 (0–7 days), 2 (7–14 days) and 3 (14–28 days) the birds' ADG increased significantly with an increase in total Lys. The point of maximum response of the broilers' ADG as a result of increasing total Lys were achieved at 1.48% for broilers 0 to 7 days old, 1.61% for 7 to 14 days and 1.37% for 14 to 21 days. For broilers 28 to 35 days of age an optimum total Lys for ADG could not be identified. A possible reason for this may have been that the total Lys levels chosen for the Experiment 4 were already above the optimum requirement and the birds had, thus, already been fed more than their requirement for optimal ADG. In experiment 1 (0–7 days), 2 (7–14 days) and 3 (14–28 days) the birds' FCR were decreased significantly with an increase in total Lys content. The point of maximum response of the broilers' FCR as a result of increasing total Lys were achieved at 1.60% for broilers 7 to 14 days and 1.34% for 14 to 21 days of age. For broilers 0 to 7 days of age and from 28 to 35 days of age an optimum total Lys for FCR could not be identified. For these two periods, the total Lys requirement for optimum FCR appeared to be even higher than that used in the experiments. These observations were very much in agreement with existing literature and support for these findings may be found in the trials conducted by Wijtten *et al.* (2001) Lemme *et al. (*2003), Plumstead (2005), Dozier *et al.* (2009) and Basurco *et al*. (2015).

The results of experiments 1 (0–7 days), and 3 (14–28 days) indicated that an increase in energy level did not have a significant effect on the broilers' ADG. Support for the finding relating to the 0 to 7-day period may be found in the trials conducted by Plumstead (2005) and Basurco *et al.* (2015), while support for the finding relating to the 14 to 28 day period may be found in the trial conducted by Basurco *et al.* (2015). An increase in energy significantly increased ADG the 7 to 14-day period. The literature review conducted in this study revealed that most studies on protein or Lys and the interaction with energy and broiler performance reported on trial periods from day old to 7 or 8 days (Sklan and Noy, 2003; Plumstead, 2005; Basurco *et al.*, 2015) and that most other studies reported only on day old to 14 to 21 days (Baghel and Pradhan 1989; Plumstead, 2007; Basurco *et al.*, 2015) or on older broilers from 14 to 28 or older (Pesti and Fletcher, 1983 Wijtten *et al.*, 2001; Lemme *et al.*, 2003). However, there has been no study conducted which reported on only the single effect of varying lysine or protein interaction with energy during the 7 to 14-day period.

It may be postulated that the broilers' requirement for energy for ADG was low during the day-old to 7-day period as a result of residual yolk absorption and that the broilers' requirement for energy for ADG in the subsequent period of 7 to 14 may be significantly more than originally estimated. The broilers' FCR was significantly reduced with an increase in energy during the 0 to 7 days, 7 to 14 days, 14 to 21 days and 28 to 35 days. There is strong support for this finding in existing literature as well as in the findings of the trials conducted by Pesti and Fletcher (1983), Wijtten *et al.* (2001), Lemme *et al.* (2003), Plumstead (2005), Leeson (2006), Urdaneta-Rincón and Leeson (2008), Dozier *et al.* (2009) and Basurco *et al.* (2015). It was only during experiment 4 (28 to 35 days) that the energy level of 3300 kcal ME/kg had a significant negative effect on the broilers' ADG. However, this may not have been due directly to the increase in energy but rather to the reduction in pellet quality as a result of the higher oil content in the feed that, in turn, had a significant negative impact on pellet durability. The reduction in pellet quality resulted in a significant reduction in ADFI and invariably had a significant negative effect in ADG. This observation is supported by the findings of the studies conducted by Briggs *et al.* (1999), Svihus *et al.* (2004), Amareh *et al.* (2007), Brickett *et al.* (2007), Mirghelenj and Golian (2009) and Zang *et al.* (2009).

It would appear that the decision regarding the optimum dietary level of ME may merely be a function of the price of the energy sources and the premium achieved for the improved feed conversion ratio (Aftab, 2009). In order to ensure the maximum utilisation of energy, Lys and every other nutrient of the diet, the correct proportion of these nutrients are necessary to ensure the optimum growth of the birds and to minimise the surplus of vital dietary components. According to Gous (2013), chickens attempt to control their FI so that they achieve a particular level of fatness. This fatness level is generally lower than that which is observed in many commercial operations and differs between strains, sexes and degrees of maturity (Gous *et al.*, 1990). The fact that this goal is not often achieved provides adequate proof that birds do not 'eat to satisfy an energy requirement' and that FI regulation is more complex than this statement suggests. To demonstrate the complexity of the process of FI regulation, birds that are fat will always attempt to reduce the excessive amounts of lipid by making use of these excessive amounts of lipid as an energy source when this is possible (Gous *et al.*, 2010). They do this because they always attempt to maintain an inherent ratio between body lipid and body protein. However, they fail to maintain this ratio when imbalanced feeds are offered but can correct the ratio if given the opportunity to do so. It would appear that strain differences exist in the extent to which broilers will utilise their body lipid reserves as an energy source. However, this may be accounted for when simulating FI and growth by assuming a higher desired lipid to protein ratio in genetically fat strains.

The results of this study revealed that the broilers' ADG increased with a decrease in the ME: total Lys ratio. Support for these findings may be found in the studies conducted by Bellaver *et al. (*2002); Lemme *et al.* (2003); Plumstead (2007) and Basurco *et al.* (2015). It may, thus, be concluded that there is no single ME: total Lys ratio suited to the modern broiler achieving maximum or optimum ADG or FCR. Instead, it would appear that the ratio changes as the broiler ages and that there is an optimum ME: total Lys ratio for each total lysine ratio. Support for this finding may be found in the conclusions drawn by Lemme (2007) who argued that, if the concept of a constant optimum protein to energy ratio worked, birds would balance their FI, thus resulting in a similar weight gain at both energy levels. The commercial practical implication is that nutritionists first need to determine the optimum Lys requirement needed to achieve the required or economical optimum ADG for each phase and then balance the lysine with the energy required to achieve the most economical FCR with the applicable ME: total Lys

The statistical evaluation of the response of broilers (0–7 days of age) to varying levels of ME: total Lys ratio gave rise to the following equations for ADG, ADFI and FCR:

$$ADG = 1.0000 + -0.9979 \text{ x (ME: total Lys ratio)} + 0.9919 \text{ x (ME: total Lys ratio}^2) \quad (R^2 = 0.37) \; [2.3]$$

$$FCR = 0.2493608 + 0.000893\text{x (ME: total Lys ratio)} + -0.0000001833 \text{ x (ME: total Lys ratio}^2) \quad (R^2 = 0.39) \; [2.4]$$

$$ADFI = 4.2932566 + 0.0099603 \text{ x (ME: total Lys ratio)} + 0.0003807 \text{ x (ME: total Lys ratio}^2) \quad (R^2 = 0.08) \; [2.5]$$

The statistical evaluation of the quadratic response of the broilers (7-14 days of age) to varying levels of ME: total Lys ratio yielded in the following equations for ADG, ADFI and FCR:

ADG = -3.108766 + 0.0389846 x (ME: total Lys ratio) + -0.00001038 x (ME: total Lys ratio^2)

(R^2 = 0.40) [3.3]

FCR =2.0683794+ -0.000729 x (ME: total Lys ratio) + 0.00000022155x (ME: total Lys ratio^2)

(R^2 = 0.39) [3.4]

ADFI =11.619103+0.0367668 x (ME: total Lys ratio) + -0.000008828 x (ME: total Lys ratio^2)

(R^2 = 0.17) [3.5]

The statistical evaluation of broilers (14-28 days of age) ADG, ADFI and FCR revealed a quadratic response to varying levels of ME: total Lys ratio:

ADG = 33.606973 + 0.0302445 x ME: total Lys + -0.00000684 x ME: total Lys ^{2}

(R^2 = 0.43) [4.3]

FCR = 4.043189 + -0.002338 x ME: total Lys + 0.00000049807 x ME: total Lys ^{2}

(R^2 = 0.31) [4.4]

ADFI = 221.00755 + -0.112043 x ME: total Lys + 0.0000233 x ME: total Lys ^{2}

(R^2 = 0.22) [4.5]

The statistical analysis of the response of the broilers (28–35 days) to total Lys for ADG, ADFI and FCR revealed a quadratic response to varying levels of ME: total Lys ratio:

ADG = 136.87248 + -0.045396 x ME: total Lys ratio + 0.0000077407 x ME: total Lys ratio^2

(R^2 = 0.30) [5.3]

$$FCR = 0.1641301 + 0.0011762 \times ME{:}\ total\ Lys\ ratio + -0.0000001848 \times ME{:}\ total\ Lys\ ratio^2$$

$(R^2 = 0.37)$ [5.4]

$$ADFI = 127.57202 + 0.003209 \times ME{:}\ total\ Lys\ ratio + 0.00000059861 \times ME{:}\ total\ Lys\ ratio^2$$

$(R^2 = 0.07)$ [5.5]

An interesting comparison that can be drawn is that the R^2 of the ADG and FCR regressions obtained from feeding the 0-21 days old broilers a range of varying ME: total Lys varied significantly between the literature review and that obtained from this study. For ADG the literature review produced a R^2 of 0.89 and for FCR the R^2 was 0.85. Experiment 2 that was conducted from 7 to 14 days produced a ADG of R^2 of 0.40 and for FCR the R^2 was 0.39. A lower R^2 indicate that the model does not describe the data set that well. A possible explanation for this anomaly is that the literature review employed a wider range of ME: total Lys values (1641 to 6275) than that what was applied in this study (1525 to 2385). The literature review presented energy values that ranged from 2547 ME kcal/kg to 3274 ME kcal/kg while for the same period of evaluation in this study (7-14 days), the energy values varied only between 2700 and 3100 ME kcal/kg. Additionally, the literature reviewed employed a total Lysine that varied between 0.5 to 1.85% while in Experiment 2, the total Lys levels varied only between 1.30 and 1.77%. It can be postulated that if wider ME: total Lysine ranges were employed the R^2 would have been higher. However, based on the trial facility design and commercial application desired form this study, more treatments were not possible.

The trials conducted during this study may be regarded as unique because the birds were fed trial diets with a combination of ME: total Lys levels for a specific period only. During the period leading up to the trial, the birds were fed similar diets to ensure that it would be possible to study the effect of the specific treatment in detail. This, in turn, prevented a carryover effect from one period to the next. It would therefore be suggested that future research be focused to quantify the effect of treatments in one period, *i.e* day old to 14 days and associated effect measured at 35 days of age given that the birds were fed the same diets from 14 to 35 days. The question that need to be answered relates to the questions if the modern broilers have the ability to exhibit compensatory growth? Additional, although birds fed higher ME levels during the day old to 14 day period during Experiment 1 and 2 have relative positive effect on FCR, would the additional energy oversupplied in this period be stored as a reserve and can it be used later in life more economically with a lower energy 28 to 35 day diets? Additionally, the experiments that were conducted and reported in this thesis have all be done with Ross 308 birds. It has been reported that different genetic lines respond differently to nutrients. It is therefore suggested that the same experiments be conducted with Cobb 500 birds. It would be of interest to know by which extent the genetic lines differ in their response to varying ME: total Lys for the individual phases of their life.

There are, however, two major limitations to the predicted regressions of the broilers' response to varying ME: total Lys ratios. Firstly, the model makes no provision for environmental conditions. If the environmental temperature were to increase above a threshold value, the ME: total Lys ratios would, in all likelihood, increase until the bird was capable of consuming an amount of energy without increasing its heat production. Secondly, the model makes no provision for economic conditions. If the price of energy sources were high relative to the price of the protein sources, then a diet with a higher ME: total Lys ratio than the biological ME: total Lys ratio could be formulated. Conversely, if the price of protein sources were low relative to the price of the energy sources, a diet with a lower ME: total Lys ratio than optimum biological ME: total Lys ratio could be formulated. This would however result in birds being fatter than their desired level because of the excess energy available. This situation often presents in broilers at market age. However, as the prices of protein sources remain high relative to the price of energy sources the latter would be the most profitable option unless the consumer were prepared to pay more for a leaner chicken than a fatter chicken.

References

Adebe, S. and Morris, T.R. 1990. Note on the effects of protein concentration on responses to dietary lysine by chicks. *Br. Poult. Sci*. 31:255-260.

Acar N, Moran Jr and ET, Bilgili SF. 1991. Live performance and carcass yield of male broilers from two commercial strain crosses receiving rations containing lysine below and above the established requirement between six and eight weeks of age. *Poult. Sci*. 70: 2315–2321.

Aftab, U. 2009. Responses of broilers to practical diets with different metabolizable energy and balanced protein concentrations. *Braz. J. Poult. Sci.* 3:169–173.

Ajinomoto Hartland Inc. 1990. Feeding broilers different lysine to apparent metabolizable energy ratios during the 40 to 2000 gram live weight period. 12:1–6.

Almquist, H.J. 1952. Amino acid requirements of chickens and turkeys: A review. *Poult. Sci.* 31:966–981.

Amerah, A.M., Ravindran, V., Lentle, R.G. and Thomas, D.G. 2007 a. Influence of feed particle size and feed form on performance, energy utilisation, digestive tract development, and digesta parameters on broiler starters. *Poult. Sci*. 86: 2615–2623.

Amerah, A.M., Ravindran, V., Lentle, R.G. and Thomas, D.G. 2007b. Feed particle size: implications on the digestion and performance of poultry. *World's Poult. Sci. J.* 63:439–455.

ASAE Standard. 1987. S269.3 *Wafers, pellets and crumbles: Definitions and method for determining density durability and moisture content*. St. Joseph, MI: ASAE.

Association of Official Analytical Chemists (AOAC). 1997. *Official methods of analysis*. 16th edition. Washington, DC: Association of Official Analytical Chemists.

Aviagen. 2014. *Broiler nutrition specifications: Ross 308*. Huntsville, AL.

Baghel, R.P.S. and Pradhan, K. 1989. Energy, protein and limiting amino acid requirements of broilers at very high ambient temperature. *Br. Poult. Sci*. 30:295–304.

Baker, D.H., Batal, A.B., Parr, T.M., Augspurger, N.R. and Parsons, M. 2002. Ideal ratio (relative to lysine) of tryptophan, threonine, isoleucine, ad valine for chicks during the second and third post hatch. *Poult. Sci.* 81:485–494.

Bartov, I., Bornstein, S. and Lipstein, B. 1973. Effects of calorie to protein ratio on the degree of fatness in broiler fed on practical diets. *Br. Poult. Sci.* 15:107–117.

Bartov, I. and Plavik, I. 1998. Moderate excess of dietary protein increases breast meat yield of broiler chicks. *Br. Poult. Sci.* 77:680–688.

Basurco, V., Viera, S.L., Serafini, N.C., Santiago, G.O, Angel, C.R. and Gonzalec-Esquerra, R. 2015. Performance and economic evaluation of feeding programs varying in energy and protein densities for broiler grillers. *J. Appl. Poult Res*. 24:304–315.

Bellaver, C.A.L., Guidoni, P.A., De Brum, A.R and Rosa, P.S. 2002. Estimates of requirements of metabolizable lysine and energy in broilers from 1 to 21 days of age, using multivariate canonical variable. *Braz. J. Poult. Sci*. 31:71–78.

Beyer, M., Chudy, A., Hoffmann, L., Jentsch,W., Laube., W, Nehring, N., and Schiemann, R. (2003). *Rostock Feed Evaluation System*, Plexus Verlag, Miltenberg- Frankfurt: 391.

Bilgili SF, Moran Jr. E, Acar N. 1992. Strain-cross response of heavy male broilers to dietary lysine in the finisher feed: Live performance and further processing yields. *Poult. Sci.* 71:850–858.

Boomgaardt, J. and Baker, D.H. 1971. Tryptophan requirement of growing chicks as affected by dietary protein level. *J. Anim. Sci.* 33:595–599.

Brickett, K.E., Dahiya, J.P., Classen, H.L. and Gomis. 2007. Influence of dietary nutrient density, feed form, and lightning on growth and meat yield of broiler chickens. *Poult. Sci.* 86:2172–2181.

Briggs, J.J. Maier, D.E., Watkins, B.A. and Behnke, K.C. 1999. Effect of ingredients and processing parameters on pellet quality. *Poult. Sci*. 78:1464–1471.

Burkhart, C.A., Cherry, J.A., Krey, H.P.V. and Siegel, P.B. 1983. Genetic selection for growth rate alters hypothalamic satiety mechanisms in chickens. *Behav. Genet*. 13:295–300.

Clark, F.A., Gous, R.M. and Morris, T.R. 1982. Responses of broiler chickens to well-balanced protein mixtures. *Br. Poult. Sci*. 23:446–449.

Classen, H.L. 2013. Responses of broiler chickens to dietary energy and its relationships to amino acids. *Proc. of the 24th Australian Poultry Science Symposium*. Sydney, pp 107–114.

Cobb-Vantress. 2012. Broiler nutrition specifications: Cobb 500. Siloam Springs, AR.

CVB. 2001. Tabellenboek veevoeding. Voedernormen landbouwhuisdieren en voederwaarde veevoeders. Centrale Veevoederbureau, Postbus 2176:8203. AD, Lelystad, the Netherlands.

Dale, N. and Villacres, A. 1988. Relationship of two-week body weight to the incidence of ascites in broilers *Avian Dis*. 32:556–560.

Dean, W.W., Binder, T.D. and Southern, L.L. 2006. Glycine supplementation of low protein, amino acid-supplementation diets supports optimal performance of broiler chicks. *Poult. Sci.* 85:288–296.

Delezie, E., Bruggeman, V., Swennen, Q., Decuypre, E. and Huyghebaert, G. 2010. The impact of nutrient density in terms of energy and/or protein on live weight performance, metabolism and carcass composition of female and male broiler chickens of two commercial broiler strains. *J. Anim. Physiol. Anim. Nutr*. 94:509–518.

Dozier III, W.A., Corzo, J., Kidd, M.T., Tillman, T.B. and Branton, S.L. 2009. Digestible lysine requirements of male and female broilers from fourteen to twenty-eight days of age. *Poultry Sci.* 88:1676–1682.

Dozier III, W.A., Kidd, M.T. and Corzo, J. 2008. Dietary amino acid response of broiler chickens. *J. Appl. Poult Res*. 17:157–167.

Dozier III, W.A. and Payne, R.L. 2012. Digestible lysine requirements of female broilers from 1 to 15 days of age. *J. Appl. Poult Res*. 21:348–357.

Dozier III, W.A., Price, C.J., Kidd, M.T., Corzo, J., Anderson, J. and Branton, S.L. 2006. Growth performance, meat yield, and economic responses of broilers fed diets varying in metabolizable energy from thirty to fifty-nine days of age. *J. Appl. Poult Res*. 15:367–382.

Durunna, C.S., Udibie, A.B.I. and Uchegbu., U. 2005. Effect of dietary inclusion of *Anthoata macrophyla* meal on the performance of started chicks. *Nig. J. Anim*. 32:268–273.

EFG software. 2017. EFG for Windows. 8th edition. Release 5.1. Fairfield, Pietermaritzburg, South-Africa.

Evonik. 2014. Standardized ileal digestible amino acid values for broilers. Hanua, Germany.

Faulkner, A.S. 1993. The influence of dietary energy on the efficiency of utilisation of dietary protein in broiler chickens. MSc dissertation. University of Natal, Pietermaritzburg, South Africa.

Fraps, G.S. 1943. Relation of the protein, fat and energy of the ration to the composition of chickens. *Poult. Sci.* 22:421–424.

Genstat. 2007. GenStat for Windows 8th Edition. GenStat Release 8.1, IACR-Rothamsted, Harpenden, Hertfordshire.

Glass, G.V. 1976. Primary, secondary and meta-analysis of research. *Educational Researcher* 5:3–8.

GfE. 1999. *Empfehlungen zur Energie- und Nährstoffversorgung der Legehennen und Masthühner (Broiler) DLG-Verlags GmbH, Frankfurt a. M., Germany: 185*.

Girish, C.K. and Payne, R.L. 2013. Effect of varying dietary metabolizable energy and protein on performance of broiler chickens. *Proc. of the 24 th Annual Australian Poultry Science Symposium.* Australia, pp. 122–124.

Gous, R.M. 2010. An effective alternative to the metabolisable energy system hens. *Proc. of the 21st Annual Australian Poultry Science Symposium.* Australia, pp. 36–43.

Gous, R.M. 2013. Predicting food intake in broilers and laying hens. *Proc. of the 24th Annual Australian Poultry Science Symposium*. Australia, pp. 99–106.

Gous, R.M., Emmans, G.C., Broadbent, L.A. and Fisher, C. 1990. Nutritional effects on the growth and fatness of broilers. *Br. Poult. Sci*. 31:495–505.

Gous, R.M. and Morris, T.R. (1983). Evaluation of a diet dilution technique for measuring the response of broiler chickens to increasing concentrations of lysine. *Br. Poult. Sci*. 26:147-161.

Gonzalez, A., M. J. und G. M. Pesti (1993). Evaluation of the protein to energy ratio concept in broiler and turkey nutrition. *Poult. Sci*. 72:2115-2123.

Grau., C.R. and Kamei, M. 1950. Amino acid imbalance and the growth requirements for lysine and methionine. Jour. Nutr. 41.89.

Greenwood, W.W., Cramer, K.R.., Beyer,R.S., Clark, P.M. and Behnke, K.C . 2005. Influence of feed form on estimated digestible lysine needs of male broilers from sixteen to thirty days of age. J. Appl. Poult. Res. 14: 130-135.

Griffiths, L.S. and Summers, J.D. 1977. Fat deposition in broilers: Effects of dietary energy to protein balance, early life caloric restriction on productive performance and abdominal fat pad size. *Poult. Sci*. 56:638–646.

Hamilton, R.M.G. and Proudfoot, F.G. 1995. Ingredient particle size and feed texture: Effects on performance of broiler chickens. *Anim. Feed. Sci. Technol*. 51:203–210.

Han, Y., Suzuki, H., Parsons, C.M. and Baker, D.H. 1992. Amino acid fortification of a low-protein corn and soybean meal diet for chicks. *Poult. Sci*. 71:1168–1178.

Havenstein, G. B., P. R. Ferket, and M. A. Qureshi. 2003. Growth, liveability and feed conversion of 1957versus 2001 broilers when fed representative 1957 and 2001 broiler diets. *Poult. Sci*. 92:1500-1508

Hidalgo, M.A., Dozier, W.A.O., Davis, A.J. and Gordon, R.W. 2004. Live performance and meat yield responses of broilers to progressive concertations of dietary energy and maintained at a constant metabolizable energy-to-crude protein ratio. *J. Appl. Poult. Res*. 13:319–327.

Jackson, S., Summers, J.D. and Leeson, S. 1982. Effects of dietary protein and energy on broiler carcass composition and efficiency of nutrient utilisation. *Poult. Sci.* 61:2224–2228.

Jafarnejad, S. and Sadegh, M. 2011. The effects of different levels of dietary crude protein, energy and using fat on the performance of broiler chicks at the end of the third week. *Asian Australes. J. Anim. Sci*. 5:35–40.

Jee, S.H., Miller, E.R., Guallar, E., Singh, V.K., Appel, L.J. and Klag, M.J. 2002. The effect of magnesium supplementation on blood pressure: a meta-analysis of randomized clinical trials. *Am. J. Hypertens.* 15:691–696.

Johnson, E.S., Lanes, S.F., Wenworth, C.E., Satterfield, M.H.M., Abebe, B.L. and Dicker, L.W. 1999. A meta regression analysis of the dose-response effect of aspirin on stroke. *Arch. Intern. Med.* 159:1248–1253.

Jones, R.L., and Wiseman, J. 1985. Effect of nutrition on broiler carcase composition: Influence of dietary energy content in the starter and finisher phases. *Br. Poult. Sci.* 26:381–388.

Kamran, Z.M., Sarwar, M., Nisa, M., Nadeem, M.A., Mahood, S. and Ahmed, S. 2008. Effect of low protein diets having constant energy-to-protein ratio on performance and carcass characteristics of broilers chickens from one to thirty-five days of age. *Poult. Sci*. 140:578–592.

Kyriazakis, I and Emmans, G.C. 1992. The effects of varying protein and energy intakes on the growth and body composition of pigs: 1: The effects of energy intake at constant, high protein intake. *Br. Poult. Sci.* 68:603-614.

Kerr, B.K. and Kidd, M.T. 1999. Amino acid supplementation of low-protein broiler diets: Formulation on an ideal amino acid basis. *J. Appl. Poult Res*. 8:310–320.

Kidd, M.T., McDanile, C.D., Branton, S.L., Miller, E.R., Boren, B.B. and Fancher, B.I. 2004. Increasing amino acid density improves live performance and carcass yields of commercial broilers. *J. Appl. Poult Res.* 13:593–604.

Kidd, M.T. and Fancher, B.I. 2001. Lysine needs of starting chicks and subsequent effects during the growth period *J. Appl. Poult Res.* 10:385–393.

Labadan, M.C., Hsu, K.N. and Austic, R.E. 2001. Lysine and arginine requirements of broiler chickens at two to three-week intervals to eight weeks of age. *Poult. Sci.* 80:599–606.

Latshaw, J.D. 2008. Daily energy intake of broiler chickens is altered by proximate nutrient content and form of the diet. *Poult. Sci.* 87:89–95.

Leclercq, B. 1983. The influence of dietary protein content on the performance of genetically lean or fat growing chickens. *Br. Poult. Sci*. 24:581–587.

Lee, C.M., Trevino, B. & Chaiyawat, M., 1996. A simple and rapid solvent extraction method for determining total lipids in fish tissue. J. AOAC Int. 79, 487-492.

Leeson, S., Caston, L. and Summers, J.D. 1996. Broiler response to energy and protein dilution in the finisher diet. *Poult. Sci.* 75:522–528.

Lemme., A, Kemp, C., Fisher, C., Kenny, M. and Petri, A. (2005). Responses of growing broilers to varying dietary energy and balanced amino acid lecvels. *Proc. of the 15th European symposium on Poultry Nutrition*. Balatonfüred, Hungary Australia, pp.447–449.

Lemme, A. 2007. How to adjust dietary amino acids to energy in broiler diets? *AMINONews* 8:13–24.

Lemme, A., Wijtten, P.J.A., Langhout, P.J.A. and Petri, A. 2003. Interaction between ideal protein and dietary energy in growing broiler chicken. *Proc. of the 14th European Symposium on Poultry Nutrition*. Norway, pp. 304–305.

Lemme, A., Wijtten, P.J., Wichen, J.V., Petri, A. and Langhout, D.J. 2006. Responses of male growing broilers to increasing levels of balanced protein offered as course mash or pellets of varying quality. *Poult. Sci.* 85:721–730.

Li, W., Chen, J., Wang, R., He, Y and Su, D. 2010. Influence of lightning schedule and nutrient density in broiler chickens: Effects of growth performance, carcass traits and meat quality. *Asian Australes. J. Anim. Sci.* 23:1510–1518.

Liu, S.Y., Raubenheimer, D., Selle, P.H., Gous, R.M., Hargreave, S.J., Cadogan, D.J. and Cowieson, A.J. (2016). Protein and energy ratios influence performance in broiler chickens.

Proc. of the 27th Annual Australian Poultry Science Symposium. Australia, pp. 170–172.

Lourdes, M., Chabela, P. 2010. Chemical analysis of poultry meat. Handbook of poultry science and technology. Economic assessment of amino acid responses in growing poultry, in *Amino acids in animal nutrition,* edited by I. Guerrero-Legarreta. 2nd edition. London: pp. 512-572.

Macleod, M.G. 1991. Fat deposition and heat production as responses to surplus dietary energy in fowls given a wide range of metabolisable energy, protein ratios. *Br. Poult. Sci*. 32:1097-1108.

Macleod, M.G. 1997. Effects of amino acid balance and energy: protein ratio on energy and nitrogen metabolism in male broiler chickens. *Br. Poult. Sci*. 38:405-411.

McDonald, P., Edwards, R.A. and Greenhalgh, J.F.D. 1988. Energy metabolism, in *Animal nutrition*. New York: John Wiley and Sons, pp. 158–168.

Meyer-Baron, M., Schaeper, M. and Seeber, A. 2002. A meta-analysis for neural behavioural results due to occupational mercury exposure. *Arch. Toxicol*. 76:127–136.

Mingbin, L., Yan, Y, Wang, Z, Wu, M. and Zunzhou, L. 2016. Effects of feed form and feed particle size on growth performance, carcass characteristics and digestive tract development of broilers. *An. Nut*. 1: 252-256.

Morris, T.R. 2004. Nutrition of chicks and layers. *World's Poult. Sci. J.* 60:5–18.

Morris, T.R., Al-Azzawi, K., Gous, R.M. and Simpson, G.L. 1987. Effects of protein concentration on responses to dietary lysine by chicks. *Br. Poult. Sci.* 28:185–195.

Namroud, N.F., Shivazad, M. and Zaghari, M. 2008. Effects of fortifying low crude protein diet with crystalline amino acids on performance, blood ammonia level, and excreta characteristics of broiler chicks. *Poult. Sci.* 87:2250–2258.

Noblet, J., J. Van Milgen, J., B. Carré, P. Dimon, S. Dubois, M. Rademacher und S. van Cauwenberghe (2003). Effect of body weight and dietary crude protein on energy utilisation in growing pigs and broilers. *EAAP Publication No*. 109: 205-208.

NRC. 1994. *Nutrient requirements of poultry*. 9th rev. edition. Washington, DC: National Academy Press.

Pack, M., Hoehler, D. and Lemme, A. 2003. Economic assessment of amino acid responses in growing poultry, in *Amino acids in animal nutrition,* edited by J.P.F.D. Mello. 2nd edition. Wallingford, UK: CABI, pp. 459–483.

Payne, J., Rattink, T.S. and Winowiski, T. 1987. *The pelleting handbook. A guide for production staff in the compound feed industry*. Germany: Borregaard, p. 62.

Pesti, G.M. 1991. Impact of dietary amino acid and crude protein levels in broiler feeds on biological performance. *Poult. Sci*. 18:477–486.

Pesti, G.M. and Fletcher, 1983. Responses of male broiler chickens to diets with varying protein and energy contents during the growing phases. *Br. Poult. Sci*. 24:91–99.

Plumstead, P.W. 2005. Effects of dietary metabolizable energy and protein on early growth responses of broilers to dietary lysine. *MSc dissertation.* University of North Carolina, Raleigh, United States of America.

Plumstead, P.W., Romera-Sanchez, H., Paton, N.D., Spears, J.W. and Brake, J. 2007. Effects of dietary metabolizable energy and protein on early growth responses of broilers to dietary lysine. *Poult. Sci*. 86:2639–2648.

Pym, R.A.E. and Nicholls, P.J. 1979. Selection for food conversion in broilers: Direct and correlated response to selection for body-weight gain, food consumption and food conversion. *Br. Poult. Sci*. 20:73–86.

Rosen, G.D. 2002. Multifactorial analysis of the effects of microbial phytase in broiler nutrition. *Proc. of the Mid-Atlantic Nutritional Conference*, University of Maryland, pp. 87–100.

Rostagno, H. S., L. F. T. Albino, J. L. Donzele, P. C. Gomes, R. F. de Oliveira, D. C. Lopes, A. S. Ferreira and S. L. de Toledo Barreto. 2005. *Brazilian tables for poultry and swine - 2nd Edition. Federal University of Viscosa, Department of Zootechnic, Vicosa, Brazil: 181.*

SAS Institute, 2015. SAS user's guide: Statistics. Version 12.0.1. SAS Institute Inc., Cary, NC.

Sanz, M., Flores, A. and Lopez-Bote, J. 2000. The metabolic use of energy from dietary fat in broilers is affected by fatty acid saturation. *Br. Poult Sci.* 41:61–68.

Sauvant, D., Schmidely, P., Daudin, J.J. and St-Pierre, N.R. 2008. Meta-analyses of experimental data in animal nutrition. *Anim*. 2(8):1203–1214.

Siegel, P.B. and Wiseman, J. 1966. Selection for body weight at eight weeks of age: 6. Changes in appetite and feed utilization. *Poult. Sci.* 5:1391–1397.

Skinner, J.T., Izat. A.L. and Waldroup, P.W. 1991. Effects of dietary amino acid levels on performance and carcass composition of broilers 42 to 49 days of age. *Poult. Sci.* 70:1223–1230.

Sklan, D., and Noy, Y. 2003. Crude protein and essential amino acids requirements in chicks during the first week post-hatch. *Br. Poult. Sci*. 44:266–274.

Smith, E.R., Pesti, G.M., Balkali, R.I., Ware, G.O. and Menten, J.F.M. 1998. Further studies on the influence of genotype and dietary protein on the performance of broilers. *Poult. Sci.* 77:1678–1687.

Sterling, K.G., Pesti, G.M. and Bakalli, R.I. 2003. Performance of broilers fed various levels of dietary lysine and crude protein. *Poult. Sci*. 82:1939–1947.

St-Pierre, N.R. 2001. Invited review: Integrating quantitative findings from multiple studies using mixed model technology. *Journal of Dairy Science* 84:741–755.

Surisdiarto, and D. J. Farrell. 1991. The relationship between dietary CP and dietary lysine requirement by broiler chicks on diets with and without the “ideal” amino acid balance. Poult. Sci. 70:830–836.

Svihus, B., Klovstad, K.H., Perez, V., Zimonja, O., Sahlstrom, S., Schüller, R.B., et al. 2004. Physical and nutritional effects of pelleting of broiler chicken diets made from wheat ground to different coarseness by the use of roller mill and hammer mill. *Anim. Feed Sci. Technol*. 177:281–293.

Swatson, H.K. 2002. The effects of excess dietary crude protein on the efficiency of utilisation of protein by broilers. PhD dissertation. University of Natal, Pietermaritzburg, South-Africa.

Thompson, S.G. and Higgins, J.P.T. 2002. How should meta-regression analysis be undertaken and interpreted? *Stat. Med.* 21:1559–1573.

Tillman, P.B. 2013. Current Amino Acid Considerations for Broilers: Requirements, Ratio’s, Economics. Proceedings of the Arkansas Nutrition Conference. September, 3rdth, Rogers.
Tukey, J. 1991. The philosophy of multiple comparisons. *Stat. Sci*. 6:100–116.

Urdaneta-Rincón, M. and Leeson, S. 2008. Evaluation of varied dietary crude protein and lysine levels at 5.7% of crude protein on productive parameters in broiler chickens. *Braz. J. Poult. Sci*. 2:154–159.

Vazquez, M. and Pesti, M. 1997. Estimation of the Lysine requirements of broilers chicks for maximum daily gain and feed efficiency. *J. Appl. Poultry. 6: 241-246.*Waldroup, P.W., Mitchell, J.R. Payne, J.R. and Hazen, K.R. 1976. Performance of chicks fed diets formulated to minimize excess levels of essential amino acids. *Poult. Sci*. 55:243–253.

Warpechowski, M. B., B., Carré, S. Dubois, Milgen, J. and Noblet, J (2004). *Energy utilization and heat production in male broilers fed normal or high fat diets. Braz. J. Poult. Sci*. 6:1-6.

Whitehead, A. 2002. *Meta-analysis of controlled clinical trials* (Internal report 201003). Chichester, UK: Wiley.

Wijtten, P.J.A., Lemme, A., Pack, M. and Langhout, P.J.A. 2001. The effects of enhanced dietary "ideal" amino acid levels in maize and wheat diets on performance of male and female broilers. *Proc. of the 13th European Symposium on Poultry Nutrition and Ascites Workshop*. Belgium, pp. 41–42.

Wijtten, P.J.A., Prak, R., Lemme, A. and Langhout, P.J.A. 2004. Effects of different dietary ideal protein concentrations on broiler performance. *Br. Poult. Sci*. 4:504–511.

WPSA. 1989. European table of energy values for poultry feedstuffs. 3rd ed.

Yamauchi, K. and Isshiki, Y. 1994. Ultra structural alterations of the duodenal epithelial cells in broiler chicks fed high protein-low energy or low protein-high energy diets. *Anim. Feed Sci. Tech*. 65:313–319.