CHAPTER 3

MATERIALS AND METHODS

3.1 Ethical considerations

The Animal Care and Ethics Committee of the University of New England (Armidale, NSW) approved this study. Health and husbandry practices complied with the *Code of Practice for the Welfare of the Domestic Fowl* issued by the Australian Bureau of Animal Health (1990).

3.2 Experimental birds

Day old mixed sex broiler birds (Cobb strain) were obtained from the Baiada Hatchery, Tamworth, and were raised on commercial broiler starter crumbles containing 12.5 ME MJ/kg and a minimum 20% CP (Fielders Agricultural Products, Tamworth, NSW), in standard chick brooders for 14 or 17 days. Four groups, each consisting of two randomly selected birds, approximately of equal weight, were selected from two different batches for each of Experiments 1 and 2. Experiment 1 began when each group of birds was 14 days old and weighed 758.8 ± 3.1 g (mean ± SE) (range 754.0 to 768.0 g). Experiment 2 commenced when each group of birds was 17 days old and weighed 945.5 ± 4.6 g (range 940.0 to 959.0g). For each experiment the birds were transferred to 4 closed circuit respiration chambers (2/cage) in a climate controlled room (25 ± 2°C) and under continuous fluorescent lighting.

3.3 Feed formulation and mixing

The "Feedmania" package (Mania Software Pty. Ltd., A.B.R.I., University of New England) was used to formulate the experimental diets, using the recommended levels of nutrients (NRC, 1994) for optimum performance. Diets for both experiments were cold-pelleted.

3.4 Respiration Chambers

The trials were conducted using 4 sets of the "closed-circuit calorimetry system" described by Farrell (1972). The system was effectively an ME cage capable of measuring oxygen intake and carbon dioxide output. Swain (1980), Pesti *et al.* (1988b) and Pesti *et al.* (1990) recorded some modifications that led to an improved oxygen flow and pressure control as illustrated in Figure 3.1. The system used water instead of mercury (Farrell, 1972) to seal the chamber (A). Pressure sensitive solenoid valves controlled the chamber pressure. The dimensions of each chamber (A) and wire mesh cage (B) were:

Chamber size: 600mm high x 380mm wide x 830mm long.
Cage size: 470mm high x 270mm wide x 480mm long.

Each chamber was constructed of poly-carbonate material and sat on a sheet-metal base containing a water trough. The cage rested on a tray (C) with sides about 10cm deep and there was sufficient room for a feeder (D) and drinker (E). Wet (G) and dry bulb (F) thermometers were located at one side of each cage. A 200 L cylinder (H), fitted with a regulator and a reducing valve (I), provided oxygen. Chamber air was circulated by a diaphragm pump (J) fitted with an electronic speed control (K), and was passed successively through a 2 L flask containing 1.5 L of potassium hydroxide (KOH) (L) and a moisture absorption train (M) containing 2 kg of calcium chloride before returning to the chamber. The KOH concentration used during Experiments 1 and 2 was adjusted

according to the live-weight of the test birds (40% KOH at 500 g up to 50% KOH at 850 g), as required to provide a safety margin as the birds grew older, i.e. the KOH concentration was maintained in excess of that required to absorb the carbon dioxide (CO_2) that was expected to be produced.

3.5 Principle of Operation

Two chicks at 14 days of age were placed in each of the 4 cages for Experiments 1 and 2 and the chambers were sealed. Initial readings of atmospheric pressure, temperature and humidity for each chamber were recorded, and an aliquot of chamber air was withdrawn through an outlet into a 0.5 L, gas-tight syringe. The air sample was subsequently analysed for O_2 and CO_2 using a Servomex digital oxygen analyser, model 570A and a Haldane gas analyser, respectively. Room temperature was controlled by an air conditioning unit at 25 ± 2°C to maintain a constant temperature within the chamber. The pump continuously circulated chamber air and as the air bubbled through the KOH solution, carbon dioxide expired by the birds was absorbed. This reduced the pressure in the system, which in turn caused a solution of sodium bicarbonate ($NaHCO_3$) in the glass manometer (N) to rise in the closed arm. When the $NaHCO_3$ solution reached the stainless steel contact (O), the electrical circuit was completed and a relay system (P) opened a solenoid valve (Q) to permit oxygen to enter the chamber so as to restore chamber pressure. As the chamber pressure rose, the solution rose in the open arm until it reached the stainless steel contact (R) and the relay system shut off the solenoid valve. At the end of each 20-22 h experimental period, pressure, temperature and humidity in the chamber were recorded and a final sample of air was withdrawn for analysis.

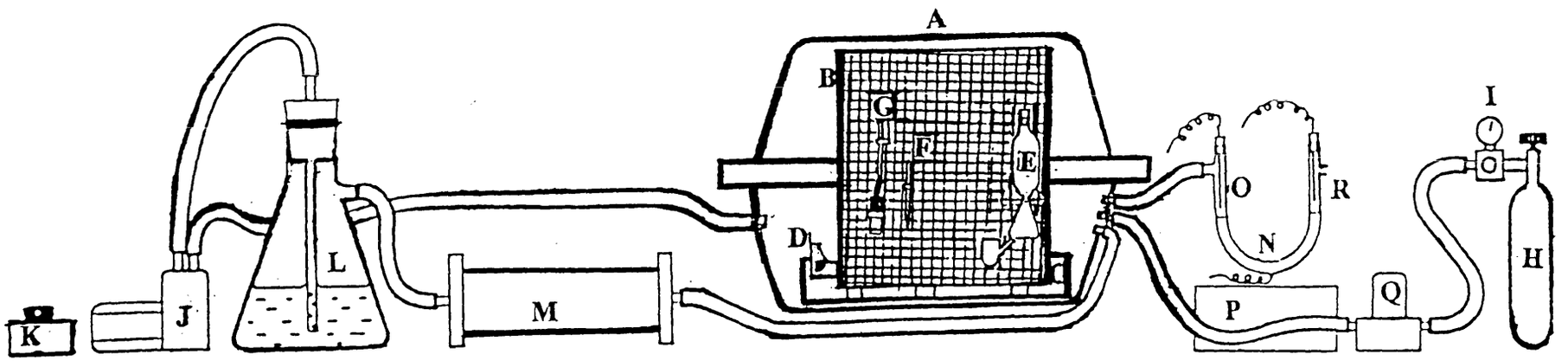

Figure 3.1: The respiration chamber and ancillary equipment.

A - Chamber
B - Wire mesh cage
C - Collection tray
D - Feeder
E - Drinker
F – Dry-bulb Thermometer
G - Wet bulb thermometer
H - Oxygen cylinder
I - Oxygen regulator and reducing valve
J – Pump
K - Motor speed control
L - Flask containing 1.5 L of KOH
M - Calcium chloride train
N – Manometer
O - Steel contact in closed arm
P - Relay system
Q - Solenoid valves
R - Steel contact in open arm

The KOH solution into which the CO_2 was absorbed during each run was washed into a 2 L volumetric flask and made up to volume. An aliquot (10 mL) was analysed gravimetrically to determine carbonate content and thus CO_2 production was determined. Corrections were made to CO_2 and O_2 values on the basis of the initial and final composition of chamber air at STP.

3.6 Analytical methods and related procedures

3.6.1 GE determination

GE of the excreta and of the diets was determined in each experiment. Live weight, daily feed intake and water consumption were recorded. Excreta were collected separately from each cage for 4 days. Spilled feed and feathers were discarded and the excreta were dried at 80°C for 24 h. The GE content of the diets and excreta were determined using an adiabatic bomb calorimeter (DDS CP 500, DIGITAL DATA SYSTEMS PTY, LTD.). Benzoic acid was used to standardise the bomb calorimeter. The dry excreta weight was adjusted to include the calculated dry weight of a sub sample that was used for VFA analysis.

3.6.2 AME determination

The GE values were then used to calculate those of the energy metabolised from the GE intake of the birds in a given period on both an as-fed and DM basis, thus:

$$\text{AME (as fed)} = \frac{GE_{in} - GE_{out}}{FI_{as\ fed}} = \frac{(GE_{feed} \times FI_{as\ fed}) - (GE_{excreta} \times \text{dry excreta wt})}{FI_{as\ fed}} \text{ MJ / kg}$$

Where FI = feed intake and GE = gross energy

$$\text{AME (DM)} = \frac{(GE_{feed} \times FI_{as\ fed} \times \text{DM feed}) - (GE_{excreta} \times \text{dry excreta})}{FI_{as\ fed} \times \text{DM feed}} \text{ MJ / kg}$$

3.6.3 Dry Matter (DM) content of feeds (%)

The dry matter content (%) of the diets was measured after drying sub-samples (range 4-5 g) at 105°C for 24 h and calculated as follows:

$$\text{DM \%} = \left[100 - \left(\frac{\text{Weight of wet feed sample (g)} - \text{Weight of dry feed sample (g)}}{\text{Weight of wet feed sample (g)}}\right)\right] \times 100$$

3.6.4 Dry Matter (DM) content of excreta (%)

Fresh excreta were dried in a force-draught oven at 80°C for 24h and the DM content was calculated as:

$$\text{DM \%} = \left[100 - \left(\frac{\text{Weight of wet excreta (g)} - \text{Weight of dry excreta (g)}}{\text{Weight of wet excreta (g)}}\right)\right] \times 100$$

3.6.5 Oxygen consumption

The difference between the weight of the oxygen cylinder at the beginning and end of each run (20-22 h) gave the O_2 consumption value by weight.

3.6.6 Carbon dioxide recoveries

The recovery of CO_2 from KOH solution was accomplished by a modified barium chloride ($BaCl_2$) precipitation technique described by Swain (1980) as a variation of the earlier method of Annison and White (1961). Modifications involved the concentrations of ammonium chloride (NH_4Cl) (200 g/l) and $BaCl_2$ (300 g/l) used in the precipitation of $BaCO_3$ from the KOH solution, as described below. The gravimetric determination of CO_2 absorbed by the KOH solution was based on the following chemical reactions:

$$CO_2 + 2KOH \rightarrow K_2CO_3 + H_2O$$

$$K_2CO_3 + BaCl_2 \rightarrow BaCO_3 + 2KCl$$

The KOH solutions (40 to 50% w/v) for both Experiments 1 and 2 were made by dissolving 400, 425, 450 or 500 g of KOH chips in 1.5 L of distilled water in a conical flask. The solution from each 20-22 h calorimeter run was made up to the 2 L volume and the dissolved CO_2 was then precipitated as follows:

1. 10 mL of the KOH solution was pipetted into a dried and weighed centrifuge tube followed by the addition of 6 mL of NH_4Cl and then 20 mL of $BaCl_2$ solution that was gently swirled and mixed thoroughly.

2. The resulting suspension was then centrifuged for 15 min at 7500 *g*.

3. The supernatant was carefully decanted and the carbonate pellet re-suspended in 20 mL of distilled water and centrifuged again for 40 min at 8000 *g*.

4. The supernatant was then decanted and the tube dried for 12 h at 105°C in a laboratory oven.

5. Finally, the centrifuge tube was cooled in a dessicator and re-weighed. The weight difference was recorded as the $BaCO_3$ recovered from the 10 mL aliquot of KOH solution. Recoveries were carried out in duplicates with an acceptable maximum difference of < 1% between duplicates. The dry weight of $BaCO_3$ was then used to calculate total CO_2 recovery as follows:

1. CO_2 recovered in sample (g) $= \frac{\text{M. Wt. } CO_2}{\text{M.Wt.}BaCO_3} \times BaCO_3 \text{ Wt.} = 0.2229 \times BaCO_3 \text{ Wt.}$

2. Total CO_2 recovered (g) $= \frac{\text{Volumetric flask mixture (2 L)}}{\text{Aliquot volume (10mL)}} \times CO_2\,(g) = CO_2\,(g) \times 100$

3. Volume CO_2 recovered at STP (litres) $= \frac{CO_2\,(g)}{\text{M.Wt. } CO_2\,(g)} \times K = CO_2 \times 0.509$

Where K is a constant (22.414) that represents the volume of 1 mole of gas at STP.

4. Combine factors: $\times \frac{\text{M.Wt.}CO_2}{\text{M.Wt.}BaCO_3} \times \frac{\text{Volume 1 mole gas at STP}}{\text{M.Wt.}CO_2} \times \frac{2000\text{mL}}{10\text{mL}} = 11.35$

5. Total CO_2 recovered (litres, STP) = $BaCO_3$ from 10mL aliquot x 11.35.

3.6.7 Heat production (HP)

The respiratory quotient (RQ) during each run refers to the ratio between the volume of CO_2 produced by the birds to the volume of O_2 used (RQ = CO_2 produced / O_2 used). The value obtained indicates the degree of oxidation of the diet on trial. By reference to the thermal equivalent of O_2 (kJ/L) for such a mixture, heat production from a known O_2 consumption was estimated, using the Brouwer equation (Johnson, 1981) incorporated into the Closed Circuit Respiratory Calorimetry (CCRC) computer program (Pesti *et al.*, 1988b). Observations of HP were made over 4 days but were suspended for about 2 h each day while the feed and water containers were replenished, excreta were collected and the system was readjusted for the next run, including increasing the KOH concentration in the CO_2 absorbed. The HP was calculated on an hourly basis and then converted to a 24 h basis.

3.6.8 Net energy (NE)

From the principle of the conservation of energy, the ME provided to a bird by its diet is either retained (ER) in the body or lost as heat (Kleiber, 1975; Sibbald, 1982; McDonald *et al.*, 1995). Thus, ME = HI + ER. The deduction of the HI component from the ME intake gave the NE value of the feed.

3.6.9 Basal metabolism

The measurement of basal metabolism as estimated by fasting heat, involved the removal of the complicating effect of the heat increment of feeding by starving the birds. The period of fasting required for the digestion and metabolism of previous meals to be completed was 2 days as recommended for poultry (Farrell, 1972; 1974c; Pym and Farrell, 1977; McDonald *et al.*, 1995). The RQ and HP from the known O_2 consumption and CO_2 produced by each pair of birds, during the starvation period were estimated using the Brouwer equation incorporated into the CCRC computer programme. By reference to the above programme, the link between basal metabolism and body weight was assumed to be (kJ/kg $^{0.75}$/day).

3.6.10 Collection of digesta and viscosity measurement

At the end of Experiment 2, the 2 birds from each cage were humanely sacrificed by cervical dislocation. The contents of the duodenum, jejunum and ileum (from Meckel's diverticulum to 4 cm above the ileo-caecal junction) were collected. Approximately 5 g of fresh digesta were spun in an induction drive centrifuge (Beckman Model, J2-21M, U.S.A) at 10,000 *g* for 15 min at 20°C. The supernatant and the residues were separated and the viscosity of the supernatant was determined immediately using a Brookfield DV-1+ Model viscometer at room temperature (25°C) with a CP40 cone and a shear rate of 2-500 s^{-1}. The samples did not exhibit shear thinning at these shear rates.

3.6.11 Determination of VFA concentration by use of the internal standard method.

A method to calculate the concentrations of VFAs from the peak areas of the chromatogram has been developed (Geissler *et al.*, 1974, cited by Forster, 1986). Gas

liquid chromatography (G.L.C: Model 427, Packard Instr. Co., USA) was used to measure the molar proportions (%) of VFAs by the use of the internal standard method which allows measurement of the volume of the sample injected. By making the proportion of the sample to the added internal standard a constant, the volume of the sample injected is proportional to the area of the internal standard peak. Sample preparation for the internal standard method was as follows:

1. A working solution comprised of 10 mL of stock internal standard solution (1.6% isocarproic acid in 90% formic acid), 10 mL of 12% stock solution of metaphosphoric acid and 30 mL of 90% formic acid.

2. Standards of 0.1 mL of the internal standard solution plus 1 mL of mixed VFAs standard and a blank of 0.1 mL of internal standard plus 1 mL of distilled water were prepared. These volumes were kept constant for each set of samples, standards and blanks. One standard and one blank were prepared for every 10-20 samples.

3. Previously frozen (-20°C) caecal and faecal samples were thawed and mixed well. To approximately 3 g of each sample, a similar weight of 0.1 M sulphuric acid (H_2SO_4) was added to solubilise the VFAs. The mixture was shaken thoroughly and centrifuged at 7000 *g* for 10 minutes.

4. 1 mL of each sample of caecal or faecal supernatant was accurately pipetted into a disposable centrifuge tube. 0.1 mL of the working internal standard solution was also pipetted into the tube, the stopper placed and the solution mixed well.

5. 1 mL of each mixture was accurately pipetted into thundberg tubes. The tubes were frozen in liquid air (-210°C) and then connected to a vacuum pump for 45 s to create a vacuum within the tubes.

6. The frozen sample in the top part of the thundberg tubes was then allowed to thaw slowly overnight in a cold room (+4°C) while the bottom parts were

suspended under liquid air in thermos flasks. This is the lyophilisation stage that involves the transfer of the VFAs in a concentrated, purified form.

7. The tubes were placed at room temperature (25 ± 2°C) to allow further thawing and then left to stand until the solution equilibrated with the room temperature. The pressure in the tubes was then released.

8. All the liquid samples in the tubes were separately pipetted into 2 mL glass vials and one drop of 10 N sodium hydroxide (NaOH) was added to each vial in order to stabilise the VFAs. A pH of 8-10 was used as the desired range for the alkalinity.

9. The open vials were placed in a rack in a dessicator that was connected to a vacuum pump under a fume hood to dry over night. The dry samples were then fed into the G.L.C.

3.6.12 Calculation of energy loss as VFAs in the excreta.

Volatile fatty acid concentration in excreta supernatant was measured and converted to kJ energy using a heat of combustion value for each VFA (McDonald *et al.*, 1995). The total VFA energy was calculated by the following equation:

$$\text{Total E loss VFA (kJ)} = E \times WL/1000$$

Where E = sum of energy from individual VFAs in sample (mMol/L)

and WL = total aqueous portion of excreta.

3.7 Statistical Analysis

All data were analysed using Repeated Measures Analysis. StatView 4.0 (FPU: 1992 Abucus Concepts, Inc.) was used to perform the analyses. The conventions below have been used to indicate statistical significance throughout the text and tables:

NS non significant; $P>0.05$

* $P<0.05$

** $P<0.01$

*** $P<0.001$

CHAPTER 4 - EXPERIMENT 1

THE EFFECT OF FEEDING MAIZE OR BARLEY ON HEAT PRODUCTION DURING METABOLISM AND LOSS OF ENERGY AS VOLATILE FATTY ACIDS IN THE EXCRETA OF BROILER BIRDS

4.1 Introduction

The nutritive value of maize (corn) and barley for poultry differs widely, especially the metabolisable energy content (SCA, 1987; NRC, 1994). Although it is well understood that the most important chemical component governing such differences in the nutritive value is the soluble NSP level (Choct *et al.*, 1996; Annison, 1991), it is the generally accepted (Close, 1990; Leeson and Summers, 1991) that energy-yielding components are the most limiting dietary ingredients. Therefore, considerable attention has been directed towards the development of systems for expressing both the energy requirements of broiler birds and the energy value of feeds.

Theoretically, the most satisfactory measure of energy in poultry feeds should be productive energy (PE) (Hill and Anderson, 1958), effective energy (EE) (Emmans, 1994; Farrell, 1996) or net energy (NE) (Close, 1990; MacLeod, 1994). However, there are no practical methods of measuring PE or EE. Moreover, Leeson and Summers (1991) pointed out that the values that have been estimated with various feedstuffs are not consistent with, or independent of, the birds' age, dietary balance, plane of nutrition or productive performance. For this reason, metabolisable energy (ME) is the current system used in description of the energy content of dietary ingredients for poultry. The system, in its variations (AME, TME, AME_n, TME_n), is relatively easy to ascertain and the values obtained reflect energy contribution under practical feeding situations. However, as stated in Section 2.3.3, the system is currently under criticism, as it does not take into account differences in the efficiency of metabolic utilisation of the ME of different feedstuffs.

In order to substantiate the extent to which the ME of the feed is utilised, it is necessary to measure the heat production and ultimately the energy retention of birds to which the feed is given (Kleiber, 1975; Emmans, 1994; McDonald *et al.*, 1995). An approach using closed circuit calorimetry with a respiration chamber is normally used (Farrell, 1972, 1974b, 1974c; MacLean and Tobin, 1987; McDonald *et al.*, 1995) to estimate heat production from the respiratory exchange of the birds. The current study was conducted to examine whether the amount of energy lost via heat production (HP) and that represented by VFAs in the excreta is influenced by the types of cereal grain type, for example maize and barley.

4.2 Materials and Methods

Chapter 3 provided a general description of the materials and methods used throughout this project.

4.2.1 Experimental length

Experiment 1 ran for 11 days and was conducted in the calorimeters described in Section 3.4. The first 6 days allowed the birds to adapt to the experimental diet (*ad libitum)* and to confinement for 20-22 h/d in the closed circuit calorimeters. The calorimeters were unsealed for 2-4 h each day to allow the feed and water containers to be replenished and to readjust the system for the next run. The following 4 days involved quantitative excreta collection. On the 11th day the birds were deprived of feed for 24 h to measure their basal metabolism as established by fasting heat production.

4.2.2 Experimental diets and design

Two experimental diets were formulated as shown in Table 4.1. Diet 1 was barley based, whereas maize was the cereal base in Diet 2. Two replicates of two birds each were used for each diet. Feed intake and excreta output were recorded for each of the 4 days in order to determine AME and HP on a daily basis. The diets were offered *ad libitum* except on the final day. Water was available at all times.

Table 4.1 Diet composition

Ingredient (g/kg)	Diet 1	Diet 2
Maize (8.6% CP)	800	-
Barley (11.5% CP)	-	800
Dicalcium phosphate	20	20
Limestone	11	11
Salt	5	5
Choline chloride (50%)	2	2
Premix*	5	5
Casein (Dried)	150	150
DL Methionine	7	7

* The active ingredients contained in each kg of the vitamin-mineral premix were as follows: retinol, 3.03 mg; cholecaliferol, 0.09 mg; all-*rac*-a-tocopherol acetate, 20 mg; menadione, 6.3 mg; riboflavin, 8 mg; pyridoxine hydrochloride, 5 mg; biotin, 0.01 mg; niacin, 30 mg; Fe, 20 mg; Cu, 5 mg; I, 1 mg; Co, 0.3 mg; Se, 0.5 mg; Mo, 0.16 mg; cyanocobalamin, 0.15 mg.

4.3 Results

The results were calculated and analysed for statistical significance according to the procedures described under Section 3.7. Table 4.4 shows a summary of the interaction between maize or barley diets and the level of VFAs as energy in the excreta of birds.

Table 4.2 shows the performance data. Feeding broiler birds (2-4 weeks of age) on maize (Diet 1) or barley (Diet 2) significantly affected feed intake per kg body weight ($P<0.05$) and the FCR ($P<0.01$); with the FCR values on the maize diet being higher than those for barley. The effects of the type of diet on feed intake per bird per day, weight gain and mean bird weight were not significant ($P>0$ 05). However, there was a numerical increase of 21.5% in weight gain and 18.8% in the overall mean weight of the birds.

Table 4.2 The effect of maize or barley diets on bird performance

	MAIZE		BARLEY		
Measure	**Mean**	**SE**	**Mean**	**SE**	**P-Value**
Feed intake (g/bird/d)	71.7	2.6	72.8	2.5	0.848
Feed intake(g/kg b wt/d)	99.3	1.7	121.8	1.4	0.025
Gain (g/bird/d)	47.5	2.9	37.3	1.6	0.220
Overall mean bird Wt (g)	722	21	586	19	0.064
FCR	1.527	0.048	1.981	0.038	0.006

The energy-related information is presented in Table 4.3. The mean RQ, HP and HI values for the birds fed different diets were not significantly different (P>0.05). A highly significant difference (P<0.01) was found between the AME value of the two diets; the mean AME value for maize was higher than that for barley. The mean NE values were also significantly affected (P<0.01) by the type of diet; birds fed maize retained more energy than birds fed barley.

Table 4.3 The effect of maize or barley diets on energy utilisation

	MAIZE		BARLEY		
Measure	**Mean**	**SE**	**Mean**	**SE**	**P-Value**
RQ	0.991	0.018	1.026	0.005	0.069
HP (MJ/kg/b wt/d)	0.760	0.009	0.792	0.005	0.510
HI (HP-FH)					
(MJ/kg b wt/d)	0.166	0.006	0.179	0.005	0.578
HI (MJ/kg feed)	1.68	0.057	1.50	0.053	0.315
AME (MJ/kg feed)	16.5	0.041	13.4	0.053	0.004
AME-HP					
(NE:MJ/kg feed)	8.66	0.192	6.71	0.141	0.005

*FH-Fasting heat

Table 4.4 summarises the effect of the two diets on loss of VFAs as energy in the excreta. The difference between diets with respect to the mean VFA energy in the excreta was

significant (P<0.05). The means of energy lost as VFAs in the excreta over the 4-day period (KJ/d) were 22.8 ± 2.6 and 63.1 ± 5.2 on the maize and barley diets, respectively.

Table 4.4 The effect of diets based on maize or barley on loss of energy as VFAs in the excreta

	Mean of total VFA energy (KJ/ chamber/d)						
Diet	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**4-day Mean**	**SE**	**P-value**
Maize	26.0	27.4	21.2	24.5	22.8	2.6	
Barley	91.7	65.8	65.9	55.9	63.1	5.3	0.035

4.4 Discussion

Maize and barley were chosen to test the hypothesis, that heat production during metabolism and energy loss as volatile fatty acids in the excreta are influenced by the type of cereal grains used in diets. The current results demonstrated that feed intake, FCR, AME, NE and energy loss as VFAs in the excreta differed markedly between the diets.

The results recorded in this experiment, in which maize and barley diets significantly affected feed intake (P<0.05) and AME (P<0.01), are in agreement with the reports from SCA (1987) and NRC (1994) that the nutritive value of maize and barley for poultry differs widely, especially in the ME contents.

Birds on the barley diet consumed (Mean ± SE) a larger amount (121.8 ± 1.4g) of feed per kg body weight each day than those on the maize diet (99.3 ± 1.7g). Leeson and Summers (1990) estimated the ME contents of maize (yellow corn) and barley as 13.93 MJ/kg and 11.7 MJ/kg, respectively which supports the concept that birds regulate intake of diet according to their energy demands. Feltwell and Fox (1978) and SCA (1987) reported that ME content of the diet is the major factor governing the amount of feed consumed.
Leeson and Summers (1991) indicated that any values obtained to measure dietary energy in poultry feeds were dependent upon the bird's dietary balance and productive performance. Similarly, it is well recognised that animals have major appetites for energy

(Leeson *et al.*, 1993) as well as protein (Forbes and Shariatmadari, 1994). Unfortunately, the experimental design was compromised in that the diets used to test these two grains were not isonitrogenous and thereby compromised the growth of the birds, and this may have confounded any results obtained and these conclusions may have limited relevance to the practical production of broilers and the use of these grains in poultry diets. It is therefore difficult to attribute the differences in feed intake and subsequently FCR, AME and NE to the grains' energy content or to the fact that the diets were vastly different in protein content and that the birds were eating to protein appetite, given their massive growth rate at the age at which they were used.

The data from the current trial showed that the type of diet did not affect weight gain. The overall mean weight of the birds was not affected although it approached significance with numerical differences of 21.5% between weight gains and 18.8% between overall mean weight. Again, the current experimental design compromised any results obtained, with only two replicates of two birds each. Similar respiration calorimetry trials performed by Jones (1990) and Jones and Farrell (1992) were conducted. The previously mentioned authors suggested the use of 8 birds (male) per group and each treatment was repeated four times to minimise the differences inherent between broilers. This is regarded (Jones pers. comm.) as the minimum number of broilers that need to be used in nutritional experimentation following the collation of a large number of broiler trials and the statistical assessment of that data according to Berndtson (1991).

If the AME and NE values were in fact markedly ($P<0.01$) different and were not artifacts of the experimental design, they may have been different due to the higher levels of starch in maize. As much as these cereals differ in value with respect to the levels of digestible starch (Classen and Bedford, 1991; McDonald *et al.*, 1995), the main differences that contributed to the variations in this experiment in ME and NE may have been influenced by the levels of NSPs present in the diets (Choct and Annison, 1990).

As already mentioned in Section 2.2, extensive work has established that soluble NSPs are the major components of dietary fibre that have anti-nutritive activities in broiler birds (Janssen and Carré, 1989; Annison *et al.*, 1991, 1992; Choct *et al.*, 1996). The results of the current project may have illustrated that the performance of birds fed a barley diet with

respect to AME and NE (13.4 ± 0.05 and 6.7 ± 0.14, respectively) was lower than that of their counterparts fed a maize diet (16.5 ± 0.04 and 8.7 ± 0.19, respectively). Although the soluble NSPs, and in this case, presumably, the water-soluble β-glucans in the endosperm of barley grain (White *et al.*, 1981; Vranjes and Wenk, 1995), make up a small percentage of the total fibre component in cereals, their influence on the nutritive value of a diet can be large. Furthermore, Choct and Annison (1990) established the levels of β-glucans + pentosans at 4.38% DM for maize and 10.87% DM for barley and with corresponding AME values (MJ/kg DM) (Mean ± SE) of 15.8 ± 0.05 and 11.9 ± 0.25. This presents further evidence that the level of NSPs in cereals like barley have an anti-nutritive effect on energy availability to birds. The action of the soluble β-glucans is to increase the viscosity of the digesta in the small intestine, thereby interfering with the action of the digestive enzymes, as well as the absorption of nutrients, including VFAs, into the portal blood system.

The results of the current experiment in which the type of diet influenced ($P<0.05$) the energy determined as VFAs in the excreta (Table 4.3) are suggestive of the significance of the VFAs as a potential source of energy to the birds, as highlighted by Annison *et al.* (1968). Mean values of energy as VFAs in the excreta were 22.8 ± 2.6 KJ/d for birds given maize and 63.1 ± 5.3 KJ/d for birds given barley. When the bird consumes diets containing high levels of soluble NSP, for example barley, the digesta transit time lengthens due to high gut viscosity, leading to a decrease in oxygen tension in the gut which then encourages the proliferation of fermentative microorganisms (Choct and Annison, 1992b; Choct *et al*, 1992; Graham *et al.*, 1993). The net effect of this may be a decrease in the efficiency of energy utilisation by the bird and an increased loss of energy as VFAs in the excreta.