# The Constraining Effect of Feed Bulk on the Voluntary Feed Intake of Laying Hens 

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## DECLARATION

The experimental work described in this thesis was carried out in the Discipline of Animal Science and Poultry Science, School of Agricultural Sciences and Agribusiness, University of KwaZulu-Natal, Pietermaritzburg, under the supervision of Professor R.M. Gous.

These studies represent original work by the author and have not otherwise been submitted in any form for any degree or diploma to any University. Where use has been made of the work of others, it has been duly acknowledged in the text.


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#### Abstract

1. Two experiments were designed to determine a suitable method of measuring and predicting feed bulk, such that this could be used to predict when the feed intake of a laying hen would be constrained by feed bulk. 2. In the first trial the diluents used were cellulose, plasterer's sand, sunflower husks, sawdust and vermiculite. These were included at 100,250 and $500 \mathrm{~g} / \mathrm{kg}$ into a commercial layer feed which was used as the basal feed. The trial was divided into three phases of 21 days each. After each phase, either the diluent fed was changed, or the inclusion level of the diluent was changed. 3. It was observed that as the water-holding capacity (WHC) of the feed increased, the feed intake decreased. The scaled feed intake (SFI) of the hens was fitted to the reciprocal of the WHC to give the relationship; SFI ( $\mathrm{g} / \mathrm{kg}$ body weight) $=313.6( \pm 8.9) \times 1 / \mathrm{WHC}$. This regression was the best fit and represents the maximum amount of feed that the laying hen can consume when the constraint measured is the reciprocal of WHC. 4. Trial 2 identifies the physical characteristics of the feed that best describe the bulkiness of the feed, and also determined the extent, and the rate at which, the laying hen can adapt to feeds that are high in bulk. The five diluents that were used were wheat bran, river sand, potter's clay, unexpanded polystyrene and sawdust, and the inclusion rates were 50,100 and $150 \mathrm{~g} / \mathrm{kg}$. The hens were fed the feeds for six weeks. The equation from Trial 1 was fitted to the data from Trial 2 and few treatments were found to be constraining. 5. The constraining feeds from both trials and Williams (1993) were combined to obtain a more accurate assessment of the relationship between the SFI and the reciprocal of WHC. This relationship was represented by the equation; $\operatorname{SFI}(\mathrm{g} / \mathrm{kg}$ body weight $)=301.4( \pm 8.9) \times$ 1/WHC. 6. The prediction of the effect of feed bulk on the voluntary feed intake of the hen is an important aspect for accurately predicting the feed intakes of the hen and formulating a "perfect" diet. The variation in constrained intakes was not accurately predicted by the WHC of the feed, although this measure of bulkiness was considerably better than any of the other measures applied.


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You have powers you never dreamed of.
You can do things you never thought you could do.
There are no limitations in what you can do
Except the limitations in your own mind as to what you cannot do.
Don't think you cannot, think you can.
Darwin P. Kingsley

## CHAPTER 1

## Literature Review

### 1.1. Introduction

### 1.1.1. Why predict feed intake in the laying hen?

To formulate a balanced feed for a laying hen, a prior knowledge of the expected feed intake of the hen is required, as well as the requirements of the hen for essential nutrients. Whereas the nutrient requirements of the laying hen have previously been extensively researched, little progress has been made (to date) in predicting voluntary feed intake, mainly because of the lack of a plausible theory (Gous, in consultation, 2004). Although it is possible to calculate the optimum economic intakes of the essential amino acids, it is not possible to convert these into dietary concentrations without knowing how much food a hen will consume in a day. Therefore, it is not possible to formulate a feed with the optimum economic nutrient density until feed intake can be accurately predicted (Gous, in consultation, 2004).

Presently, the Reading model (Fisher et al., 1973) may be used to predict the amino acid requirements of the laying hen. This model considers the mean egg production and body weight of a flock of laying hens, their inherent variability in the flock, and economic factors such as the marginal cost of the amino acids and the marginal revenue for eggs. The theory that is used by the Reading model is that the requirement of the flock for each amino acid is a single point on a response curve - known as the optimum economic intake of that amino acid. A simple factorial model can explain this response for an individual (Fisher, 1976):

$$
\begin{equation*}
\mathrm{AA} \text { intake }=\mathrm{a} \cdot \mathrm{E}+\mathrm{b} \cdot \mathrm{~W} \tag{1}
\end{equation*}
$$

Where AA intake is amino acid intake in mg/day, E is egg output in $\mathrm{g} /$ day, W is body weight in kg , and a and b are constants, where a and b are constants specific to each amino acid (Table 1.1).

Two constraints can be applied. When the AA intake is less than b .W then E is equal to zero, and when the E is maximal, then $\mathrm{E}=\mathrm{E}_{\text {max }}$. The response curve for the flock is then derived from the average of the individuals that differ in $W$ and $E_{\text {max }}$. The constants $a$ and $b$ are assumed to be constant for all hens (Fisher, 1976), for a specific amino acid.

Table 1.1: $\quad$ The estimated coefficients for egg output and maintenance for the essential amino acids for laying hens (McDonald and Morris, 1985) for the equation $y=a . E+b . W$, where $y$ gives the amino acid requirements for a specific egg output ( $E$ in $\mathrm{g} /$ day) and body weight ( $W$ in kg ).

| Amino acid | $\mathbf{a}$ | $\mathbf{b}$ |
| :--- | :---: | :---: |
| Lysine | 9.99 | 73 |
| Methionine | 4.77 | 31 |
| Tryptophan | 2.62 | 11 |
| Isoleucine | 7.97 | 67 |
| Valine | 8.90 | 76 |
| Methionine and Cystine | 8.30 | 80 |
| Arginine | 8.90 | 53 |
| Histidine | 3.30 | 16 |
| Leucine | 12.50 | 32 |
| Phenylalanine and Tyrosine | 13.50 | 32 |
| Threonine | 6.90 | 32 |

The most important limitation of the Reading model is that it can only be used for flocks of young laying pullets in which $\mathrm{E}_{\text {max }}$ is normally distributed. Although the optimum intakes are accurately predicted, the model leads to an overestimation of requirements when the intake is divided by the mean feed intake of the flock. The model also assumes that egg value is directly proportional to egg mass, which may not always hold true (Fisher, 1976). This latter problem has been addressed by Morris and Gous (1988).

A model is required that will accurately predict the requirements of the mature laying hen so that overfeeding is minimized and an economical diet can be formulated easily and accurately. This can only be done when the feed intake of the hen is accurately predicted and the hen's requirements can be accurately converted into dietary concentrations. This will lead to less fat deposition during the laying period and, therefore, more efficient egg production.

### 1.1.2. Modelling feed intake

Emmans (1981a) proposed that an animal would eat to attain its genetic potential, through satisfying its requirement for maintenance and growth. The genetic potential for laying hens is defined by its egg output, which is the product of the number of eggs weighed and the egg weight. To meet this potential, defined by her genotype, the hen must consume sufficient quantities of the nutrients she requires from the feed provided to her. There are factors that exist that may prevent the hen from consuming sufficient nutrients. These factors are referred to as constraints and include the bulkiness of the feed, environmental temperature, nutrient excesses and toxins, and the health status of the hen (Emmans and Oldham, 1988).

The nutrient requirements for laying hens to sustain egg production and maintenance are defined by nutritional constants (Figure 1.1). The feed and the environment provide the resources to meet these requirements. The hen has a certain capacity to eat sufficient nutrients to meet these requirements. If the requirements can be met the hen will have achieved a desired feed intake (DFI). If the hen's capacities prevent the consumption of sufficient nutrients from the feed provided, feed intake will be constrained (CFI). The actual feed intake (AFI) is the lesser of the DFI and the CFI and will define the performance of the laying hen. This is illustrated in Figure 1.1.


Figure 1.1: A scheme proposed by Emmans and Oldham (1988) showing the regulation of feed intake in the laying hen, where DFI $=$ desired feed intake, $A F I=$ actual feed inatke, and CFI = constrained feed intake.

### 1.2. Maintenance Requirements of the Laying Hen

In broiler chickens to market age, maintenance is an almost negligibly small proportion of the total requirement for nutrients and a relatively small proportion of the requirement for energy. Conversely, in laying hens, even those producing at a high rate, maintenance energy is a major part of the total energy requirement and maintenance represents an appreciable proportion of the total requirement for protein and energy (Emmans and Fisher, 1986, and Young, 1998). The importance of quantifying maintenance requirements is thus much more important in laying hens than in commercial broilers. Armsby and Moulton (1925) describe the concept of maintenance as conserving the existing status of the animal, while doing no work and producing no product. There should be an exact balance between the in- and outflow of ash, nitrogen, heat and energy. This means that there is neither a loss nor gain of protein, fat, carbohydrate or mineral matter (Emmans and Fisher, 1986). Production animals are rarely kept in this non-productive state, so it might seem only of academic interest to derive the maintenance requirements of the laying hen. However, summing the requirements calculated separately for maintenance and production arrives at the total requirements of several classes of animals. Consequently knowledge of
the maintenance requirements is of practical and theoretical significance (McDonald et al., 1995).

The nutrient requirements of the laying hen will depend on the state of the hen (maintenance) and the potential rate of production. The animal requires resources to maintain its current state. These resources are defined as the animal's maintenance requirements. The maintenance requirements are reflected by the chemical composition of the body and feathers and the weights of these two components of the hen. Armsby and Moulton, 1925, defined the body of an animal as the gut-fill and the empty body. The empty body is calculated as:

$$
\begin{equation*}
\text { Empty body }(\mathrm{g})=\text { protein }+ \text { ash }+ \text { water }+ \text { lipid } \tag{2}
\end{equation*}
$$

To predict the composition of an animal in a certain state the values of eight parameters must be known and substituted into Equation 3 to determine the rate of change of the body composition (dEW/dt) (Emmans and Fisher, 1986).

$$
\begin{equation*}
\mathrm{dEW} / \mathrm{dt}=\left[\mathrm{P}_{\mathrm{m}} \cdot \mathrm{~B} \cdot \ln (1 / \mathrm{u})\right]\left(\mathrm{u}+\mathrm{z}^{1} \cdot \mathrm{u}^{\mathrm{c}}+\mathrm{z}^{2} \cdot \mathrm{u}^{\mathrm{c} 2}+\mathrm{z}^{3} \cdot \mathrm{u}^{\mathrm{c} 3}\right) \tag{3}
\end{equation*}
$$

Where $z^{1}, c^{1}$ are for ash, $z^{2}, c^{2}$ are for water, $z^{3}, c^{3}$ are for lipid, $P_{m}$ is the mature protein weight and $B=1 / k$, where $k$ is a characteristic of the animal. The value of $u$ is calculated as the ratio of the protein weight against the protein weight at maturity, and is referred to as the degree of maturity. The growth and composition of the hen depend on her genotype and are, therefore, inherited. It can be assumed that the parameters of the equation are then inherited. The values for $\mathrm{c}_{\text {ash }}=0, \mathrm{z}_{\text {ash }}=0.19, \mathrm{c}_{\text {water }}=-0.11$, and $\mathrm{z}_{\text {water }}=3$ are constant across all genotypes. The values for $\mathrm{c}_{\text {lipid }}$ and $\mathrm{z}_{\text {lipid }}$ are highly correlated across genotypes, but vary widely between genotypes. The gut fill can be predicted from the rate of feed intake and the feed composition (Emmans and Fisher, 1986).

Maintenance energy is mainly obtained by the oxidation of body fat if the animal is given no food (McDonald et al., 1995). This production of energy is transferred to the nutrients
in a diet if food is provided. No heat is produced if the efficiency of conversion of the nutrients to energy is the same as when body fat is converted to energy.

| For maintenance: | $\mathrm{PR}=\mathrm{LR}=0$ |
| :--- | :--- |
| Therefore: | $\mathrm{ER}=0$ |

Where PR is the protein retention, LR is the lipid retention, and ER is the energy retention (Emmans, 1994).

The protein requirements for maintenance can be described as the loss of nitrogen in the faeces and urine once the protein reserves of the animal have been depleted (McDonald et al., 1995). The nitrogen in the faeces arises from the enzyme and cell residues of the digestive tract. The nitrogen in the urine represents nitrogen which has been incorporated into materials, subsequently expended, and which cannot be recovered by the body for reuse. The majority of the nitrogen in the urine is in the form of urea, the by-product of amino acid catabolism. The turnover rate of body proteins varies from one tissue to another. The amino acids released when body proteins are broken down form a pool from which replacement proteins are synthesised. Therefore, the body proteins exchange amino acids amongst themselves. Amino acids liberated from one protein may fail to be incorporated into another and are catabolised. The amino groups yield urea, which is excreted in the urine (McDonald et al., 1995).

Once the protein reserves of an animal have been depleted, the urinary nitrogen excretion reaches a minimal and rather constant level. The nitrogen excreted at this minimal level is known as the endogenous urinary nitrogen. The endogenous urinary nitrogen excretion can therefore be used to estimate the nitrogen, and protein, required by the animal for maintenance (McDonald et al., 1995).

When the animal is eating a maintenance diet, the rates of protein and lipid retention are zero, but the animal is consuming feed and is, therefore, producing heat ( $\mathrm{HM}, \mathrm{kJ} /$ day $)$ (Emmans, 1994). The heat increment of a maintenance diet (HIM) relative to the maintenance heat (MH) can be defined as Equation 4.

$$
\begin{equation*}
\mathrm{HIM}(\mathrm{~kJ} / \mathrm{day})=\mathrm{HM}-\mathrm{MH} \tag{4}
\end{equation*}
$$

The general scaling rule of maintenance (Equation 5) relates the supply of nutrients to the animal's state and genotype so that the lipid and protein retention is zero.

$$
\begin{equation*}
\mathrm{MH}=\mathrm{M}_{\mathrm{E}} \times \mathrm{P}_{\mathrm{m}}{ }^{0.73} \times \mathrm{u} \quad(\mathrm{MJ} / \mathrm{d}) \tag{5}
\end{equation*}
$$

Where $\mathrm{P}_{\mathrm{m}}$ is the mature protein weight in kg and u is the degree of maturity. The degree of maturity is calculated by dividing the body protein weight by the mature protein weight. The value of $\mathrm{M}_{\mathrm{E}}$ is constant over all values of $\mathrm{P}_{\mathrm{m}}$ and degrees of maturity. The maintenance scaling rule implies that no energy is needed to maintain body lipid. Work done with pigs has shown that body lipid has a zero rate of turnover (Emmans, 1987). Emmans (1987) defines $\mathrm{M}_{\mathrm{E}}$ as a constant equal to $1.63 \mathrm{MJ} / \mathrm{unit}$ per day in a thermally neutral environment with the maintenance unit of the function equal to $\mathrm{P}_{\mathrm{m}}{ }^{0.73} \times \mathrm{u}$ per day (Emmans, 1987). The amino acid requirements for maintenance can then be calculated from the maintenance protein requirement and the amino acid composition of body protein (Emmans, 1987).

Emmans (1994) assumes that the heat increment produced over the MH is related to two activities:

- The hen's consumption of organic matter (OM).
- The excretion of nitrogen in the urine (UN).

The maintenance diet of $O M(\mathrm{~g} / \mathrm{d})$ will lead to the excretion of $0.16 \times$ digestible crude protein (DCP) to give the amount of urinary nitrogen (UN) in grams of nitrogen ( $g N$ ) in the urine/day. The maintenance diet will also lead to the production of methane (MTHE, $\mathrm{kg} / \mathrm{d}$ ) (Emmans, 1994). It is assumed that the heat increment over the maintenance heat, due to the animal eating a maintenance diet, is related to just three activities: its consumption of organic matter, its excretion of N in the urine, and its production of methane. It is further assumed that the rates of heat production associated with these activities are linearly related to the quantities of OM, UN and MTHE (Emmans, 1994). Therefore, the heat increment (HI), due to the animal consuming a maintenance diet, is given by Equation 6.

$$
\begin{equation*}
\operatorname{HIM}(\mathrm{kJ} / \mathrm{d})=\left(\mathrm{w}_{\mathrm{d}} \cdot \mathrm{OM}\right)+\left(\mathrm{w}_{\mathrm{u}} \cdot \mathrm{UN}\right)+\left(\mathrm{w}_{\mathrm{m}} \cdot \mathrm{MTHE}\right) \tag{6}
\end{equation*}
$$

Where $w_{d}$ is the heat associated with the digestibility of the OM in the diet, $w_{u}$ is the heat associated with the excretion of UN , and $\mathrm{w}_{\mathrm{m}}$ is the heat associated with the production of methane (Emmans, 1994). For most cases the MTHE value can be considered zero for single stomached animals (Emmans, 1994). Therefore, the HIM can be estimated from the digestible OM, know as the faecal organic matter (FOM), and the UN in Equation 7.

$$
\begin{equation*}
\mathrm{HIM}(\mathrm{~kJ} / \mathrm{day})=\left(\mathrm{w}_{\mathrm{d}} \cdot \mathrm{FOM}\right)+\left(\mathrm{w}_{\mathrm{u}} \cdot \mathrm{UN}\right) \tag{7}
\end{equation*}
$$

Combining Equations 5 and 7 allows the amount of ME required for maintenance (MEM) to be defined in Equation 8 (Emmans, 1994).

$$
\begin{equation*}
\operatorname{MEM}(\mathrm{kJ} / \text { day })=\mathrm{MH}+\mathrm{HIM} \tag{8}
\end{equation*}
$$

Equation 8 defines the energy requirements of the laying hen for maintenance which can be converted to nutrient concentrations when the feed intake is predicted.

### 1.2.1. White layers vs. brown layers

The Hy-line variety Brown hens lay slightly less hen-housed and hen-day eggs than the Silver Brown variety, but on average they lay slightly heavier eggs. The Hy-line variety Brown requires less feed per kg eggs produced than the Silver Brown variety, the difference being 20 g (Hy-line Commercial Management Guide, 2004). Therefore the Brown variety has slightly higher production requirements than the Silver variety, but the maintenance requirements are similar.

### 1.2.2. Feathering

The feather conditions of the ben will affect her maintenance requirements and her feed efficiency. Leeson and Morrison (1978) found that feed efficiency was significantly correlated with feather weight and could be described by the linear equation:

$$
\begin{equation*}
\mathrm{Y}(\mathrm{FE}(\mathrm{~g} \mathrm{feed} / \mathrm{g} \text { egg mass }))=-0.04(\mathrm{FW})+5.4 \tag{9}
\end{equation*}
$$

Where $y$ is the feed efficiency ( $\mathrm{P}<0.05$ ) (Leeson and Morrison, 1978). This suggests that feed efficiency deteriorates for every $0.04 \mathrm{~g}(\mathrm{SE} \pm 0.01)$ for each gram loss in feather cover. The effect of feather cover in relation to feed efficiency is likely to increase at low ambient temperatures.

### 1.3. The Requirements for Egg Production

The hen requires nutrients to sustain her egg output. The egg output is defined as the product of the rate of egg production and weight of the eggs being produced. Therefore, by analysing these factors, the requirements of the hen for production can be estimated.

### 1.3.1. Composition of the hen's egg

The nutrient composition of the egg will depend on the relative proportions of yolk, albumen and shell of which the egg is composed. Table 1.2, Table 1.3 and Table 1.4 describe the average characteristics of these three components from the work of Romanoff and Romanoff (1949), Cunningham et al. (1960), Jaffé (1964), and Shenstone (1968). These characteristics may be used to define the composition of an average egg.

Table 1.2: $\quad$ The yolk, albumen and shell as a percentage of the whole egg, also showing the percentage of water and solids in the three components. Taken from Romanoff and Romanoff (1949), Cunningham et al. (1960), Jaffé (1964), and Shenstone (1968)

| Component | Percentage of whole <br> egg | Water percentage | Solids percentage |
| :--- | :---: | :---: | :---: |
| Yolk | 32 | 23.6 |  |
| Albumen | 58 | 76.2 | 20.0 |
| Shell | 10 | 0.2 | 29.0 |

Table 1.3: $\quad$ The weight in grams of the components of the egg. Taken from Romanoff and Romanoff (1949), Cunningham et al. (1960), Jaffé (1964), and Shenstone (1968)

| Component | Solid (g) | Water (g) | Total (g) |
| :--- | :---: | :---: | :---: |
| Yolk | 9.9 | 9.1 | 19.0 |
| Albumen | 3.8 | 29.4 | 33.2 |
| Shell | 5.7 | 0.1 | 5.8 |
| Total | 19.4 | 38.6 | 58.0 |

Table 1.4: The percentage composition of the yolk, albumen and shell. Taken from Romanoff and Romanoff (1949), Cunningham et al. (1960), Jaffé (1964), and Shenstone (1968)

| Chemical class | Yolk | Albumen | Shell |
| :--- | :---: | :---: | :---: |
| Water | 47.5 | 88.5 | 1.0 |
| Protein | 17.4 | 10.5 | 4.0 |
| Lipid | 33.0 | - | - |
| Carbohydrate | 0.2 | 0.5 | - |
| Inorganic ions | 1.1 | 0.5 | 95.0 |
| Other | 0.8 | - | - |

### 1.3.1.1. Composition of the yolk

The yolk is made up of two parts, namely the white and yellow yolk. These two parts are chemically different to each other. The white yolk makes up 1-2\% of the total yolk mass and the rest is made up of the yellow yolk. The white and yellow yolk are laid down in layers, which has lead to the development of the 'alternating-layer' theory (Romanoff and Romanoff, 1949 and Bellairs, 1964). Because the yellow yolk is not homogenous the yolk granules may become layered resulting in the apparent layered effect (Bellairs, 1964). White yolk contains only $10-13 \%$ solids and is described by Bellairs (1964) as an oil-water emulsion. White yolk has a higher proportion of protein to lipid than yellow yolk (Bellairs, 1964).

In general, yolk contains just over 50\% solids (Romanoff and Romanoff, 1949). These proteins are divisible into three classes depending on their behaviour in the ultra-centrifuge (Gilbert, 1972). The protein classes are low density (LD), water soluble and granular
fractions. The LD fraction is the most abundant, making up $34 \%$ of the yolk, and containing $90 \%$ of the lipids and nearly $20 \%$ of the phosphorus as phospholipid. The water-soluble proteins form $10 \%$ of the yolk solids and contain $30 \%$ of the yolk protein (Gilbert, 1972). The granular fraction is divided into two more fractions, namely the highdensity fraction (lipovitellins) and the phosvitin fraction (Gilbert, 1976). The phosvitin fraction contains nearly $70 \%$ of the yolk phosphorus as phosphoprotein, but only forms $4 \%$ of the yolk solids. The high-density fraction forms $16 \%$ of the yolk solids (Connelly and Taborsky, 1961) and is $25 \%$ lipid (Gilbert, 1972).

The amino acid composition of the yolk is shown in Table 1.5. This can represent the requirement to produce an egg.

Table 1.5: $\quad$ The amino acid composition of the yolk of a hen's egg in $\mathrm{mg} / \mathrm{g}$ nitrogen (Lunven and Le Clement De St Marcq, 1973)

| Amino Acid | Standard average amino acid composition $(\mathrm{mg} / \mathrm{g}$ <br> Nitrogen $)$ |
| :--- | :---: |
| Arginine | 434 |
| Cysteine | 163 |
| Histidine | 148 |
| Isoleucine | 348 |
| Leucine | 548 |
| Lysine | 477 |
| Methionine | 175 |
| Phenylalanine | 273 |
| Threonine | 313 |
| Tryptophan | 121 |
| Tyrosine | 253 |
| Valine | 378 |

Yolk lipids are restricted to the lipovitellins and the low-density fractions of the yolk. In total yolk lipids form $30 \%$ of the yolk and $60-70 \%$ of the yolk solids (Romanoff and Romanoff, 1949).

The composition of the yolk is summarized by Gilbert (1972) and is shown in Table 1.6.

Table 1.6: $\quad$ Composition of the yolk of a hen's egg (Gilbert, 1972)

| Protein class | \% yolk <br> weight | \% yolk <br> solids | \% yolk <br> proteins | \% yolk lipids |
| :--- | :---: | :---: | :---: | :---: |
| LD fraction | 33.7 | 65.0 | 22.0 | 93.0 |
| Livetins | 5.3 | 10.0 | 30.0 | - |
| Granules: |  |  |  |  |
| $\quad$ Phosvitin | 2.1 | 4.0 | 12.0 | - |
| $\quad$ Lipovitellins | 8.3 | 16.0 | 36.0 | 7.0 |
| $\quad$ Other | 2.6 | 5.0 | - | - |

### 1.3.1.2. Composition of the albumen

The albumen weighs just less than two thirds of the egg and consists of one part protein to two parts water. The solid portion makes up $11 \%$ of the total, of which $92 \%$ is protein. The non-protein solids are made up of carbohydrates and inorganic ions (Gilbert, 1972).

Egg albumen is not homogenous, but consists of two fractions; a thin, watery solution (thin albumen) and a thick gelatinous material (thick albumen) (Gilbert, 1972). The protein content of each layer differs only slightly (Feeney and Allison, 1969). The thick albumen forms $60 \%$ of the total albumen and is rich in the albumin protein ovomucin (Shenstone, 1968). This seems to be the only difference between the thick and thin albumen fractions.

There are a vast number of different proteins present in the egg albumen. What is of importance when predicting the requirements of the laying hen is the amino acid composition of these proteins. This is shown in Table 1.7.

The amino acid composition of the hen's egg can be used to predict the amino acid requirements of the hen for egg production. Amino acid requirements are a function of the rate of production, the weight and composition of the egg, and the efficiency with which the hen converts dietary amino acids into egg protein (Fisher, 1976).

Table 1.7: $\quad$ The amino acid composition of the albumen of a hen's egg in $m g / g$ Nitrogen (Lunven and Le Clement De St Marcq, 1973).

| Amino Acid | Standard average amino acid composition (mg/g <br> Nitrogen) |
| :--- | :---: |
| Arginine | 330 |
| Cysteine | 178 |
| Histidine | 132 |
| Isoleucine | 331 |
| Leucine | 521 |
| Lysine | 378 |
| Methionine | 240 |
| Phenylalanine | 368 |
| Threonine | 272 |
| Tryptophan | 116 |
| Tyrosine | 257 |
| Valine | 429 |

### 1.4. Efficiency of Utilization of Nutrients

Dietary amino acids are not converted directly to egg protein: there is some loss in efficiency in the process that must be taken into account when calculating the amino acid requirements for egg production. McDonald and Morris (1985) examined the efficiency of conversion of five dietary amino acids into egg protein. The efficiencies ranged from 0.74 to 0.83 (Table 1.8). Combining these estimates gives an average efficiency of 0.77 (McDonald and Morris, 1985). Differences in efficiency may result from uncertainties in the estimates of $a$ or of the amino acid content of egg protein or both. A contributing factor may be differences in the digestibility of amino acids, or in the case of methionine, amino acid being diverted to an alternative output-dependent process (McDonald and Morris, 1985). The calculated efficiencies for the conversion from feed into egg protein are given in Table 1.8.

Table 1.8: $\quad$ The calculation of efficiency of utilization of amino acids to produce Ig of egg protein

|  | AA present in egg <br> (mg/g nitrogen) <br> Amino acid <br> (Lunven and Le <br> Clement De St <br> Marcq, 1973) | AA AA/g egg <br> requirement <br> per 1 g egg <br> output <br> $(\mathrm{mg} / \mathrm{g} \mathrm{egg)}$ | Efficiency of <br> utilization <br> (McDonald and <br> Morris, 1985) |  |
| :--- | :---: | :---: | :---: | :---: |
| Lysine | 378 | 7.93 | 10.04 | 0.79 |
| Methionine | 240 | 3.90 | 5.27 | 0.74 |
| Isoleucine | 331 | 6.33 | 8.33 | 0.76 |
| Tryptophan | 116 | 2.21 | 2.99 | 0.74 |
| Valine | 429 | 7.55 | 9.10 | 0.83 |

Determining what quantity of the limiting amino acid is required per gram of egg output, and then comparing this with the amount of that amino acid deposited in the egg can yield the efficiency of utilization of an amino acid. The amount of amino acid deposited in the egg can be calculated from the constants in Table 1.7. The average protein in the albumen and yolk are calculated separately and then added together to give the total protein in the egg. The weight of the albumen is calculated based on the fact that the albumen constitutes $58 \%$ of the total weight of the egg (Romanoff and Romanoff, 1949, Cunningham et al. 1960, and Shenstone, 1968). The protein weight of the albumen is then calculated as $10.5 \%$ of the total albumen weight (Table 1.4). This is then converted into mg of nitrogen by dividing by the constant 6.25 . The yolk protein is then calculated by substituting $17.4 \%$ for the $10.5 \%$ (Table 1.4). The proportion of the weight that the yolk makes up is $32 \%$ (Romanoff and Romanoff, 1949, Cunningham et al. 1960, and Shenstone, 1968). These values are then compared with the amount of amino acids that the hen requires to produce 1 g of egg output. By calculating the ratio between the mg of amino acid per gram of egg and the mg amino acid required to form a gram of egg the efficiency of utilization of the amino acid can be calculated. Table 1.8 illustrates the amount of each amino acid required to produce 1 g of egg.

When referring to efficiencies the limiting amino acid is always referred to - there is no value in determining to what extent the non-limiting amino acids are utilized, as this
depends on the amount of feed consumed relative to the first limiting amino acid. It is only really important to know to what extent the first limiting amino acid is utilized by the bird, as this is then applied to all amino acids, assuming that each will be first limiting - which is the case in a perfectly balanced feed (Fisher et al. 1973). Knowing the requirements of the laying hen and her rates of maintenance and production, her total requirements related to these two factors can be calculated for the individual.

### 1.4.1. Energy requirements of egg production

A diet that leads to positive retentions of protein and lipid, at the rates of $P R$ and $L R(g / d)$, will be associated with the production of faecal organic matter (FOM) and UN (Emmans, 1994). It is assumed that there are heat increments (HI), in addition to the HI associated with maintenance, with the positive retention of protein and lipid. These HI are assumed directly proportional to PR and LR. Therefore, a diet that leads to positive protein and lipid retention has a heat increment of the feed (HIF) relative to the maintenance heat (MH) given as shown in Equation 10.

$$
\begin{equation*}
\operatorname{HIF}(\mathrm{kJ} / \mathrm{d})=\left(\mathrm{w}_{\mathrm{d}} \cdot \mathrm{FOM}\right)+\left(\mathrm{w}_{\mathrm{u}} \cdot \mathrm{UN}\right)+\left(\mathrm{w}_{\mathrm{p}} \cdot \mathrm{PR}\right)+\left(\mathrm{w}_{\mathrm{l}} \cdot \mathrm{LR}\right) \tag{10}
\end{equation*}
$$

Where $\mathrm{w}_{\mathrm{p}}$ is the heat associated with the rate of PR and $\mathrm{w}_{1}$ is the heat associated with the rate of LR.

The laying hen also has heat production associated with egg production. This is related to the rate of heat produced per egg, or the heat of combustion of the egg, and the average energy content of the egg produced (Emmans, 1994). Hoffman et al. (1973), as cited by Young (1998), stated the heat of combustion of eggs $\left(\mathrm{w}_{\mathrm{e}}\right)$ as $0.48 \mathrm{~kJ} / \mathrm{kJ}$ where the average energy content of eggs is $251 \mathrm{~kJ} / \mathrm{g}$ egg (Emmans, 1994). Therefore the heat increment (HI) of egg production can be defined as:

$$
\begin{equation*}
\mathrm{HI}(\mathrm{egg} \text { production })=0.48 \times 251=120.48 \mathrm{~kJ} / \mathrm{g} \text { egg } \tag{11}
\end{equation*}
$$

This can be included into the classical metabolisable energy (ME) equation so that:

$$
\begin{equation*}
\mathrm{ME}(\mathrm{~kJ} / \text { day })=\mathrm{FHP}+\mathrm{h}_{\mathrm{p}} \cdot \mathrm{PR}+\mathrm{h}_{1} \cdot \mathrm{LR}+\mathrm{w}_{\mathrm{e}} \cdot(\mathrm{EW} \cdot \mathrm{ROL})+\mathrm{H} \tag{12}
\end{equation*}
$$

Where FHP is the fasting heat production, PR and LR are the rates of retention of protein and lipid in $g / d$ and $h_{p}$ and $h_{i}$ are their heats of of combustion in $\mathrm{kJ} / \mathrm{g}$, EW is the egg weight, $w_{e}$ is the heat of combustion of an egg, ROL is the rate of lay, and H is the rate of heat production/loss (Emmans, 1994).

There is an obvious increase in the heat production (HP) when the hen is fed above maintenance requirements. This increase in heat production is due to the deposition of protein and lipid, the excretion of UN and FOM, and the formation of eggs. Therefore; $\mathrm{HP}(\mathrm{kJ} /$ day $)=\mathrm{MH}+\mathrm{HI}($ excretion $)+\mathrm{HI}($ defæcation $)+\mathrm{HI}($ fattening $)+\mathrm{HI}($ egg prod. $)$
1.4.1.1. The effective energy (EE) scale for the laying hen

The heat increment of feeding (HIF) can be broken down as the heat increment of maintenance (HIM) and the heat increment of the feed. These are defined as (Emmans, 1994):

$$
\begin{align*}
& \operatorname{HIM}(\mathrm{kJ} / \text { day })=\left(\mathrm{w}_{\mathrm{u}} \cdot \mathrm{UN}\right)+\left(\mathrm{w}_{\mathrm{d}} \cdot \mathrm{FOM}\right)  \tag{13}\\
& \operatorname{HIF}(\mathrm{kJ} / \text { day })=\left(\mathrm{w}_{\mathrm{p}} \cdot \mathrm{PR}\right)+\left(\mathrm{w}_{\mathrm{l}} \cdot \mathrm{LR}\right) \tag{14}
\end{align*}
$$

But, $\mathrm{UN}=0.16(\mathrm{DCP}-\mathrm{PR})$, therefore;

$$
\begin{equation*}
\operatorname{HIM}(k J / d a y)=\left(w_{u} \cdot 0.16(D C P-P R)\right)+\left(w_{d} \cdot F O M\right) \tag{15}
\end{equation*}
$$

By subtracting the HIM from ME in the diet leaves the energy supply scale:

$$
\begin{equation*}
\mathrm{EE}(\mathrm{~kJ} / \mathrm{day})=\mathrm{ME}-\left[\left(\mathrm{w}_{\mathrm{u}} \cdot 0 \cdot 16 \cdot \mathrm{DCP}\right)+\left(\mathrm{w}_{\mathrm{d}} \cdot \mathrm{FOM}\right)\right] \tag{16}
\end{equation*}
$$

Where FOM is the faecal organic matter and DCP is the digestible crude protein. The term relating to the excretion of methane is assumed zero for single stomached animals and not included for the laying hen. The requirement for effective energy (EERQ) is the sum of the maintenance heat (MH) and the heat increment (HI) of the positive protein and lipid retentions:

$$
\begin{equation*}
\operatorname{EERQ}(\mathrm{kJ} / \text { day })=\mathrm{MH}+\operatorname{PR}\left[\left(\mathrm{h}_{\mathrm{p}}-\mathrm{a}\right)+\left(\mathrm{w}_{\mathrm{p}}-0.16 \mathrm{w}_{u}\right)\right]+\operatorname{LR}\left(h_{1}+w_{1}\right) \tag{17}
\end{equation*}
$$

By substituting in the constants $\mathrm{w}_{\mathrm{U}}=29.2 \mathrm{~kJ} / \mathrm{g}, \mathrm{w}_{\mathrm{d}}=3.8 \mathrm{~kJ} / \mathrm{g}, \mathrm{w}_{\mathrm{p}}=36.5 \mathrm{~kJ} / \mathrm{g}$, and $\mathrm{w}_{\mathrm{l}}=16.4$ $\mathrm{kJ} / \mathrm{g}$ the effective energy scale is defined as:

$$
\begin{equation*}
\mathrm{EERQ}(\mathrm{~kJ} / \mathrm{day})=\mathrm{MH}+50 \mathrm{PR}+56 \mathrm{LR} \tag{18}
\end{equation*}
$$

The EERQ defines the effective enegy requirement of the animal. In the laying hen, growth is not a major consideration once point of lay has been reached. It is for this reason that Emmans (1994) developed another equation for laying hens.

$$
\begin{equation*}
\text { EERQ (kJ/day) HENS }=\mathrm{MH}+8.8 \mathrm{EO} \tag{19}
\end{equation*}
$$

Where EO is the egg output in $\mathrm{g} / \mathrm{day}$ and $8.8 \mathrm{~kJ} / \mathrm{g}$ is the effective energy required per gram of egg. The effective energy required per gram of egg was calculated by Emmans (1994), using the information of Hoffman et al. (1973), as cited by Young (1998). The average energy of the eggs recorded by Hoffman et al. was $6.71 \mathrm{~kJ} / \mathrm{g}$ and 0.1117 g protein per g egg, compared to the 0.12 g protein per g of egg calculated from Tables 1.3 and 1.4. The heat increment was calculated as $0.48 \times 6.71=3.22 \mathrm{~kJ} / \mathrm{g} \mathrm{egg}$ (Young, 1998).

On the effective energy scale, the energy retained in eggs needs to be reduced by $5.63 \mathrm{~kJ} / \mathrm{g}$ protein and the heat increment by $4.67 \mathrm{~kJ} / \mathrm{g}$ protein (Emmans, 1987). This means that the effective energy requirement per egg becomes:

$$
\begin{equation*}
\operatorname{EERQ}=(6.71+3.22)-0.1117(5.63+4.67)=8.8 \mathrm{~kJ} / \mathrm{g} \text { egg. } \tag{20}
\end{equation*}
$$

### 1.4.2. Predicting the production requirements of the laying hen

The protein and energy requirements for egg production can be predicted considering the characteristics of the egg being formed (Emmans and Fisher, 1986). Analysing the egg produced and including an efficiency factor to account for the efficiency with which feed nutrients are converted to egg protein can predict the quantity of nutrients required to form the egg. However, the composition of every egg is not identical and factors like the age of
the hen and the size of the egg will affect the requirements. The degree to which these factors affect the egg composition is important when predicting the requirements for producing an egg (Emmans and Fisher, 1986).

Egg production is more difficult to describe than growth because a longer period is involved and the hen passes through many periods in her productive life. The laying hen's productive life can be divided into three periods (Gilbert, 1969). The first period represents the onset of reproductive activity. The laying pattern of the hen during this period is often erratic and irregularities are common. These irregularities include laying more than one egg a day, production of soft-shelled eggs, the production of double yolked eggs, and irregular laying intervals. Egg weight is often low during this period, but increases exponentially to sexual maturity (Gilbert, 1969). The second period represents the main period of lay after sexual maturity has been reached and should be the longest period in the productive life of the hen. Egg weight and egg output should be constant during this period (Gilbert, 1969). The third period represents the end of lay for the hen. Egg production declines rapidly as the production of ova ceases. The ideal choice of inputs would involve the simultaneous consideration of all stages, even though the main laying period is considered the most economically important period (Gilbert, 1969).

Egg is a mixture of yolk, albumen and shell, of which each can be regarded as having a constant composition. If we can predict the production requirements of producing the yolk component, then the production requirements of producing the albumen and shell components can be calculated by difference. Emmans and Fisher (1986) have done this in three steps.

## 1. Predicting the rate of production of yolk material

If $y$ is taken as the rate of yolk production, $t$ the time from first egg and $G_{0}$ the initial state the prediction of yolk production is as follows,

$$
\begin{equation*}
Y=a e^{-c . t} \exp -\left[\exp \left(G_{0}-b . t\right)\right] \tag{21}
\end{equation*}
$$

Where, $a$ is a decay parameter, $c$ is the initial state parameter at $t=0$ and a growth parameter, $b$ (Emmans and Fisher, 1986).

## 2. Predicting the partition of yolk material into individual yolks

If the function in Equation 21 predicts the potential rate of yolk deposition in the ovary, then the mean yolk weight (MYW) is,

$$
\begin{equation*}
\mathrm{MYW}=y / \mathrm{R} \tag{22}
\end{equation*}
$$

Where R is the rate of ovulation and lay (Emmans and Fisher, 1986). Here it is important to remember that not all ovulated yolks will become eggs (Gilbert, 1972). At a given time a hen has an internal cycle length of ICL hours. The environment also has a cycle defined by the light length and is referred to as a length of EXCL hours. Therefore, when ICL = EXCL then the hen is expected to ovulate once in each environmental cycle (Emmans and Fisher, 1986). In this case the rate of lay, R, per 24 hours is given by:

$$
\begin{equation*}
\mathrm{R}=24 / \mathrm{EXCL} \quad(\text { when } \mathrm{ICL}=\mathrm{EXCL}) \tag{23}
\end{equation*}
$$

The two other cases to consider are when EXCL > ICL and where EXCL $<$ ICL. In the first of these cases the hen becomes entrained to the external cycle and the rule is that it cannot lay more than one egg per external cycle. In the second case, where EXCL < ICL, at each ovulation the hen will incur a 'lag' of ICL - EXCL hours. This lag is accumulated and when this value attains a value of $L$ hours, the bird does not ovulate, and misses a day. This develops a clutch pattern and the rate of lay ( R ) is defined as (Emmans and Fisher, 1986):

$$
\begin{equation*}
\mathrm{R}=\mathrm{L} /[(\mathrm{ICL}-\mathrm{EXCL})(1+[\mathrm{L} /(\mathrm{ICL}-\mathrm{EXCL})])] \tag{24}
\end{equation*}
$$

## 3. Predicting the albumin and shell weights from yolk weight

In non-limiting conditions it is reasonable to think that mean albumen output per yolk, MAW, is related to mean yolk weight (MYW) and that mean shell weight (MSW) is related to mean egg contents weight, MECW $=(M Y W+M A W)$. From this assumption we can then calculate the mean egg weight (MEW) as MYW + MAW + MSW and mean egg output is given by MEW.R (Emmans and Fisher, 1986).

The proportions of the albumen, yolk and shell in the egg are given in Table 1.2 as $58 \%$, $32 \%$ and $10 \%$ respectively. If the average weight of the egg is known then the amino acid requirements for each component of the egg can be calculated. These are then added together to give the total requirement for the egg produced (Emmans and Fisher, 1986).

If the albumen proportion of the egg is $58 \%$ and the weight of the egg is 60 g then the weight of the albumen in the egg is:

$$
60 \mathrm{~g} \times 0.58=34.8 \mathrm{~g}
$$

The protein content of the albumen is given as $10.5 \%$. The total weight of protein in the albumen of a 60 g egg is given as:

$$
34.8 \mathrm{~g} \times 0.105=3.654 \mathrm{~g}
$$

The nitrogen content of this albumen protein can then be calculated by dividing the protein weight by 6.25 .

$$
3.654 \mathrm{~g} / 6.25=0.585 \mathrm{~g} \mathrm{~N}
$$

The average amino acid composition of the albumen of a hen's egg is in Table 1.7. If these figures are multiplied by the weight of nitrogen in the albumen then the average for each amino acid in the albumen is given. If these are then added up for each component the total amino acid requirement for egg production is given.

### 1.4.3. Factors affecting production requirements

### 1.4.3.1. Rate of lay

The rate of lay and the timing of albumen and shell formation will be influenced by the hen's individual ovulatory cycle. A hen produces a sequence of ovulations on successive days, occurring slightly later each day, until the total lag exceeds eight to ten hours. This is when no ovulation occurs and a pause day occurs (Emmans and Fisher, 1986). This will not affect the deposition of yolk, which is a continuous process (Gilbert, 1972, Emmans and Fisher, 1986). The mean rate of lay in a flock of hens at a specific age is determined by the individual laying patterns of sequential laying at that time. The slopes of the initial rise in egg production and peak rate of lay are influenced by the distribution of the ages at sexual maturity and by the lengths of the individual sequences (Johnston and Gous, 2003).

### 1.4.3.2. Egg weight

Morris (1985) suggested that egg weight is related to the age of the hen and her genotype. This suggestion corresponds with the fact that, at the commencement of lay, egg weight is low (Morris, 1985). The environment has more of an indirect effect on egg weight. The direct effect is the feed intake. In warmer climates, feed intake may be lower, which may lead to lighter eggs (Morris, 1985).

Nutritional means may be used to alter the egg weight slightly. Early in the egg production cycle, this may prove essential to increase the egg weight. In a study done by Leeson and Summers (1983), the weight of eggs from pullets was not affected by increases in dietary levels of methionine, linoleic acid or protein above the established requirement (Morris, 1985). Another study showed that by increasing the linoleic acid content of the diet from $0.6 \%$ to $4.3 \%$ the egg weight during the first 14 weeks of production increased. This did not affect the average daily egg yield.

Morris and Blackburn (1982) showed that as the dietary protein content is increased the increase in the egg weight response gradually decreases and approaches an asymptotic
value. This resulting response curve is consistent with the Reading Model, which assumes that the shape of the curve is a function of individual variation in body weight and potential egg output (Morris and Blackburn, 1982).

Sell and Johnson (1974) showed in a different study that by adding 3 to $6 \%$ fat to diets, fed to laying hens during early egg production, increased the egg weight by increasing the yolk weight whether the diets were isocaloric or not. When egg weight is increased by fat supplementation of diets, it is not known if the response is due to the fat in general, or if it is a specific response to linoleic acid. Increasing the percentage of fat or oil in isoenergetic diets caused hens to lay heavier eggs (Sell and Johnson, 1974). Decreasing the dietary energy level may decrease the egg weight. Diet costs may increase when supplemental fats are used to obtain higher dietary fat and energy concentrations (Sell and Johnson, 1984).

Decreasing the dietary levels of the most limiting amino acid can affect egg weight (Morris and Gous, 1988). A review of 12 scientific papers indicated that; as the most limiting amino acid level decreased below the required level, egg weight and the rate of egg production decreased proportionately. This reduction occurred until the egg weight decreased to about $90 \%$ of the maximum. Decreases beyond this level only caused a decrease in the rate of egg production. An exception to the general effects of an amino acid inadequacy occurs with tryptophan, as a deficiency fails to decrease the egg weight (Morris and Gous, 1988).

### 1.4.3.3. Moulting

Moulting is induced in commercial laying hens by imposing a period of fasting on the hens. The length of this period can vary, although the most common periods are between 4 and 10 days. During this period the hen receives no food, and sometimes no water. The lighting is often decreased to that of a normal day length or less. The period of moulting in wild birds coincides with the incubation of eggs and brooding of offspring (Summers and Leeson, 1977).

Feather loss and regrowth will cause the maintenance requirements of the hen to increase and, therefore, her feed intake will increase (Emmans and Charles, 1977, as cited by Hughes, 1980). Hughes found the maintenance requirements to increase by an increment of $7 \%$.

The requirements of the laying hen have been defined in this chapter, but it is important to remember that the requirements are only met through the feed intake of the hen, which can be constrained by either the feed or the environment. This subject will be dealt with next.

## Chapter 2

## Constraints on Feed Intake

In an ideal environment, if the hen were provided with an ideal feed, she would be able to eat to meet her requirements and, therefore, achieve the potential defined by her genotype. This is rarely the case in practice, as the feed is not always perfect, and constraints occur in most environments. The actual feed intake consumed will define her performance. This actual intake must be predicted to maximize the hen's performance in a specific environment. In order to do this, the requirements of the hen must be known, and the feed must be formulated to account for these requirements as well as the constraints that may exist. Figure 2.1 represents the effect that constraints are proposed to have on the feed intake of a laying hen.


Figure 2.1: Graph showing the proposed relationship between the desired feed intake (DFI, broken line), constrained feed intake (CFI, solid line) and actual intake (AFI, dotted line) as feed is diluted with an inert filler (Gous, in consultation, 2004).

Three kinds of constraints can operate to limit feed intake (Emmans, 1981(a)). The environmental temperature can limit the heat loss of the animal, thereby constraining feed intake; the gut capacity of the hen may not allow sufficient food to be consumed due to the quantity required or the bulkiness of the feed; and the presence of toxins or imbalances in the feed may limit the feed intake by the hen.

CFI can be calculated as (Emmans, 1987):

$$
\begin{equation*}
\mathrm{CFI}=\mathrm{CAP} / \mathrm{FCON} \quad(\mathrm{~kg} / \text { day }) \tag{25}
\end{equation*}
$$

Where CAP = Capacity for the first limiting constraint (units/day).
FCON $=$ Feed's yield of the first limiting constraint (units/day).

Each constraint will be dealt with briefly in turn. However, the bulk constraint of the feed will be dealt with in more detail, being the subject of this thesis.

### 2.1. Environmental Heat Demand

Hens are classed as homeotherms and a homeotherm is defined by Mount (1979) as an animal that usually maintains a stable deep body temperature within narrow limits, even though the environmental temperature fluctuates and the animal's activity fluctuates. The thermal physiological features of birds are that they usually have a core temperature above $40^{\circ} \mathrm{C}$, they have an insulating layer of feathers that lower their capacity for a high rate of heat loss, and under hot conditions their body temperature is controlled mainly by evaporative heat loss from the respiratory tract (Marsden and Morris, 1987). Several components of the physical environment affect the heat loss of the animal. In order of importance these are:

- Dry bulb temperature
- Radiant temperature
- Air speed
- Wet bulb temperature (Marsden and Morris, 1987).

A heat balance exists in the animal between the animal's heat production and heat loss. The result of this balance is that the core temperature of the animal is maintained. Feeding increases the heat produced. Therefore, when feed intake is high the level of heat
production in the zone of thermal neutrality is higher than when feed intake is low (Marsden and Morris, 1987).

The feed supplied to the animal is that animal's sole source of energy and substrate for the formation of energy reserves. The chemical energy in the feed is oxidized by the animal and utilized. The heat of combustion produced by the feed measures the amount oxidized. However, some of this chemical energy can escape unutilised in two ways (Emmans, 1994):

1. Energy in the excreta and gases.
2. Energy through heat production.

The energy in the excreta and gases can be predicted by the calculation of the metabolisable energy (ME) in the feed available to the animal. The energy in the heat of production can be calculated through the prediction of the heat increment produced through feeding (Emmans, 1994), explained in Section 1.4.1.

The ME is the potential energy supplied to the animal by the feed it receives. The ME of the diet is predicted through the heat of combustion of the feed. The organic matter (OM) component of the feed yields the heat produced by combustion; therefore, the potential energy of the diet depends on the rate of OM intake multiplied by the heat of combustion. Some of this potential energy is lost as OM in the excreta (Emmans, 1994). The useful amount of ME in the diet can be defined by Equation 26.

$$
\begin{equation*}
\mathrm{ME}(\mathrm{~kJ} / \mathrm{day})=\mathrm{GE}-(\mathrm{FE}+\mathrm{UE}+\mathrm{MTHE}) \tag{26}
\end{equation*}
$$

Where ME is the rate of supply of metabolisable energy and FE, UE and MTHE are the rates of loss of energy in the faeces, urine and combustible gases respectively (Armsby (1903) as cited by Emmans, 1994). The laying hen is classed as a monogastric, meaning that the MTHE value can be considered zero.

The ME in Equation 26 is known as the classical ME $\left(\mathrm{ME}_{\mathrm{c}}\right)$. It can be useful to correct this ME value to reflect the ME if the rate of nitrogen retention is zero (Emmans, 1994). This is termed the nitrogen-corrected $\mathrm{ME}\left(\mathrm{ME}_{\mathrm{N}}\right)$ and can be estimated as:

$$
\begin{equation*}
\mathrm{ME}_{\mathrm{N}}=\mathrm{ME}_{\mathrm{c}}-\mathrm{a}(6.25 \mathrm{NR}) \tag{27}
\end{equation*}
$$

Where NR is the nitrogen retention in $g /$ day and the value of $a$ is constant and equal to 5.63 kJ/g (Emmans, 1994).

It has been defined previously that at maintenance the rate of protein retention is zero. If this is the case then $\mathrm{ME}_{\mathrm{N}}$ is the ME measured at maintenance (Emmans, 1994). The ME in the diet is either retained by the animal or lost as heat. The ME retained is in the form of either protein or lipid (Emmans, 1994). This means that the $\mathrm{ME}_{\mathrm{c}}$ can be defined as:

$$
\begin{equation*}
\mathrm{ME}_{\mathrm{c}}(\mathrm{~kJ} / \mathrm{day})=\mathrm{h}_{\mathrm{p}} \cdot \mathrm{PR}+\mathrm{h}_{1} \cdot \mathrm{LR}+\mathrm{H} \tag{28}
\end{equation*}
$$

Where H is the rate of heat production, PR and LR are the rates of protein and lipid retention (g/day), respectively, and $h_{p}$ and $h_{1}$ are their respective rates of combustion (Emmans, 1994). The values of $h_{p}$ and $h_{1}$ are constant and estimated as 23.8 and 39.6 $\mathrm{kJ} /$ day respectively (Emmans, 1994). If $\mathrm{ME}_{\mathrm{c}}$ is corrected for zero nitrogen retention the Equation 28 becomes:

$$
\begin{equation*}
\mathrm{ME}_{\mathrm{c}}(\mathrm{~kJ} / \text { day })=\left(\mathrm{h}_{\mathrm{p}}-\mathrm{a}\right) \cdot \mathrm{PR}+\mathrm{h}_{1} \cdot \mathrm{LR}+\mathrm{H} \tag{29}
\end{equation*}
$$

The problem now lies in predicting the heat production. The heat production can be divided into two components (Emmans, 1994):

1. $\mathrm{FHP}=$ the fasting heat production which is the rate at which the animal produces heat when given no food.
2. $\mathrm{HIF}=$ the heat increment resulting from feeding.

Fasting heat production is only produced from the catabolism of protein and lipid within the body. The heat produced by lipid catabolism is equal to its heat of combustion. However, protein catabolism occurs with the loss of some energy in the form of nitrogencontaining compounds in the urine. This means that the heat produced by protein catabolism is less than its heat of combustion. This reduction in the potential energy supplied to the hen can be calculated by multiplying the protein retention in the hen by the
energy value of the nitrogen-containing compounds in the urine ( $34.4 \mathrm{~kJ} / \mathrm{g}$ ) multiplied by the amount of nitrogen in protein $(0.16)$. [ $\mathrm{PR} \times(34.4 \times 0.16)]$ (Emmans, 1994). The FHP is given as:

$$
\begin{equation*}
\operatorname{FHP}(\mathrm{kJ} / \text { day })=\left(\mathrm{h}_{\mathrm{p}}-\mathrm{a}\right) \cdot \mathrm{PR}+\mathrm{h}_{1} \cdot \mathrm{LR} \tag{30}
\end{equation*}
$$

Where PR and LR are the rates of loss of reserves during the fast, considered here as positive quantities (Emmans, 1994).

The synthesis and excretion of the nitrogen-containing compounds, produced during protein catabolism, produces a specific 'heat of excretion' (HEX, kJ/day) (Emmans, 1994). This is produced at a rate of $w_{U}$ in the urine and is defined as:

$$
\begin{equation*}
\operatorname{HEX}(\mathrm{kJ} / \text { day })=\mathrm{w}_{\mathrm{U}} \cdot \mathrm{FUN} \tag{31}
\end{equation*}
$$

Where FUN (g/day) is the rate of nitrogen excretion during fasting (Emmans, 1994).

Animal behaviour is also important when calculating heat production. It can change heat loss in the short-term. Animals fed at less than ad libitum show a lower heat loss at low temperatures than those fed ad libitum (Emmans, 1981(a)). This appears to be due to behavioural rather than biochemical adaptation (Emmans, 1981(a)). With ad libitum feeding, it is assumed that the environmental heat demand (EHD), can be represented by the equation:

$$
\begin{equation*}
\mathrm{EHD}=\mathrm{MBW}(\mathrm{a}-\mathrm{bT}) \tag{32}
\end{equation*}
$$

Where MBW is the metabolic body weight and T is the effective temperature. The values of $a$ and $b$ are regarded as being independent of the environment and feed composition. As they reflect the insulation value of the animal's coat, they can be considered as functions of the degree of maturity.

A feed that allows production of either protein and lipid, or eggs, will also produce heat increments which are referred to as the HIF. The HIF is assumed to be directly proportional to the rate of production, and the amount of energy needed to digest the feed. This will affect the rate of production of a hen on a specific feed. The equation that represents the HIF is Equation 10, and Equation 11 represents the specific HIF equation for egg production.

Marsden and Morris (1987) showed that egg production could be maintained up to an environmental temperature of $30^{\circ} \mathrm{C}$ by adjusting the composition of the diet. Higher dietary protein concentrations were needed to maintain egg output at higher temperatures. At $30^{\circ} \mathrm{C}$ the reduction in egg output was not attributable to inadequate protein intake. Increasing the dietary energy concentration was attempted to counteract the decline in egg production at $30^{\circ} \mathrm{C}$. The increase in dietary energy resulted in some increase in egg output at all experimental temperatures, but it did not prevent the reductions in egg output at $30^{\circ} \mathrm{C}$.

Marsden and Morris (1987) found that the relationship between temperature and ME intake is curvilinear. Feed intake decreased more rapidly as the environmental temperature approached body temperature. The adapted heat production of the bird was also found to be a curvilinear function of temperature and tended toward a value of zero as the environmental temperature approached the body temperature. Energy intake and heat output can be expressed linearly between 15 and $30^{\circ} \mathrm{C}$ when they are expressed as functions of metabolic size $\left(\mathrm{kg}^{0.73}\right)$. It was estimated that the energy available for egg production was at a maximum at $23^{\circ} \mathrm{C}$ for brown Leghorns and at $24^{\circ} \mathrm{C}$ for white Leghorns. Gross energetic efficiency is at a maximum at $30^{\circ} \mathrm{C}$, but egg output is greatly reduced at this temperature (Marsden and Morris, 1987).

### 2.2. Bulk Constraint

### 2.2.1. Relationship between feed bulk and feed intake

When a highly digestible feed is diluted with a "bulkier" feed it can be assumed that the hen will increase her feed intake so that her intake of required nutrients remains relatively
constant and, therefore, her performance remains unaffected. Emmans (1981(b)), states that an animal has a potential rate of performance at a given time and it seeks to eat an amount that will allow this potential rate of performance to be achieved. If the dilution of the feed continues, a critical point will be reached when the hen's feed intake will decrease and performance will be reduced. This critical point needs to be defined so that feed intake can be accurately predicted as it reflects the capacity of the hen for 'bulk'. It is this equilibrium value that will be used in prediction equations (Whittemore et al., 2003). The time taken to adapt to a new feed also needs to be known. This time can be affected by the previous diet that the hen was consuming (Tsaras et al., 1998, and Whittemore et al., 2003).

The digestive tract of the hen is responsible for the digestion and absorption of the feed and nutrients. The most obvious constraint that the feed bulk can impose here is the physical limitation to the quantity of food that the digestive tract can contain. The factors that affect the gut capacity, extent of stretch of the organ and the rate of emptying are important in providing an accurate measurement of this constraint. Feed intake is regulated through the hypothalamus, through the ventromedial hypothalamic satiety centre and the lateral hypothalamic feeding centre, and is therefore a homeostatic function (Sykes, 1986). It is thought that the distention of the gut plays a large role in regulating feed intake. Polin and Wolford (1973) showed that the continuous distention of the crop inhibited feed intake. Richardson (1972) found that feed intake was reduced with the physical restriction of the duodenum of cockerels. This may be due to the fact that physical factors within the duodenum appear to inhibit gastric motility and, therefore decrease the feed intake of the bird.

The amount of a 'bulky' feed that a hen can consume depends on her capacity for bulk and the bulk content of the feed. Tsaras et al. (1998) found that in pigs the maximum capacity for food bulk was directly proportional to the live weight of the animals.

Savory (1980) found that the density of the feed affects the amount of time that the birds spend eating. He found that by altering the density of the feed, the amount of time that the birds spend eating and, therefore, feed intake is altered. The results showed that birds fed a
diluted feed consumed more than the birds on a non-diluted feed. The feeding patterns also varied according to the nutrient density of the feed. Birds on non-diluted feeds increase their intake of feed throughout the day, while birds fed a diluted feed have a consistent high feed intake throughout the day. If the values making up the feed intake are limited by bulk, the actual feed intake is fixed by the volume intake and ad libitum feeding is no longer in operation (Emmans, 1981(a)).

### 2.2.2. The digestive system

The components of the digestive system that are important to the hen in the digestion of the feed are the crop, the proventriculus, the gizzard, the small intestine and the colon. The feed is ingested and swallowed without mastication and is stored in the crop until it can be ground and mixed with digestive juices (Morris, 2004).

In the crop, the feed is moistened with ingested water and liquid secretions. Feed moves from the crop, small quantities at a time, into the proventriculus. Here the digestive juices are added to the feed. The feed then passes into the gizzard, which grinds the feed before it enters the small intestine. More gastric juices are added to the feed in the small intestine and the absorption of nutrients occurs here (Morris, 2004).

Jorgensen et al. (1996) found that broiler chickens can adapt to high fibre diets by increasing the size of the digestive tract and increasing the length of the small intestine. The partitioning of retained energy between body protein and body fat also changes in favour of body protein. The changes in the size of the digestive tract will have an effect on the maintenance requirements of the animals because of the relationship between the maintenance requirements of an animal and the animal's metabolic weight.

### 2.2.3. Measurement of feed bulk

Feeds and feed ingredients are often analysed to obtain detailed information about their nutritional composition. This information should not only allow the nutritionist to check
that the nutrient requirements of the hen are satisfied, but also that she is able to consume sufficient of the feed to meet these requirements.

It is important to find a suitable method to determine the bulk density of a specific feed. The method applied should be an accurate measurement of the in vivo environment and it should be quick and easy to perform or calculate. The best method would, of course, be a calculation. This could be possible by determining if a correlation exists between: the chemical components of the feed and the density of the feed.

### 2.2.4. Methods of expressing feed bulk

### 2.2.4.1. Water-displacement method

This method was described by Moughan et al. (1999). A 50 g sample is taken as fed, i.e. no further grinding, and analysed using a 250 ml volumetric flask and a $37^{\circ} \mathrm{C}$ water bath. The 50 g sample is placed in the 250 ml volumetric flask and 100 ml of $37^{\circ} \mathrm{C}$ distilled water is added. The sample is then mixed and 50 ml distilled water is added. It is then allowed to equilibrate for 15 minutes. Finally an additional 50 ml of distilled water is added and again the sample is allowed to equilibrate for 15 minutes. The flask is then filled to volume by adding the distilled water via a pipette. The density is calculated by dividing the weight of the sample added at the beginning by the total amount of distilled water added to the volumetric flask. The wet-density is expressed in $\mathrm{g} / \mathrm{ml}$.

### 2.2.4.2. Dry-density determination

Cherry et al. (1983) described this rather inaccurate, but quick, method of feed density determination. A 10 g sample is placed into a graduated cylinder and the cylinder is then tapped 10 to 12 times to remove any air pockets. The sample volume is then recorded and the dry-density is calculated and expressed as $\mathrm{g} / \mathrm{ml}$.

### 2.2.4.3. Water-holding capacity

The bulk characteristics of the feed may change when the feed enters the digestive tract. The main effect of this change will be the water-holding capacity of the feed. Kyriazakis and Emmans (1994), and Whittemore et al. (2003) have used the water-holding capacity (WHC) of the feed as a measurement of the bulk of the feed. The WHC of the feed has been determined by two different methods in the past, the filtration method and the centrifugation method.

The filtration method was described by Kyriazakis and Emmans (1994). A 1 g sample of the feed, as fed, is soaked in 500 ml of distilled water for 24 hours. The samples are then filtered through filter paper (Whatman \#2 filter paper, Whatman International Ltd., Maidstone, Kent) and the wet sample weight is determined. The samples are then freezedried and the dry sample weight is recorded. The WHC of the feed is calculated and expressed as g water/g dry feed.

The centrifugation method involves placing a 0.5 g sample of oven-dried feed into a 25 ml centrifugation tube. The sample is then soaked for 24 bours in distilled water before being centrifuged at $6000 G$ for 15 minutes. The supernatant fraction is decanted and the fresh weight of the feed is determined. The samples are then freeze dried and the WHC is expressed as g water/g dry feed (Robertson and Eastwood, 1981).

### 2.2.4.4. Crude fibre, NDF and ADF

The bulk volume of the feed can be measured by the fibre content of the feed, or the indigestible portion of the feed. Lehmann (1941), as cited by Whittemore et al. (2003), proposed that a suitable scale for bulk would be to measure the undigested dry matter. Voluntary feed intake models that have previously been developed have used dry matter as a measure of bulk (Whittemore et al. 2003), or undigested organic matter as a measure of bulk (Lehmann (1941), as cited by Whittemore et al. (2003)).

The determination of dry matter as a measure of bulk is inadequate across the complete range of feeds that exist (Kyriazakis and Emmans, 1994). Mraz et al. (1957) determined that the dry matter from different feeds has different filling effects in chickens. It has also been suggested that undigested dry matter may also have different bulk equivalents. This was suggested after Brouns et. al. (1991) discovered that the voluntary feed intake of sows is depressed far more by feeds based on sugar-beet pulp than by other, more indigestible materials, like straw and rice bran. Brouns et al. concluded from this that there must be another property of feeds that could be responsible for the reduction of feed intake.

This leads to the question of which method describes the bulk density of the feed best? This will be dealt with in Chapter 3.

### 2.3. Toxins and Nutrient Excesses or Imbalances

At some level in a feed, all chemicals, including nutrients, are toxic because feed is a mixture and, therefore, must obey the geometry of mixtures (Emmans, 1981(a)). Simply this means that as the amount of a single ingredient in the feed increases, so one or more of the others must decrease. Nutrient excesses can be seen as an example of how an animal reacts to toxins (Emmans, 1981(a)). Faced with the intake of some material an animal has several options:

- It can refrain from absorbing it.
- It can excrete it.
- It can alter it to a less harmful form.
- It can store it (Emmans, 1989).

The process of altering the ingredient to a less harmful form will increase as the exposure to the ingredient increases. The excretion and storage of the ingredient will eventually reach an upper limit. If the way in which the animal deals with the toxin is understood and can be quantified it should be possible to predict the level at which feed intake will be depressed (Emmans, 1981(a)).

### 2.4. OTHER FEED INTAKE CONTROL MECHANISMS

Neural control, both peripheral and central, serve as important regulators of feed intake. Peripheral receptors in the upper digestive tract are important regulators of feed intake. These receptors are presumed to be interrelated and in contact with the hypothalamus via neurons (Polin and Wolford, 1973). Five theories exist that have been based on a variable monitored by the central nervous system. Each of these theories will be discussed briefly. The theories that exist are those of the glucostatic theory, thermostatic theory, lipostatic theory, aminostatic theory and the ionostatic theory.

The glucostatic theory has been reported to exist in mammals, but it is not readily detectable by normal protocols in birds (National Research Council, 1987). Feeding has not been altered after the manipulation of blood glucose levels. (National Research Council, 1987).

The thermostatic theory is based on the exchange of heat between the animal and its environment. Several peripheral detectors exist that sense temperature changes and stimulate the central controller in the brain to increase or decrease feed intake (National Research Council, 1987).

The lipostatic theory is based on a feed-back system from fat depots to the brain and results in long-term control of feed intake. Polin and Wolford (1973) have shown this mechanism to exist in poultry and influence feed intake, but these effects are not consistent with the normal functioning of a lipostatic mechanism in mammals, and it is therefore questionable whether or not a negative feed-back exists from adipose tissue in poultry (National Research Council, 1987).

The balance of amino acids markedly influences feed intake and this is the basis of the aminostatic theory. Imbalanced diets cause rapid decreases in feed intake and a reduction of the first limiting amino acid in the blood. This may be the signal that causes the reduction in feed intake (National Research Council, 1987).

The role of $\mathrm{Na}^{+}$and $\mathrm{Ca}^{++}$within the brain, specifically the hypothalamus, has effects on the body temperature of the hen and feed intake and is the basis of the ionostatic theory. It is this effect on body temperature, caused by an imbalance of ions, which affects the feed intake (National Research Council, 1987).

The factors that affect the feed intake of the laying hen have been summarized in the above chapters. The effect of the feed bulk on feed intake will be investigated to determine a suitable measure of feed bulk and to quantify the upper limit of feed bulk that the hen can consume before intake is constrained.

## Chapter 3

## The Effect of Feed Bulk on Feed Intake in the Laying Hen

### 3.1. Introduction

For a hen to attain her genetic potential, she must eat sufficient of the food offered to her to meet her requirements for all essential nutrients. She may be constrained from doing so if the feed is too bulky, if the environmental temperature is too high that she is incapable of losing sufficient heat to the environment in which she is placed, or if there are toxins in the feed that she can detect (Williams, 1993). The research reported in this thesis deals with the first of these constraints, namely, feed bulk. The objectives of the research are to define the physical characteristics of the feed that best describe the bulkiness of the feed, and to define the maximum capacity of the hen to consume feed bulk.

As a means of finding a suitable method of describing feed bulk, diluents used in such a study should vary in physical properties such that the most suitable measure(s) can be found for predicting when feed intake by the hen would be constrained by bulk. Characteristics such as wet and dry density, water-holding capacity, crude fibre, aciddetergent fibre, neutral-detergent fibre, and even dry matter and undigested organic matter have been used for this purpose, with different degrees of success (Williams, 1993).

The most obvious constraint that feed bulk can impose is the physical limitation to the quantity of feed the hen can consume. However, although birds are capable of increasing crop capacity, as a means of storing food prior to its being processed (Williams, 1993), this is not an adequate measure of gut capacity, as it does not account for the rate at which the food is processed (Williams, 1993). Because food is not stored in the true stomach (proventriculus) of birds, as it is in simple stomached animals, the extent of stretch of the stomach is also unlikely to be the factor that constrains intake (Williams, 1993). The rate of flow of digesta is clearly important in providing an accurate measure of this constraint (Williams, 1993). Whatever the mechanism for constraining feed intake, the effect is an intake lower than would be predicted, given the extent to which the food has been diluted.

For example, a hen would be thought to attempt to consume twice her characteristic intake if the food were diluted $1: 1$ with an inert filler, in order to satisfy her requirement of nutrients to attain her genetic potential.

The physico-chemical properties of the feed, such as fibre content and water-holding capacity, are likely to relate the feed composition to the feed intake. A good measure of feed bulk would be a measure of one of these physico-chemical properties that would allow an accurate prediction of feed intake for both bulky and dense feeds. The measure needs to predict the effect of the digestive tract and juices on the feed and the change that these factors will cause on the feed. For example, a feed that is dense may have a high water-holding capacity when wet, which will increase the bulk and viscosity of the digesta (Williams, 1993).

Apart from the constraint on feed intake, it is likely that changes in reproductive performance of the hen may be attributed to the constraint that the feed imposes on the hen. The equilibrium value of feed intake, as defined by Whittemore (2003), is defined as the point when increased dilution of the feed will cause a reduction in performance, and it is this point that needs to be predicted from knowledge of the bulkiness of the food and the gut capacity of the hen. Hence there is an advantage in measuring the reproductive traits in addition to feed intake in such trials.

The objectives of this trial were to define the physical characteristics of the feed that best describe the bulkiness of the feed, and to define the maximum capacity of the hen to consume feed bulk. This should lead to the definition of the capacity of the laying hen for feed bulk such that it would be possible to predict, by means of a feed intake model, when the hen will be constrained by food bulk; and therefore no longer be capable of consuming sufficient of the food to attain her potential reproductive performance.

### 3.2. Materials and Methods

### 3.2.1. Animal description

One hundred and sixty laying hens were randomly allocated to the individual laying cages. The hens selected were Hy-line Variety Silver. They were hatched on the $19^{\text {th }}$ of September 2002 and were transferred to group pens in an adjacent laying house on the $27^{\text {th }}$ of February 2003 at the start of their $24^{\text {th }}$ week. At the commencement of the trial the hens were 49 weeks old. The hens were kept on a commercial layer feed, to which they had become accustomed, for one week after being moved to the individual cages, before the test treatments were offered. The commercial layer feed was used as the basal feed and an adaptation period of 1 week was allowed between treatments, when the hens were fed the basal feed.

### 3.2.2. Facilities

The birds were housed individually in wire floored laying cages, arranged in two tiers, in an open-sided laying house with adequate ventilation. Each cage was equipped with a nipple drinker located at the rear of the cage and an individual feed trough at the front.

### 3.2.3. Basal feed and diluents used

A commercial layer feed (Table 3.1), known to sustain good egg production, was used as the basal feed in the trial. This was sourced from a local feed company. This basal feed was diluted with one of five diluents, each being included at four levels, resulting in 20 dietary treatments in total. The diluents used were cellulose, sand, sawdust, sunflower husks and vermiculite, and the rates of dilution were $0,100,250$ and $500 \mathrm{~g} / \mathrm{kg}$. The diluents were included into the feed as a percentage of the final weight required, for example, to mix 50 kg of a sand diluted feed at $100 \mathrm{~g} / \mathrm{kg}, 5 \mathrm{~kg}$ of sand were added to 45 kg of the commercial feed. The addition of the diluent into the basal feed would not alter the balance of nutrients within the feed, but would increase the bulkiness and dilute the nutrient density
of the feed. The physical properties and the daily consumption of the diluted feed will provide information on the gut capacity of the laying hen.

Table 3.1: $\quad$ The composition and analysis of the basal feed ${ }^{\prime}(\mathrm{g} / \mathrm{kg})$, purchased from a local feed company, and offered to Brown and White Hy-line hens in all three Phases of the trial.

| Ingredient | Inclusion $(\mathrm{g} / \mathrm{kg})$ |
| :--- | :---: |
| Yellow maize | 512.0 |
| Bran | 150.0 |
| Hominy chop | 53.0 |
| Molasses | 20.0 |
| Soya oilcake $(47.0 \mathrm{~g} / \mathrm{kg}$ protein $)$ | 70.5 |
| Sunflower oilcake (38.0 g/kg protein) | 100.0 |
| Limestone | 81.5 |
| Monocalcium phosphate | 5.9 |
| Salt | 4.0 |
| DL methionine | 0.6 |
| Lysine HCl | 1.3 |
| Layer premix and Phytase | 1.6 |
|  |  |
| Analysis of basal feed | Determined $(\mathrm{g} / \mathrm{kg})$ |
| Protein $(\mathrm{g} \mathrm{N} \mathrm{x} \mathrm{6.25} \mathrm{kg)}$ | 145.0 |
| ME (MJ/kg) | 11.3 |
| Lysine (g/kg) | 6.9 |
| Ca (g/kg) | 34.0 |
| P (g/kg) | 5.0 |

The diluents sourced for this trial were chosen so that they would have contrasting physiochemical properties. Diluents with high fibre contents, e.g. cellulose, were contrasted with a diluent with little or no fibre content, e.g. sand. Diluents with differing densities were chosen, but all diluents were chosen so that they would combine well with the basal feed and not be unattractive to the hens.

[^0]
### 3.2.4. Design

Three phases were used in each trial, Phase 1, Phase 2, and Phase 3, each lasting three weeks. In the Phase 1, 20 dietary treatments were randomly allocated, using the random function in Microsoft Excel Software, between one hundred and sixty laying hens, each treatment, therefore, being offered to eight hens. In the Phase 2, the hens whose feed was diluted at the rate of $100 \mathrm{~g} / \mathrm{kg}$ in Phase 1 were switched to a dilution rate of $500 \mathrm{~g} / \mathrm{kg}$, and the hens that received a feed diluted at the rate of $500 \mathrm{~g} / \mathrm{kg}$ in Phase 1 were switched to a dilution rate of $100 \mathrm{~g} / \mathrm{kg}$. Those hens receiving feed diluted at a rate of $250 \mathrm{~g} / \mathrm{kg}$ remained on that feed. This was designed to confirm the effect that different dilutions of the same diluent had on the feed intake of the laying hen.

In the Phase 3, four of the diluents were switched: hens on the cellulose and sand treatments were swapped, as were the hens on the sawdust and sunflower husk treatments, all hens received the same dilution as was used in Phase 2. This design would confirm that different diluents had a different effect on the feed intake. Hens initially on the vermiculite treatment were kept on vermiculite for the entire nine weeks; hence those hens fed vermiculite-diluted at a rate of $250 \mathrm{~g} / \mathrm{kg}$ received the same feed for the entire nine weeks. The details of the dietary treatments are presented in Table 3.2. The design's purpose was to give sufficient information of the effects of the different bulk densities on feed intake. All data was analysed using the Genstat release 6.1.

Feed was available to the hens ad libitum. The feed was weighed into the self-feeding trough at the start of the week, and the amount remaining at the end of the week was weighed in order to calculate feed intake, by difference, for the week. The feed was weighed into buckets for each individual hen, from which that hen was fed throughout the week. Feed was weighed in on a Monday and the orts were weighed out the following Monday.

Table 3.2: Details of the dietary treatments offered to Silver Hy-line variety hens in Phases 1, 2 and 3, giving the dilution level in $\mathrm{g} / \mathrm{kg}$

| Diluent | Symbol | Dilution level |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | Phase 1 | Phase 2 | Phase 3 |  |
|  |  |  |  | Diluent | Level |
| Cellulose | C | 0 | 0 | S | 0 |
| Cellulose | C | 100 | 500 | S | 500 |
| Cellulose | C | 250 | 250 | S | 250 |
| Cellulose | C | 500 | 100 | S | 100 |
| Plasterer's sand | S | 0 | 0 | C | 0 |
| Plasterer's sand | S | 100 | 500 | C | 500 |
| Plasterer's sand | S | 250 | 250 | C | 250 |
| Plasterer's sand | S | 500 | 100 | C | 100 |
| Sawdust | SD | 0 | 0 | SF | 0 |
| Sawdust | SD | 100 | 500 | SF | 500 |
| Sawdust | SD | 250 | 250 | SF | 250 |
| Sawdust | SD | 500 | 100 | SF | 100 |
| Sunflower husks | SF | 0 | 0 | SD | 0 |
| Sunflower husks | SF | 100 | 500 | SD | 500 |
| Sunflower husks | SF | 250 | 250 | SD | 250 |
| Sunflower husks | SF | 500 | 100 | SD | 100 |
| Vermiculite | V | 0 | 0 | V | 0 |
| Vermiculite | V | 100 | 500 | V | 500 |
| Vermiculite | V | 250 | 250 | V | 250 |
| Vermiculite | V | 500 | 100 | V | 100 |

Due to the biological nature of the theory being investigated a dose-response analysis of the data was adopted. The output is expected to increase with increasing inputs, leading to an asymptotic response (Morris, 1999). This response may initially be linear, but becomes curvilinear as the ceiling is reached. Eventually, a plateau is reached where there is no
response to further increases in input (Figure 3.1). At the high end of the scale there may, or may not, be a reduction in output when the input is supplied in excess (Morris, 1999).


Figure 3.1: A general model of response to increasing inputs, applicable to many animal experiments. The model implies that some positive input is required before any output can be obtained, although it may be difficult to investigate the responses to very small inputs. Some inputs may lead to 'toxicity'; others may not (Morris, 1999).

For a single animal two straight lines can be fitted to data in the form of a bent-stick model (Morris, 1999). The two components of this model represent the maintenance and production requirements of the animal. The maintenance requirement is fitted to a horizontal line and the production requirement is fitted by linear regression. The production requirement is the subject of focus in this trial and, therefore, only linear regressions will be fitted to the data. For the bent-stick model, the residual sum of squares is then calculated about the pair of fitted lines and the interception is moved one place both sides and the residual sum of squares is calculated as well. The lowest residual sum of squares value represents the best pair of fitting lines (Morris, 1999). The regression model adopted to determine the response of the interactions between the period, diluent and level of diluent inclusion was:
Response $=\mu+$ Group effect $+[$ Period effect + Diluent effect + Level effect $]$

### 3.2.5. Feed and diluent analyses

The methods used to analyse the feeds are described below, and all analyses were done in duplicate. All feeds and ingredients were analysed using the following methods.

### 3.2.5.1. Water-displacement method

This method was described by Moughan et al. (1999). A 50 g sample is taken as fed, i.e. no further grinding, and analyzed using a 250 ml volumetric flask and a $37^{\circ} \mathrm{C}$ water bath. The 50 g sample is placed in the 250 ml volumetric flask and 100 ml of $37^{\circ} \mathrm{C}$ distilled water is added. The sample is then mixed and 50 ml distilled water is added. It is then allowed to equilibrate for 15 minutes. Finally an additional 50 ml of distilled water is added and again the sample is allowed to equilibrate for 15 minutes. The flask is then filled to volume by adding the distilled water via a pipette. The density is calculated by dividing the weight of the sample added at the beginning by the total amount of distilled water added to the volumetric flask. The wet-density is expressed in $\mathrm{g} / \mathrm{ml}$.

### 3.2.5.2. Dry-density determination

Cherry et al. (1983) described this rather inaccurate, but rapid method of feed density determination. A 10 g sample is placed into a graduated cylinder and the cylinder is then tapped 10 to 12 times to remove any air pockets. The sample volume is then recorded and the dry-density is calculated and expressed as $\mathrm{g} / \mathrm{ml}$.

### 3.2.5.3. Water-holding capacity

The bulk characteristics of the feed may change when the feed enters the digestive tract. The main effect of this change will be that water will combine with the feed thereby increasing its density, so it is useful to know the water-holding capacity (WHC) of the feed. Kyriazakis and Emmans (1994) and Whittemore et al. (2003) have both used this characteristic as a measurement of the bulk of a feed. The water-holding capacity of the feed represents the property of the non-starch polysaccharides of the feed to trap water
within its matrix and swell to form gels of high water content. This is relevant to the specific type of polysaccharide and it can also be influenced by the preparation of the feed before measuring the WHC (Kyriazakis and Emmans, 1994). The formation of a gel within the gastrointestinal tract will increase the viscosity of the feed, as well as increase the bulk. The WHC of the feed has been determined by two different methods in the past: the filtration method and the centrifugation method.

The filtration method was described by Kyriazakis and Emmans (1994). A 1 g sample of the feed, as fed, is soaked in 500 ml of distilled water for 24 hours. The samples are then filtered through filter paper (Whatman \#4 filter paper, Whatman International Ltd., Maidstone, Kent) and the wet sample weight is determined. The samples are then freezedried and the dry sample weight is recorded. The WHC of the feed is calculated and expressed as g water/g dry feed.

The centrifugation method involves placing a 0.5 g sample of oven-dried feed into a 25 ml centrifugation tube. The sample is then soaked for 24 hours in distilled water before being centrifuged at 6000 g for 15 minutes. The supernatant fraction is decanted and the fresh weight of the feed is determined. The samples are then freeze-dried and the WHC is expressed as g water/g dry feed (Robertson and Eastwood, 1981).

Only the centrifugation method was used since previous authors have found the two methods to be highly correlated and the filtration method posed the problem of separating the sediment from the filter paper (Robertson and Eastwood, 1981).

### 3.2.5.4. Crude fibre, NDF and ADF

The fibre content of the feed or its indigestible portion may be used as a measure of the bulkiness or volume of the feed. Lehmann (1941), as cited by Whittemore et al. (2003), proposed that a suitable scale for bulk would be to measure the undigested dry matter (Kyriazakis and Emmans, 1994).

All the fibre analysis was done by the Nutrient Analysis Laboratory, Discipline of Animal and Poultry Science, University of KwaZulu-Natal. Crude fibre (CF) and acid detergent fibre (ADF) were analysed on a Dosi-Fibre machine using the AOAC Official Method 920.39 and AOAC Official Method 973.18, respectively. Neutral netergent fibre (NDF) was analyzed on a Dosi-Fibre machine according to the method described by Van Soest et al. (1991).

### 3.2.6. Measurements

Rate of lay was measured daily for each hen and egg weight was recorded three times a week. Feed intake was recorded weekly. The body weights of the hen at the start, and after each three-week period, were recorded, and the change in body weight of the hen was calculated by difference. The body weight of the hens was recorded to check the feed intake and the welfare of the birds. Those hens whose body weight had dropped below 1000 g were removed from the trial and returned to the basal feed. One mortality was recorded and this was due to the hen having a split beak and being unable to eat the feed in a mash form.

### 3.3. Results and Discussion

### 3.3.1. Bulk content analysis

The calculated analysis of all the feeds offered in the trial is given in Table 3.3. Measurements were done for protein, ash, fat, moisture, crude fibre (CF), neutral detergent fibre (NDF) and acid detergent fibre (ADF) in the Feed Analyses Laboratory at the University of KwaZulu-Natal.

Table 3.3: $\quad$ The composition of the mixed feeds (Basal feed + diluent) offered to the laying hens during the trial, including the measurements of crude protein (CP), lipid, ash and moisture contents (g/kg)

| Diluent | Diluent inclusion <br> level $(\mathrm{g} / \mathrm{kg})$ | CP | Fat | Ash | Moisture |
| :--- | :---: | :---: | :---: | :---: | :---: |
| Cellulose | 0 | 144.1 | 31.8 | 129.6 | 112.0 |
|  | 100 | 138.4 | 22.4 | 146.6 | 108.0 |
|  | 250 | 104.8 | 13.0 | 164.4 | 100.0 |
|  | 500 | 67.8 | 5.3 | 237.8 | 94.0 |
| Sand | 0 | 144.1 | 31.8 | 129.6 | 112.0 |
|  | 100 | 129.3 | 24.5 | 242.9 | 92.0 |
|  | 250 | 108.2 | 17.0 | 320.1 | 98.0 |
|  | 500 | 75.0 | 10.0 | 616.8 | 54.0 |
| Sawdust | 0 | 144.1 | 31.8 | 129.6 | 112.0 |
|  | 100 | 135.3 | 27.5 | 97.0 | 116.0 |
|  | 250 | 114.2 | 25.7 | 90.3 | 114.0 |
|  | 500 | 77.0 | 22.4 | 64.2 | 119.0 |
| Sunflower | 0 | 144.1 | 31.8 | 129.6 | 112.0 |
| husk | 100 | 125.1 | 34.9 | 111.1 | 107.0 |
|  | 250 | 123.9 | 33.9 | 91.3 | 111.0 |
|  | 500 | 99.1 | 32.9 | 65.2 | 109.0 |
| Vermiculite | 0 | 144.1 | 31.8 | 129.6 | 112.0 |
|  | 100 | 128.7 | 24.0 | 202.5 | 105.0 |
|  | 250 | 108.2 | 17.0 | 320.1 | 98.0 |
|  | 500 | 73.5 | 15.3 | 483.1 | 79.0 |

The bulk characteristics for each of the feeds used in the trial, as well as each diluent, were measured, as described above. Values for each of the feeds used are presented in Table 3.5, and for the diluents in Table 3.4.

Table 3.4: $\quad$ The measured bulk characteristics of the basal feed and the individual diluents used in the trial, including mean water-holding capacity (WHC) ( $g$ water/g feed), dry and wet density ( $g / m l$ ), crude fibre $(C F)$, acid detergent fibre (ADF) and neutral detergent fibre (NDF) (g/kg)

| Diluent | WHC <br> $(\mathrm{g}$ water/g <br> Feed $)$ | Dry <br> density <br> $(\mathrm{g} / \mathrm{ml})$ | Wet <br> density <br> $(\mathrm{g} / \mathrm{ml})$ | CF <br> $(\mathrm{g} / \mathrm{kg})$ | ADF <br> $(\mathrm{g} / \mathrm{kg})$ | NDF <br> $(\mathrm{g} / \mathrm{kg})$ |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: |
| Basal feed | 4.55 | 0.66 | 1.34 | 43.2 | 67.0 | 145.9 |
| Cellulose | 25.00 | 0.37 | 0.67 | 158.6 | 336.0 | 437.4 |
| Sand | 1.49 | 1.43 | 1.99 | - | - | - |
| Sawdust | 7.14 | 0.23 | 0.93 | 689.0 | 768.7 | 874.5 |
| Sunflower | 6.25 | 0.25 | 0.54 | 519.9 | 648.8 | 751.6 |
| husk |  |  |  |  |  |  |
| Vermiculite | 6.67 | 0.16 | 0.55 | - | - | - |

Table 3.5: $\quad$ The bulk characteristics of the feeds including the mean water-holding capacity (WHC) (g water/g feed), dry and wet density (g/ml), crude fibre (CF), acid detergent fibre (ADF) and neutral detergent fibre (NDF) $(\mathrm{g} / \mathrm{kg})$ of feeds used in the trial

| Diluent | Level <br> $(\mathrm{g} / \mathrm{kg})$ | WHC <br> $(\mathrm{g}$ <br> water/g <br> Feed $)$ | Dry <br> density <br> $(\mathrm{g} / \mathrm{ml})$ | Wet <br> density <br> $(\mathrm{g} / \mathrm{ml})$ | CF <br> $(\mathrm{g} / \mathrm{kg})$ | ADF <br> $(\mathrm{g} / \mathrm{kg})$ | NDF <br> $(\mathrm{g} / \mathrm{kg})$ |  |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  |  |  |  |  |  |
|  | Cellulose | 100 | 5.00 | 0.57 | 1.31 | $*$ | 91.0 |  |
|  | 250 | 5.88 | 0.55 | 0.98 | $*$ | 126.4 | 257.1 |  |
|  | 500 | 7.69 | 0.50 | 0.60 | $*$ | 192.3 | 264.7 |  |
| Sand | 100 | 4.55 | 0.70 | 1.43 | 34.6 | 396.7 | 132.4 |  |
|  | 250 | 3.03 | 0.78 | 1.53 | 25.3 | 341.9 | 111.9 |  |
|  | 500 | 2.27 | 0.92 | 1.81 | 20.4 | 560.8 | 79.8 |  |
| Sawdust | 100 | 4.76 | 0.56 | 1.32 | 105.1 | 137.9 | 224.6 |  |
|  | 250 | 5.00 | 0.48 | 1.29 | 165.9 | 228.6 | 342.3 |  |
|  | 500 | 5.56 | 0.32 | 1.14 | 305.3 | 386.6 | 513.3 |  |
| Sunflower | 100 | 4.76 | 0.48 | 1.20 | 145.6 | 187.5 | 297.0 |  |
| husk | 250 | 5.00 | 0.47 | 1.15 | 134.4 | 190.0 | 294.4 |  |
|  | 500 | 5.26 | 0.39 | 0.95 | 288.2 | 330.9 | 443.6 |  |
| Vermiculite | 100 | 4.76 | 0.51 | 1.13 | 40.8 | 83.3 | 145.7 |  |
|  | 250 | 5.00 | 0.41 | 1.04 | 38.1 | 100.0 | 126.2 |  |
|  | 500 | 5.26 | 0.37 | 0.86 | 28.4 | 112.6 | 97.4 |  |

*Sample was impossible to filter to obtain results for crude fibre, due to the nature of the
substance.

Each measure of bulk will be discussed in turn.

### 3.3.1.1. Crude fibre

The values used for crude fibre were determined from researched values (Williams, 1993). Crude fibre content was negatively related to the feed intake, but different diluents
expressed different slopes (Figure 3.2), making a standard measurement difficult to calculate and the relationship could not be represented by one equation. The relationship between crude fibre content in the feed and feed intake was linear but this relationship was significantly different ( $p<0.05$ ) for each diluent.


Figure 3.2: $\quad$ The feed intake of hens ( $g /$ /day) fed feeds varying in diluent inclusion percentage and diluent, giving feeds with a series of different crude fibre levels. The observed mean values for each level of diluent inclusion are shown as points: $\square=C,=S, \Delta=S D, x=S F$, and $*=V$, where $C$ represents cellulose, $S$ sand, $S D$ sawdust, $S F$ sunflower husks, and $V$ vermiculite. $S E=15.6$ and the percentage variation accounted for is $80.4 \%$ obtained over the data set.

As the crude fibre content of the feed increased, so feed intake decreased. The rate of decrease in feed intake was not the same between diluents, suggesting that a factor other than crude fibre was directly responsible for altering the feed intake of the hen, i.e. palatability of the feed or environmental temperature. The rate of decrease in feed intake was significantly greater for the sand-diluted feeds than for the sawdust-, vermiculite- and sunflower husk-diluted feeds.

The determination of dry matter as a measure of bulk is inadequate across the complete range of feeds that exist (Kyriazakis and Emmans, 1994). Mraz et al. (1957) determined that the dry matter from different feeds had different filling effects in chickens. It has also been suggested that undigested dry matter may have different bulk equivalents. This was suggested after Brouns et al. (1991) discovered that the voluntary feed intake of sows was depressed far more by feeds based on sugar-beet pulp than by other, more indigestible materials like straw and rice bran. Brouns et al. (1991) concluded from this that there must be another property of feeds that could be responsible for the reduction of feed intake.

The coefficients of the regression equation are recorded in Table 3.6.

Table 3.6: The coefficients of the linear regressions equations (of the form $y=a x+c$ ) of feed bulk on the feed intake illustrated in Figure 3.2 through 3.5

| Diluent |  | CF |  | Dry Density | Wet Density | $1 / \mathrm{WHC}$ |  |  |  |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | \% variance | $71.7 \%$ |  |  | $71 \%$ | $83.3 \%$ | $59.8 \%$ |  |  |
|  | SE | 13.5 |  | 18.9 |  |  | 14.3 | 26.6 |  |
|  | Coefficient | $a^{I}$ | $c^{\prime}$ | $a^{I}$ | $c^{I}$ | $a^{I}$ | $c^{I}$ | $a^{I}$ | $c^{I}$ |
| Cellulose |  | $*$ | $*$ | 604 | -276.8 | 115.6 | -54.4 | 72.8 | 46.04 |
| Sand |  | -26.8 | 224.8 | 604 | -276.8 | 115.6 | -54.4 | 72.8 | 41.73 |
| Sawdust |  | -1.3 | 128.7 | 97 | 58.2 | 115.6 | -54.4 | 72.8 | 49.52 |
| Sunflower |  |  |  |  |  |  |  |  |  |
| husk |  | -1.2 | 129.3 | 60 | 80.2 | 115.6 | -23.4 | 72.8 | 50.16 |
| Vermiculite |  | 33.1 | -18.1 | 114 | 51.2 | 115.6 | -23.4 | 72.8 | 49.98 |

[^1]
### 3.3.1.2. Dry density

Feed intake was directly proportional to the dry density of the feed. As the dry density of the feed increased, feed intake increased. However, the slopes of the responses differed between diluents. As with crude fibre, this again suggests that the dry density of the food is not a good measure for predicting the constraining effects of the food on feed intake, and that another measure must be acting to restrain the feed intake of the hens.

No significant difference ( $\mathrm{p}<0.05$ ) was found between the sand- and cellulose-diluted feeds when a simple linear regression was fitted to the data (Figure 3.3). There was a significant difference observed between the sand- and cellulose-diluted feeds and all the other treatments.


Figure 3.3: $\quad$ The feed intake of hens (g/day) fed feeds varying in diluent inclusion percentage. and diluent, giving feeds with a series of different dry density levels. The observed mean values for each level of diluent inclusion are shown as points: $\square=C, \downarrow=S, \Delta=S D, \times=S F$, and ${ }^{*}=V$, where $C$ represents cellulose, $S$ sand, $S D$ sawdust, $S F$ sunflower husks, and $V$ vermiculite. $S E=18.9$ and the percentage variation accounted for is $71 \%$.

The coefficients of the regression equation are recorded in Table 3.6.

### 3.3.1.3. Wet density

Figure 3.4 shows the slopes of all diluents to be the same when feed intake is plotted against the wet density of the feed. The cellulose-, sand- and sawdust-diluted feeds lay on one line, while the sunflower husk- and vermiculite-diluted feeds lay on another line. There was no significant difference ( $p<0.05$ ) in feed intake between the cellulose-, sand- or sawdust-diluted feeds. A significant difference ( $\mathrm{p}<0.05$ ) existed between the feeds that lay on the same line and the sunflower- and vermiculite-diluted feeds. The feed intake of the hen is directly positively related to the wet density of the feed. Feed intake was highest on the feed with the highest wet density.


Figure 3.4: $\quad$ The feed intake of hens ( $g /$ day) fed feeds varying in diluent inclusion percentage and diluent, giving feeds with a series of different wet density levels. The observed mean values for each level of diluent inclusion are shown as points: $\square=C,=S, \boldsymbol{\Delta}=S D, \times=S F$, and $*=V$, where $C$ represents cellulose, $S$ sand, $S D$ sawdust, $S F$ sunflower husks, and $V$ vermiculite. $S E=14.3$ and the percentage variation accounted for is $83.3 \%$

The coefficients of the regression equation are recorded in Table 3.6.

### 3.3.1.4. Water-holding capacity

The feed intake of the hen is positively related to the inverse of the measured waterholding capacity (WHC). Kyriazakis and Emmans (1994) found the relationship between the scaled feed intake (SFI) and the reciprocal of water-holding capacity to be linear in pigs and Figure 3.5 shows this to be true in laying hens. The feed intake was converted to scaled feed intake, by calculating the feed intake per kilogram of body weight (BW). However, the y-intercept differs between all treatments; therefore, there is a significant difference in feed intake between feeds that differ in WHC.

The separation between the lines has been illustrated in Figure 3.5, although there was no significant difference ( $\mathrm{p}<0.05$ ) between the scaled feed intakes of the different feeds. The separation between lines may exist because of the difference in the intakes of the basal feed, which can be attributed to the differences in the average weight of the hens in each treatment. A common line was fitted to the data (Figure 3.6) in order to predict the SFI for the entire group. The coefficients of the regression equation are recorded in Table 3.6.

The relationship between SFI and 1/WHC was linear and is shown as:

$$
\begin{equation*}
\mathrm{SFI}(\mathrm{~g} \text { feed } / \mathrm{kg} \text { BW.d })=46.3( \pm 12.2)+83.5( \pm 60.8) \mathrm{x} \tag{33}
\end{equation*}
$$

Equation 33 was adjusted to pass through the origin (represented in Figure 3.6 as the broken line) and gives the equation:

$$
\begin{equation*}
\mathrm{SFI}(\mathrm{~g} \text { feed } / \mathrm{kg} \text { BW. } \mathrm{d})=272.4( \pm 8.91) \mathrm{x} \tag{34}
\end{equation*}
$$



Figure 3.5: $\quad$ The feed intake of hens (g/day) fed feeds varying in diluent inclusion percentage and diluent, giving feeds with a series of different inverse water-holding capacity (1/WHC) levels. The observed mean values for each level of diluent inclusion are shown as points: $\square=C, \leqslant=S=S D, \times=S F$, and $*=V$, where $C$ represents cellulose, $S$ sand, $S D$ sawdust, $S F$ sunflower husks, and $V$ vermiculite.

The constant term was suppressed because, although it is the best fit, it is not significantly different from zero. This is equivalent to saying that ( $\mathrm{SFI} \times \mathrm{WHC} \mathrm{)} \mathrm{is} \mathrm{constant} \mathrm{at} \mathrm{the} \mathrm{value}$ of $272 \mathrm{~g} / \mathrm{kg}$ per d which can be assumed to be the limit of laying hens for WHC (Figure 3.6).


Figure 3.6: The mean relationship between the scaled feed intake of the laying hen in g feed/kg body weight. d and I/ WHC of the feed (g water/g dry feed), where C represents cellulose, $S$ sand, $S D$ sawdust, $S F$ sunflower husks, and $V$ vermiculite. Solid line includes a constant term $(y=46.3( \pm 12.2)+83.5( \pm 60.8) x)$ and take account of the two outliers; the dotted line is fitted through the origin and ignores the two outliers $(y=272.4( \pm 8.91) x)$.

### 3.3.2. Feed intake

The mean feed intakes for each diluent and period are presented in Table 3.7. Feed intake was analysed according to the main effects of the Phase, diluent and level of diluent inclusion. Each of these were analysed separately and then the interactions between the Phase and diluent, diluent and level of inclusion, and then the phase and diluent and level of inclusion were analysed. Over all diluents, feed intake was significantly higher ( $\mathrm{p}<0.05$ ) in Phases 2 and 3 than in Phase 1. The highest mean feed intake was recorded in Phase 2.

The feed intake was highest in the sand-diluted treatments, this being significantly higher ( $p<0.05$ ) than on the sawdust, sunflower husk and vermiculite treatments. Feed intake was lowest on the cellulose treatments. On the cellulose treatments feed intake declined rapidly from the zero to $25 \%$ inclusion rates, but then the rate of decline decreased between the $25 \%$ and the $50 \%$ cellulose inclusion rates.

Table 3.7: $\quad$ The calculated mean feed intakes ( $g /$ bird. $d$ ), over all dilution levels of the mixed feeds, for the diluent $\times$ level $\times$ period, diluent $\times$ level, and diluent $\times$ period interactions of the model, with corresponding standard error (SE) values and significance shown by the letter superscripts.

| Diluent | Period | Dilution rate ( $\mathrm{g} / \mathrm{kg}$ ) |  |  |  | Period $x$ Diluènt (SE $=5.66$ ) |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | 0 | 100 | 250 | 500 |  |
| Cellulose$(S E=2.83)$ | 1 | $107.8{ }^{\text {c }}$ | $79.3{ }^{\text {b }}$ | $27.5{ }^{\text {c }}$ | $21.1{ }^{\text {c }}$ | $58.9{ }^{\text {b }}$ |
|  | 2 | $128.3{ }^{\text {a }}$ | $107.9^{\text {a }}$ | $44.5{ }^{\text {b }}$ | $31.3{ }^{\text {b }}$ | $78.0{ }^{\text {a }}$ |
|  | 3 | $114.9{ }^{\text {a }}$ | $98.5{ }^{\text {a }}$ | $29.9{ }^{\text {b }}$ | $23.3{ }^{\text {b }}$ | $66.7{ }^{\text {b }}$ |
| $\begin{aligned} & \text { Diluent } \times \text { Level } \\ & (S E=4.90) \end{aligned}$ |  | $117.0^{\text {a }}$ | $95.2{ }^{\text {b }}$ | $34.0{ }^{\text {c }}$ | $25.2^{\text {c }}$ |  |
| Sand$(S E=2.83)$ | 1 | $102.4{ }^{\text {ab }}$ | $117.0^{\text {a }}$ | $113.7{ }^{\text {ab }}$ | $137.6^{\text {a }}$ | $117.7^{\text {c }}$ |
|  | 2 | $116.5{ }^{\text {bc }}$ | $135.7^{\text {b }}$ | $152.9{ }^{\text {b }}$ | $187.7^{\text {a }}$ | $148.2^{\text {b }}$ |
|  | 3 | $119.8{ }^{\text {c }}$ | $136.9^{\text {c }}$ | $166.7^{\text {b }}$ | $217.2^{\text {a }}$ | $160.1^{\text {a }}$ |
| Diluent $x$ Level $(S E=4.90)$ |  | $112.9{ }^{\text {d }}$ | $129.9^{\text {c }}$ | $144.4{ }^{\text {b }}$ | $180.8^{\text {a }}$ |  |
| Sawdust$(S E=2.83)$ | 1 | $96.1{ }^{\text {a }}$ | $107.1^{\text {a }}$ | $104.2^{\text {a }}$ | $78.2{ }^{\text {ab }}$ | $96.4{ }^{\text {b }}$ |
|  | 2 | $108.9{ }^{\text {ab }}$ | $131.9^{\text {a }}$ | $125.5^{\text {a }}$ | $80.7{ }^{\text {b }}$ | $111.8^{\text {a }}$ |
|  | 3 | $119.0^{\text {a }}$ | $135.6^{\text {a }}$ | $126.9^{\text {a }}$ | $79.1{ }^{\text {b }}$ | $115.1^{\text {a }}$ |
| Diluent $x$ Level $(S E=4.90)$ |  | $108.0^{\text {ab }}$ | $124.9{ }^{\text {a }}$ | 118.9a | $79.3{ }^{\text {b }}$ |  |
| Sunflower husk$(S E=2.83)$ | 1 | $105.9^{\text {a }}$ | $104.2^{\text {a }}$ | $112.6^{\text {a }}$ | $63.4{ }^{\text {b }}$ | $96.5{ }^{\text {b }}$ |
|  | 2 | $121.9^{\text {a }}$ | $136.5^{\text {a }}$ | $129.0^{\text {a }}$ | $97.9{ }^{\text {b }}$ | $121.3^{\text {a }}$ |
|  | 3 | $108.1^{\text {a }}$ | $124.8{ }^{\text {a }}$ | $122.8{ }^{\text {a }}$ | $95.1{ }^{\text {ab }}$ | $112.7^{\text {a }}$ |
| Diluent $x$ Level $(S E=4.90)$ |  | $112.0^{\text {a }}$ | $121.8^{\text {a }}$ | $121.5^{\text {a }}$ | $85.5{ }^{\text {b }}$ |  |
| Vermiculite$(S E=2.83)$ | 1 | $113.8{ }^{\text {a }}$ | $110.7^{\text {a }}$ | $91.2^{\text {ab }}$ | $67.0{ }^{\text {c }}$ | $95.7^{\text {b }}$ |
|  | 2 | $115.2^{\text {a }}$ | $134.7^{\text {a }}$ | $116.0^{\text {a }}$ | $74.3{ }^{\text {b }}$ | $110.0^{\text {a }}$ |
|  | 3 | $120.3^{\text {a }}$ | $134.9{ }^{\text {a }}$ | $119.6{ }^{\text {a }}$ | $81.0{ }^{\text {b }}$ | $113.9^{\text {a }}$ |
| Diluent $x$ Level $(S E=4.90)$ |  | $116.4^{\text {a }}$ | $126.8^{\text {a }}$ | $108.9^{\text {ab }}$ | $74.1{ }^{\text {c }}$ |  |

In Figure 3.7 the trend lines showing feed intake for each diluent over the entire 9 -week period are illustrated by lines of best fit. Mean feed intake was significantly higher $(\mathrm{p}<0.05)$ at the $100 \mathrm{~g} / \mathrm{kg}$ inclusion levels than on the undiluted feed, except for cellulose, where intake was lower at a higher dilution. As the inclusion level increased to $250 \mathrm{~g} / \mathrm{kg}$, feed intake decreased significantly ( $p<0.05$ ), except for sand and sunflower husks where
intake continued to increase, and remained the same, respectively. The lowest mean feed intake was recorded at the $500 \mathrm{~g} / \mathrm{kg}$ inclusion level for all diluents, other than sand, which had the highest intake at that rate of dilution.


Figure 3.7: $\quad$ The relationship between mean feed intake and the level of inclusion of the diluent in the feed over all three phases. The observed mean values are shown as points: $\square=C, \downarrow=S, \boldsymbol{\Delta}=S D, x=S F$, and *= $V$, where $C$ represents cellulose, $S$ sand, $S D$ sawdust, $S F$ sunflower husks, and $V$ vermiculite.

In Figure 3.8 the cellulose graph shows that hens increased their feed intake between Phases 1 and 2, but in Phase 3 the feed intake decreased. Hens receiving cellulose-diluted feeds in this period were previously fed sand-diluted feeds, so their gut capacity would not have increased to the same extent as those hens previously on cellulose-diluted feeds, and this is likely to be the reason for the lower intake of food by these birds. This statement is supported by the fact that the feed intake of hens fed the sand-diluted feeds increased over all three phases.

The feed intake of the hens on the sawdust-diluted feeds increased between Phases 1 and 2. In Phase 3 the hens were switched to sunflower husk-diluted feeds and intake increased during this phase. The opposite occurred for the hens fed sunflower husk-diluted feeds,
where feed intake increased between Phases 1 and 2, but then feed intake decreased when they were switched onto sawdust-diluted feeds.

Feed intake on the vermiculite-diluted feeds increased over all phases, but the increase in feed intake was greater between Phases 1 and 2 than between 2 and 3. This can be attributed to the fact that between Phases 1 and 2 the hens were swapped between $100 \mathrm{~g} / \mathrm{kg}$ and $500 \mathrm{~g} / \mathrm{kg}$ diluted feeds. The hens that moved onto the $100 \mathrm{~g} / \mathrm{kg}$ diluted feeds from the $500 \mathrm{~g} / \mathrm{kg}$ diluted feeds were able to consume more of the feed than the hens previously on the $500 \mathrm{~g} / \mathrm{kg}$ diluted feeds, due to a small amount of bulk capacity adaptation, and may have compensated better than the previous group for the lower nutrient density of the previous feed.

The general trend of feed intake of the cellulose, sawdust, sunflower and vermiculite diluted feeds was a decrease in the voluntary feed intake of the hen as the dilution level increased from the $25 \%$ inclusion level. This could indicate that the feed intake of the hens was restricted by these feeds from this level of dilution. The sawdust, sunflower and vermiculite treatments showed an initial increase in the feed intake of the hens between the basal feed and the $10 \%$ diluted feed, perhaps indicating that the hen was increasing feed consumption to sustain nutrient intake, however the feed intake then decreased over the $25 \%$ and $50 \%$ dilution levels.

When statistically analysed the intake of the sand- and vermiculite-diluted feeds was linearly related to the level of the diluent in the feed while, with the other diluents, feed intake was quadratically related to the feed intake and the level of dilution of the feed (Table 3.8 and Figure 3.8). This could indicate that the other diluents did not restrict feed intake, or completely restricted the feed intake leading to a linear or quadratic increase or decrease in feed intake over all levels of inclusion.


Diluent Inclusion Level (\%)
Figure 3.8: $\quad$ Mean feed intake for each diluent in each of the three phases and at the four rates of dilution of the feed (\%) for each individual diluent. P1 represents Phase 1, P2 Phase2, P3 Phase 3, FI1 feed intake in Phase I, F12 feed intake in Phase 2, and FI3 feed intake in Phase 3.

Table 3.8 shows the coefficients for the regression equations for the graphs in Figure 3.8. The form of the regression equation is $y=a x+b x^{2}+c$. Where, $y$ is the feed intake and $x$ is the level of diluent included in the feed.

Table 3.8: $\quad$ The coefficients of the components of the regression equations represented in Figure 3.8

| Diluent | Period | SE | a | b | c |
| :--- | :---: | :---: | :---: | :---: | :---: |
| Cellulose | 1 | 3.58 | -4.5 | 0.05 | 113.92 |
|  | 2 | 3.79 | -4.5 | 0.05 | 133.10 |
|  | 3 | 3.76 | -4.5 | 0.05 | 121.52 |
| Sand | 1 | 6.62 | 1.326 | 0 | 89.52 |
|  | 2 | 7.85 | 1.326 | 0 | 120.03 |
|  | 3 | 7.85 | 1.326 | 0 | 131.95 |
| Sawdust | 1 | 4.83 | 1.56 | -0.04 | 98.51 |
|  | 2 | 5.07 | 1.56 | -0.04 | 113.90 |
|  | 3 | 5.07 | 1.56 | -0.04 | 117.26 |
| Sunflower husk | 1 | 5.02 | 1.30 | -0.04 | 98.46 |
|  | 2 | 5.27 | 1.30 | -0.04 | 123.30 |
|  | 3 | 5.27 | 1.30 | -0.04 | 114.65 |
| Vermiculite | 1 | 4.63 | -1.32 | 0 | 127.13 |
|  | 2 | 5.48 | -1.32 | 0 | 145.85 |
|  | 3 | 5.48 | -1.32 | 0 | 149.35 |

### 3.3.3. Egg weight

The mean egg weights recorded for Trial 1 are represented in Table 3.9. Mean egg weight was significantly higher ( $\mathrm{p}<0.05$ ) in Phases 1 and 2 than in Phase 3 , which had the lowest mean egg weight.

The highest mean egg weights were recorded on the sunflower husk treatments. Significantly lower ( $p<0.05$ ) mean egg weights compared to all other diluents were recorded on the cellulose treatments.

Table 3.9: $\quad$ The mean egg weights ( $g /$ bird.d), over all dilution levels of the mixed feeds, for the diluent $\times$ level $\times$ period, diluent $\times$ level, and diluent $\times$ period interactions of the model, with corresponding standard error (SE) values and significance shown by the letter superscripts.


The highest mean egg weight was recorded for the $0 \mathrm{~g} / \mathrm{kg}$ treatments. The lowest mean egg weight was recorded for the $500 \mathrm{~g} / \mathrm{kg}$ treatments. The $100 \mathrm{~g} / \mathrm{kg}$ treatments had a significantly lower ( $\mathrm{p}<0.05$ ) mean egg weight than the $250 \mathrm{~g} / \mathrm{kg}$ treatments. This is
presented in Figure 3.9. Production relates to feed intake, and feed intake was higher with the hens consuming the $100 \mathrm{~g} / \mathrm{kg}$ treatment, therefore some other factor is influencing the fact that the egg weight was higher in the $250 \mathrm{~g} / \mathrm{kg}$ treatment, perhaps environmental factors or fat deposits.


Figure 3.9: The relationship between egg weight and the level of inclusion of diluent in the feed. The observed values are shown as points: $\square=C S, \boldsymbol{\Delta}=S D, x=S F$, and ${ }^{*}=V$, where CS represents cellulose and sand, SD sawdust, SF sunflower husks, and $V$ vermiculite.

### 3.3.4. Rate of lay

The mean rate of lay recorded for Trial 1 is represented in Table 3.10. The rate of lay was significantly higher ( $\mathrm{p}<0.05$ ) in Phase 3 than in Phases 1 and 2, which had the same mean rate of lay.

The sawdust treatments had the highest rate of lay, followed by the sand, sunflower husk, vermiculite and cellulose treatments respectively. The sawdust and sand treatments produced a significantly higher ( $\mathrm{p}<0.05$ ) rate of lay when compared to the sunflower husk and vermiculite treatments. The cellulose treatments rates of lay were significantly lower than the sunflower husk and vermiculite treatments ( $\mathrm{p}<0.05$ ).

Table 3.10: $\quad$ The calculated mean rate of loy per 100 birds per day, over all dilution levels of the mixed feeds, for the diluent $\times l$ level $\times$ period, diluent $\times$ level, and diluent $\times$ period interactions of the model, with corresponding standard error (SE) values and significance shown by the letter superscripts.

| Diluent | Period | Dilution Rate ( $\mathrm{g} / \mathrm{kg}$ ) |  |  |  | Periodx <br> Diluent |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | 0 | 100 | 250 | 500 | (SE=3.98) |
| Cellulose$(S E=1.99)$ | 1 | $75.60^{\text {a }}$ | $55.95{ }^{\text {b }}$ | $17.26^{\text {c }}$ | $14.88^{\text {c }}$ | $40.92^{\text {b }}$ |
|  | 2 | $86.31^{\text {a }}$ | $8.93{ }^{\text {b }}$ | $1.19{ }^{\text {b }}$ | $8.93{ }^{\text {b }}$ | $26.34^{\text {c }}$ |
|  | 3 | $84.52^{\text {a }}$ | $87.50^{\text {a }}$ | $85.71{ }^{\text {a }}$ | $76.79^{\text {a }}$ | $83.63^{\text {a }}$ |
| $\begin{gathered} \text { Diluent x Level } \\ (S E=3.45) \end{gathered}$ |  | $82.14{ }^{\text {a }}$ | $50.79^{\text {b }}$ | $34.72^{\text {c }}$ | $33.53{ }^{\text {c }}$ |  |
| Sand <br> ( $S E=1.99$ ) | 1 | $85.71{ }^{\text {a }}$ | $67.86{ }^{\text {b }}$ | $20.84{ }^{\text {c }}$ | $20.84^{\text {c }}$ | $48.81{ }^{\text {b }}$ |
|  | 2 | $80.95{ }^{\text {a }}$ | $66.67{ }^{\text {ab }}$ | $77.38^{\text {a }}$ | $84.52^{\text {a }}$ | $77.38^{\text {a }}$ |
|  | 3 | $79.76{ }^{\text {a }}$ | $25.00^{\text {c }}$ | $17.26^{\text {c }}$ | $49.41^{\text {b }}$ | $42.86{ }^{\text {c }}$ |
| $\begin{gathered} \text { Diluent } x \text { Level } \\ (S E=3.45) \end{gathered}$ |  | $82.14{ }^{\text {a }}$ | $53.17{ }^{\text {b }}$ | $38.49^{\text {c }}$ | $51.59{ }^{\text {b }}$ |  |
| Sawdust$(S E=1.99)$ | 1 | $68.45{ }^{\text {a }}$ | $77.38^{\text {a }}$ | $67.26^{\text {a }}$ | $22.03^{\text {b }}$ | $58.78{ }^{\text {a }}$ |
|  | 2 | $72.02^{\text {a }}$ | $48.81{ }^{\text {b }}$ | $65.48^{\text {a }}$ | 22.62 c | $52.23{ }^{\text {ab }}$ |
|  | 3 | $89.88^{\text {a }}$ | $37.50^{\text {c }}$ | $84.52^{\text {a }}$ | $52.98{ }^{\text {b }}$ | $66.22^{\text {a }}$ |
| Diluent $x$ Level ( $S E=3.45$ ) |  | $76.79^{\text {a }}$ | $54.56{ }^{\text {b }}$ | $72.42^{\text {a }}$ | $32.54^{\text {c }}$ |  |
| Sunflower husk (SE=I.99) | 1 | $75.00^{\text {a }}$ | $60.12^{\text {a }}$ | $69.05^{\text {a }}$ | $20.24{ }^{\text {b }}$ | $56.10^{\text {a }}$ |
|  | 2 | $81.55^{\text {a }}$ | $26.79{ }^{\text {c }}$ | $68.45{ }^{\text {b }}$ | $72.03^{\text {a }}$ | $62.20^{\text {a }}$ |
|  | 3 | $70.83{ }^{\text {a }}$ | $30.95{ }^{\text {b }}$ | $61.31^{\text {a }}$ | $21.43{ }^{\text {b }}$ | $46.13^{\text {b }}$ |
| $\begin{gathered} \text { Diluent x Level } \\ (S E=3.45) \end{gathered}$ |  | $75.79^{\text {a }}$ | $39.29^{\text {c }}$ | $66.27^{\text {b }}$ | $37.90^{\text {c }}$ |  |
| Vermiculite$(S E=1.99)$ | 1 | $80.95^{\text {a }}$ | $79.17^{\text {a }}$ | $65.48{ }^{\text {ab }}$ | $21.43^{\text {b }}$ | $61.76{ }^{\text {a }}$ |
|  | 2 | $75.00^{\text {a }}$ | $38.10^{\text {c }}$ | $58.33{ }^{\text {b }}$ | $21.43{ }^{\text {d }}$ | $48.21{ }^{\text {b }}$ |
|  | 3 | $82.14{ }^{\text {a }}$ | $37.50{ }^{\text {b }}$ | $49.40^{\text {b }}$ | $20.83{ }^{\text {c }}$ | $47.47^{\text {b }}$ |
| $\begin{gathered} \text { Diluent x Level } \\ (S E=3.45) \end{gathered}$ |  | 79.36a | $51.59^{\text {b }}$ | $57.74{ }^{\text {b }}$ | $21.23{ }^{\text {c }}$ |  |

The highest rate of lay was recorded at the $0 \mathrm{~g} / \mathrm{kg}$ level of diluent inclusion. The $250 \mathrm{~g} / \mathrm{kg}$ level of inclusion had a significantly higher ( $\mathrm{p}<0.05$ ) rate of lay than the $100 \mathrm{~g} / \mathrm{kg}$ inclusion level. Again, as with egg weight, production relates to feed intake, but here another factor must be influencing the rate of lay, other than feed intake. The lowest rate of lay was recorded for the $500 \mathrm{~g} / \mathrm{kg}$ inclusion level. This is presented in Figure 3.10.


Figure 3.10: A graphic representation of the effect of the level of inclusion of the diluent on mean rate of lay of the hen. The observed values are shown as points: $\square=C$ and $S, \Delta=S D, x=S F$, and ${ }^{*}=V$, where $C$ represents cellulose, $S$ sand, $S D$ sawdust, $S F$ sunflower husks, and $V$ vermiculite.

### 3.4. Conclusion

The objective of this trial was to determine the effect of feed bulk on the voluntary feed intake of the laying hen, so as to determine a suitable measure of feed bulk that would assist in determining the constraining effect of the feed on voluntary intake. Of the bulk characteristics measured on each of the feeds neither the fibre content (CF) nor did the density (dry and wet) expressed a suitable relationship for the prediction of the upper limit of voluntary feed intake and the formation of a single equation. However, the waterholding capacity of the feeds appeared to describe the effect of bulkiness on the voluntary feed intake (Section 3.3.1.4), as scaled feed intake declined linearly as the WHC of the feed increased (Figure 3.11) across dilution levels and diluents.


Figure 3.11: $\quad$ The scaled feed intake (SFI; g/kg live weight per d) on each of the experimental feeds vs. their water-holding capacity determined by centrifugation (WHC; $g$ water/g dry feed). The line shown is that for $S F I(g / k g$ per $d)=272.4 x$

The effect of the WHC of the feed on the feed intake of the hen can be seen when the hens were switched between diluents between Phases 2 and 3. The most noticeable change was that observed in Table 3.7, when the hens on the cellulose-diluted feeds were switched onto sand-diluted feeds and the feed intake of the hens increased significantly ( $P<0.05$ ). This is shown in the change in the feed intake between Phase 2 and 3 . The opposite was true for the hens that were changed from a sand-diluted feed to a cellulose-diluted feed. In this case, the hens that had a mean feed intake of $114 \mathrm{~g} /$ bird d of hens on the $250 \mathrm{~g} / \mathrm{kg}$ sand feed in Phase 1 dropped to $45 \mathrm{~g} /$ bird d on $250 \mathrm{~g} / \mathrm{kg}$ cellulose feed in Phase 3. The failing of the design were that there was no allowance for the possibility for carry-over effects that may have occurred when the hens were switched between diets. Whether a carry-over effect has occurred can be deduced by looking at individual hens SFI for each week of a phase and comparing that to her intake in the other phases. Comparing week for week for each individual hen will determine if there were some effects of the previous treatment on the present intake. The other method of increasing the accuracy of the analysis of the collected FI data would be to use the mean FI, for each diluent, of the previous phase as a
covariate in the analysis of the data from the next phase. This would also decrease the effect of the carry-over effects.

The body weight data was collected essentially to monitor the welfare of the hens, but could be used to determine a more accurate measure of SFI by using the lean body mass to scale the feed intake, thereby removing the variable of energy deposits and their use for egg production. If this was done the use of the egg data could be used more effectively to show where the FI constrained egg production.

The effect of WHC could also be seen with the change from the sunflower husk- to sawdust-diluted feeds. Sawdust has a higher WHC than sunflower husk, which explains the significant decrease ( $P<0.05$ ) in voluntary feed intake when hens were changed onto sawdust-diluted feeds from sunflower husk-diluted feeds. The opposite reaction was observed when hens were changed onto sunflower husk-diluted feeds from sawdust-diluted feeds and the voluntary feed intake increased significantly ( $P<0.05$ ).

If no constraints existed, the desired feed intake of the hens could be calculated the following way. The basal feed was formulated according to a daily feed intake of 110 g . If this feed were diluted at a rate of $100 \mathrm{~g} / \mathrm{kg}$ the hen would have to increase her feed intake by $10 \%$ to sustain her required level of production. The calculated feed intakes are represented in Table 3.11.

Table 3.11: $\quad$ The desired feed intake of the basal feed used and scaled feed intakes of hens weighing 1.8 kg when offered feeds diluted to the levels used in the trial

|  | 0 | 100 | Dilution rate (g/kg) |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | 110 | 121 | 138 |  |
| Desired feed intake <br> $(\mathrm{g} / \mathrm{bird}$ d) <br> Scaled feed intake | 59 | 64 | 73 | 165 |  |
| (g/kg BW per d for <br> a 1.8 kg hen $)$ |  |  | 88 |  |  |

Hens on the cellulose treatments (Table 3.7) did not consume the desired amount of feed (Table 3.11) at any of the dilution levels; i.e. their intake was constrained even by the lowest dilution of cellulose.

Feed intakes by hens on the sand-diluted treatments increased sufficiently to achieve the desired feed intake at each dilution, although production decreased, as illustrated in Figure 3.9 and Figure 3.10. This decrease in egg production could be due to the decreased intakes in Phase 1 and the changing of the feeds every three weeks. The interval between changing the treatments may have been too short, one week, to allow complete adaptation to the feed which would explain the decrease in egg production even though the nutrient intake was sufficient to maintain the original level of production.

Hens managed to compensate and consume sufficient feed on the sawdust-diluted feeds up to the $100 \mathrm{~g} / \mathrm{kg}$ level of inclusion in Phases 2 and 3. Feed intakes in Phase 1 were below the desired levels. Feed intakes of hens on feeds diluted at the levels of 250 and $500 \mathrm{~g} / \mathrm{kg}$ were below the desired intakes and were not sufficient to consume sufficient nutrients to maintain the desired level of production. The same pattern occurred for the sunflower husks and vermiculite treatments.

The feed intakes by hens on all treatments in Phase 1 were lower than the predicted feed intake of $110 \mathrm{~g} /$ bird.d. This hampered the hens from the beginning of the trial and may be the reason why the egg production decreased, even though some hens managed to compensate for the shortfall in nutrients in the diluted feeds in later phases. The hens would have had to use their body reserves in Phase 1 to maintain production which is evident in the decrease in body weights after the first three weeks. This reflects the importance of an adaptation period when changing feeds. The hens used in the trial did receive a week adaptation period to the basal feed before the start of the trial and intakes during this week were satisfactory and did not decrease.

The measurement of the WHC of feed fulfils the experimental objective of identifying an accurate measure of the bulkiness to predict the voluntary feed intake in the laying hen. The actual feed intake is the lower of the constrained and desired feed intakes. The desired
feed intake may be calculated from knowledge of the nutrient requirements of the hen and the nutrient content of the feed offered; the first-limiting nutrient in the feed dictating the constraining effect of the feed on intake appears to be $1 /$ WHC. However, it was felt that insufficient data were available from this trial to derive an equation that could be relied upon to describe this constraining effect. A second trial was, therefore, conducted using different diluents, in an attempt to gather further information that might be used when predicting the constraining effect of a feed on voluntary feed intake.

The effect that the feed bulk had on the laying hens may be more accurately and more convincingly proved if the statistical design of the trial is revisited. More adaptation periods are required to completely eliminate the possibility of carry-over effects between phases, and perhaps it would be easier and better to look at each phase of this trial separately. If done correctly this method would be a more accurate assessment of the effect of bulk on the feed intake of laying hens, and this would be supported by two other phases of experimentation. By running each phase for an experimental period of 4 weeks, followed with a period of 2 weeks between the end and the start of the next experimental phase when the hen is fed the basal feed. The hen would then start the next phase, which could be referred to as a completely different trial. The first week of feeding the experimental feed would be the adaptation period, and measurements would be taken from the following 3 weeks. Trial 1 was originally designed as it was because of time and space constraints. The hen's that were supplied were already past their peak and egg production was starting to decline so it was important that the trial happened in a time space that minimised the effect of this decrease on the collected data.

## CHAPTER 4

## The Extent to which Laying Hens Adapt to High Bulk Feeds

### 4.1. Introduction

A change to a feed of higher bulk content will initially cause a hen to increase her feed intake to compensate for the nutrient shortfall in a diluted feed. Intake may then gradually increase if the hen adapts to the bulkiness of the diet or, if the feed is too 'bulky', the hen may not have the capacity to increase her intake sufficiently and therefore her intake will decrease. This adaptation will continue until an equilibrium intake, appropriate to the feed, is reached. The adaptation may be as a result of dealing better with the direct effect of the bulk, or by an increasing ability of the hen to process the nutrients in the bulky feed (Whittemore et al., 2003). On a constraining feed the equilibrium level of intake will be less than the desired level of feed intake, which is that required for the genetic potential to be achieved (Kyriazakis and Emmans, 1999). In the first trial the inclusion of the diluents was at too high a level, causing most of the diets to be constraining on feed intake. Trial 2 was designed with lower inclusion rate and different diluents to determine the effect on the FI of the hens, and increase the investigation of the effect of WHC on FI.

Jorgenson et al. (1996) undertook a study to determine if the development of the digestive tract of broilers, from 12 days of age, was affected by the amount of fibre in the diet. The effect of the higher dietary crude fibre levels was evident through the increases in size of the digestive tract, particularly the length and weight of the small intestine and caecum. These findings supported those of Kondra et al. (1974). Summers and Leeson (1986) found that Leghorn's gut size increased when higher levels of crude fibre were fed, particularly the length of the intestine and the weight of the gizzard.

The objectives of this trial were to collect additional data that would assist in defining the physical characteristics of the feed that best describe the bulkiness of the feed, and to determine the extent to which, and the rate at which the laying hen can adapt to feeds that have a high bulk and/or low density. This should lead to a more accurate definition of the
capacity of the laying hen for feed bulk such that it would be possible to predict, by means of a feed intake model, at what stage the hen would no longer be capable of adapting to a high bulk feed and, therefore, no longer be able to consume sufficient of the feed to meet her requirements for her potential performance. This required different levels of inclusion of diluents to be examined, as well as diluents that provided different bulk properties, so that the results from the two trials can be combined to give a final conclusion.

### 4.2. Materials and Methods

### 4.2.1. Facilities

The birds were housed individually in wire floored laying cages, arranged in two tiers, in an open-sided laying house with adequate ventilation. Each cage was equipped with a nipple drinker located at the rear of the cage and an individual feed trough at the front. The lighting program consisted of 16 hours of light and 8 hours of dark.

### 4.2.2. Animal description

Two hundred laying hens were randomly allocated to the laying cages. The hens selected were the Hy-line Variety Silver. They were hatched on the $4^{\text {th }}$ of September 2003 and were transferred to group pens in the adjacent laying house on the $8^{\text {th }}$ of January 2004. At the commencement of the trial, the hens were 32 weeks old. The hens were offered the formulated basal feed for one week after being moved into the individual cages, before the test treatments were offered.

### 4.2.3. Basal feed and diluents used

A basal feed was formulated using the WinFeed feed formulation programme (© EFG Software). The raw material composition of the basal feed is given in Table 4.1. The nutrient specifications used in formulating the basal feed were based on an expected feed intake of $100 \mathrm{~g} / \mathrm{d}$, thereby ensuring a nutrient-dense feed. The ME of the feed was 11.5
$\mathrm{MJ} / \mathrm{kg}$ and the amino acid composition was based on the optimum amino acid intakes for a 1.9 kg hen with a mean egg output of 56 g per bird day.

Table 4.1: The formulated composition of the basal feed used throughout Trial 2, and fed to Hy-line Variety Silver birds.

| Ingredient | Inclusion $(\mathrm{g} / \mathrm{kg})$ |
| :--- | :---: |
| Maize | 530.0 |
| Wheat bran | 57.9 |
| Soybean (Full-fat) | 248.0 |
| Sunflower (37.0g/kg protein) | 50.0 |
| L-lysine HCl | 0.10 |
| DL methionine | 1.70 |
| Vitamin premix | 1.50 |
| Limestone | 95.4 |
| Salt | 3.30 |
| Monocalcium phosphate | 9.50 |
| Sodium bicarbonate | 2.50 |
| Analysis of basal feed | Calculated (g/kg) |
| Protein (g N $\times 6.25 \mathrm{~kg}$ ) | 170.1 |
| ME (MJ/kg) | 11.50 |
| Lysine | 8.30 |
| Calcium | 35.00 |
| Phosphorus | 3.50 |

The basal feed was diluted with one of five diluents. The diluents were included at four levels, resulting in twenty dietary treatments in total. The diluents used were river sand, potter's clay, sawdust, unexpanded polystyrene ${ }^{2}$ and wheat bran. The diluents were changed from that of Trial 1 to use a range of different diluents with different bulk properties. The treatments were supplemented with synthetic amino acids and minerals to maintain similar calculated ratios as in the basal food. The inclusion rates of the diluents into the basal feed were $0,50,100$ and $150 \mathrm{~g} / \mathrm{kg}$.

[^2]
### 4.2.4. Design

The trial ran for a total of seven weeks. During the first week, the hens were fed the basal feed to adapt to the change in feed. The twenty treatments were then randomly assigned to individual hens, using a random number generator, for the remaining six weeks of the trial.

Feed was available to the hens ad libitum. The feed was weighed into the self-feeding trough at the start of the week, and the amount remaining at the end of the week was weighed in order to measure feed intake. Feed was weighed in on a Monday and the orts were weighed out the following Monday. At the end of the six-week feeding period, three hens were randomly selected from each treatment group and slaughtered. The hens were eviscerated and the gut contents examined. The weights of the de-feathered carcass and the entire gastrointestinal tract (GIT) were determined. The gizzard, small intestine, caecum and colon were weighed while still containing digesta and again after cleaning. The fat pad was removed and weighed. The lengths of the small intestine, caecum and colon were measured.

### 4.2.5. Feed and diluent analysis

The feeds were analysed with the same methods used in the first trial (Chapter 3) and all analyses were done in duplicate. The methods used to analyse the feeds were:

- Water-displacement method,
- Dry-density method,
- Centrifugation to determine the water-holding capacity.

These are described in detail in Chapter 3. The feeds were also analysed for CF, ADF and NDF. All the fibre analysis was done by the Nutrient Analysis Laboratory, Discipline of Animal and Poultry Science, University of KwaZulu-Natal. Crude fibre (CF) and acid detergent fibre (ADF) were analysed on a Dosi-Fibre machine using the AOAC Official Method 920.39 and AOAC Official Method 973.18, respectively. Neutral netergent fibre (NDF) was analyzed on a Dosi-Fibre machine according to the method described by Van Soest et al. (1991).

### 4.2.6. Measurements

The weekly feed intake, daily rate of lay, egg weight on three days each week and body weights of the hen at the start, and after each three-week period were recorded. From these measurements, the change in body weight of the hen could be determined. Those hens whose body weight had dropped below 1000 g were removed from the trial. On the day of slaughter, the feed was removed from the hens in the morning. The hens were then tagged for carcass identification. The hens were rendered unconscious by stunning before exsanguination. The carcasses were bled out before evisceration. The carcasses were eviscerated and the components examined.

### 4.3. RESULTS AND DISCUSSION

### 4.3.1. Physical and chemical properties of dietary treatments

Measurements were made of crude fibre (CF), acid detergent fibre (ADF) and neutral detergent fibre (NDF) as well as the mean water-holding capacity (WHC) and dry and wet densities of each feed and the diluents used. These values for each of the feeds used are presented in Table 4.3, and for the diluents, in Table 4.2.

Table 4.2: The bulk measurements of the individual, unmixed diluents used.

| Diluent | WHC <br> (g water/g <br> dry feed) | Dry <br> density <br> $(\mathrm{g} / \mathrm{ml})$ | Wet <br> density <br> $(\mathrm{g} / \mathrm{ml})$ | CF <br> $(\mathrm{g} / \mathrm{kg})$ | ADF <br> $(\mathrm{g} / \mathrm{kg})$ | NDF <br> $(\mathrm{g} / \mathrm{kg})$ |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: |
|  | 3.80 | 0.744 | 1.39 | 39.7 | 49.4 | 118.4 |
|  | 9.43 | 0.318 | 1.05 | 112.2 | 139.6 | 428.5 |
| Potters clay | 1.33 | 0.748 | 2.39 | 0 | 0 | 0 |
| Unexpanded polystyrene | 2.87 | 0.668 | 0.96 | 0 | 0 | 0 |
| River Sand | 1.29 | 1.403 | 2.55 | 0 | 0 | 0 |
| Sawdust | 8.07 | 0.212 | 0.93 | 689.0 | 768.7 | 874.5 |

Table 4.3: Mean water-holding capacity (WHC) ( $g$ water $/ g$ dry feed), dry and wet density $(g / m l)$, crude fibre (CF), acid detergent fibre (ADF) and neutral detergent fibre (NDF) (g/kg) of the mixed feeds.

| Diluent | Level <br> $(\mathrm{g} / \mathrm{kg})$ | WHC <br> $(\mathrm{g}$ water/g <br> dry feed $)$ | Dry <br> density <br> $(\mathrm{g} / \mathrm{ml})$ | Wet <br> density <br> $(\mathrm{g} / \mathrm{ml})$ | CF <br> $(\mathrm{g} / \mathrm{kg})$ | ADF <br> $(\mathrm{g} / \mathrm{kg})$ | NDF <br> $(\mathrm{g} / \mathrm{kg})$ |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Wheat bran | 50 | 4.00 | 0.67 | 1.37 | 4.27 | 5.93 | 13.22 |
|  | 100 | 4.17 | 0.67 | 1.36 | 4.39 | 6.32 | 14.17 |
|  | 150 | 3.85 | 0.59 | 1.34 | 4.64 | 7.03 | 16.84 |
| Potters clay | 50 | 3.57 | 0.70 | 1.49 | 3.77 | 4.69 | 11.25 |
|  | 100 | 3.70 | 0.67 | 1.49 | 3.57 | 4.46 | 10.66 |
|  | 150 | 3.33 | 0.72 | 1.39 | 3.37 | 4.20 | 10.06 |
| Unexpanded | 50 | 4.00 | 0.67 | 1.39 | 3.77 | 4.69 | 11.25 |
| polystyrene | 100 | 4.00 | 0.71 | 1.31 | 3.57 | 4.46 | 10.66 |
|  | 150 | 3.45 | 0.72 | 1.29 | 3.37 | 4.20 | 10.06 |
| River sand | 50 | 2.94 | 0.73 | 1.34 | 3.77 | 4.69 | 11.25 |
|  | 100 | 4.17 | 0.73 | 1.45 | 3.57 | 4.46 | 10.66 |
|  | 150 | 3.33 | 0.78 | 1.49 | 3.37 | 4.20 | 10.06 |
| Sawdust | 50 | 4.76 | 0.60 | 1.35 | 7.12 | 9.27 | 15.34 |
|  | 100 | 4.76 | 0.51 | 1.30 | 9.62 | 12.87 | 20.22 |
|  | 150 | 4.76 | 0.49 | 1.28 | 14.37 | 16.09 | 23.39 |

Each measure of bulk will be discussed in turn.

### 4.3.1.1. Crude fibre

Three of the diluents used in this trial had no crude fibre (CF) content, so it is unlikely that this measure of feed bulk could be used to predict when a feed would become too bulky for the bird to be able to meet its desired feed intake. As the CF contents of bran and sawdust are higher than that of the basal feed, the CF contents of the feeds diluted with these two diluents would have increased with each increment of the diluent. However, the relationship between the crude fibre content and feed intake (Figure 4.1) indicates that this chemical measure of bulk bears little relationship with feed intake, and is therefore not a worthwhile predictor of the bulkiness of the feed.

As the crude fibre content of the feed increased, so feed intake increased. The rate of increase in feed intake was not the same between diluents, suggesting that a factor other than CF was directly responsible for altering the feed intake of the hen.


Figure 4.1: $\quad$ The relationship between the fibre content of the feed and the feed intake in $g / b i r d$ d. The observed values are shown as points: $\square=B$, and $\boldsymbol{\Delta}=S D$. Where $B$ and $S D$ refer to wheat bran and sawdust respectively. $S E=6.15$.

### 4.3.1.2. Dry density

As the dry density of the feed increased, feed intake decreased for bran, clay and unexpanded polystyrene treatments, but increased for the sand and remained the same for sawdust (Figure 4.2). The sand was denser than all the other diluents which may explain why the intake increased and palatability may have played a role. As with crude fibre, this again suggests that the dry density of the food is not a good measure for predicting the constraining effects of the food on feed intake as there seems to be no explanatory pattern.


Figure 4.2: $\quad$ The relationship between the dry density of a feed and feed intake in g/bird $d$. The observed values are shown as points: $\square=B, \downarrow=C, \Delta=S D, \times=S$ and ${ }^{*}=P$, where $B, C, S D, S$ and $P$ refer to wheat bran, potter's clay, sawdust, river sand and unexpanded polystyrene, respectively, $S E=5.97$.

A significant difference ( $\mathrm{p}<0.05$ ) was observed between the dry density of the bran-diluted diets and the rest of the diluents.

### 4.3.2.3. Wet density

Figure 4.3 illustrates that no relationship exists between the wet density of the feed and the feed intake in Trial 2. In Trial 1 the relationship was distinctly positive, i.e. as the wet density of the feed increased, so the intake of the feed increased. Once again there was no significant difference ( $\mathrm{p}<0.05$ ) between the feed intakes of the different diluents. This indicates that the wet density of the feed had little effect on the feed intake of the hens at the diluent level of inclusion in Trial 2.


Figure 4.3: The relationship between the wet density of the feed and feed intake in g/bird d. The observed values are shown as points: $\square=B, \star=C, \Delta=S D, x=S$ and $*=P$, where $B, C, S D, S$ and $P$ refer to wheat bran, potter's clay, sawdust, river sand and unexpanded polystyrene, respectively.

### 4.3.1.4. Water-holding capacity

In the previous chapter, it was found that the scaled feed intake of the hen is positively related to the inverse of the measured water-holding capacity (WHC). Kyriazakis and Emmans (1994) found the relationship between the scaled feed intake and the reciprocal of
water-holding capacity to be linear in pigs and, in Trial 1, Figure 3.5 shows this to be true in laying hens. The y-intercept differs between all treatments, but the regression analysis showed that no significant difference ( $\mathrm{p}<0.05$ ) in feed intake existed between diluents (Figure 3.5).

In Figure 4.4 the separation between lines exists because of the difference in the intakes of the basal feed, which can be attributed to the differences in the average weight of the hens in each treatment. Another improvement of data analyses, as mentioned earlier, may be to include the hen's lean mass as a covariate, which may then show a better adaptation pattern to the treatments and decrease the separation between the lines.


Figure 4.4: A graphic representation of the relationship between $1 /$ WHC of the feed and the scaled feed intake in $\mathrm{g} /$ bird $d$. The observed values are shown as points: $\square=B, \star=C, \Delta=S D, \times=S$ and $*=P$, where $B, C, S D, S$ and $P$ refer to wheat bran, potter's clay, sawdust, river sand and unexpanded polystyrene, respectively.

### 4.3.2. Feed intake

The mean feed intake ( $\mathrm{g} / \mathrm{bird} \mathrm{d}$ ) on each of the dietary treatments is presented in Table 4.4 in $\mathrm{g} / \mathrm{bird}$ d. Feed intake increased for all diluents and levels from the average feed intake. The relationship between feed intake and the level of inclusion of diluent in the feed was linear in all cases and the coefficients of the regressions are shown in Table 4.5.

Table 4.4: $\quad$ The mean feed intakes in g/bird d for each treatment over the six week period, showing the SE values, the main effects of the diluent and level, the interaction between diluent and level of inclusion, and comparing the mean recorded feed intakes with the calculated desired feed intakes

| Diluent | Dilution rate (g/kg) |  |  |  | Main effect- |
| :--- | :---: | :---: | :---: | :---: | :---: |
| (SE=4.17) | 0 | 50 | 100 | 150 | diluent (SE=2.08) |
| Wheat bran | $112.2^{\mathrm{ab}}$ | $111.0^{\mathrm{a}}$ | $110.6^{\mathrm{a}}$ | $115.4^{\mathrm{b}}$ | $112.3^{\mathrm{a}}$ |
| Potter's clay | $114.0^{\mathrm{a}}$ | $117.4^{\mathrm{ab}}$ | $119.6^{\mathrm{b}}$ | $119.9^{\mathrm{b}}$ | $117.7^{\mathrm{b}}$ |
| Unexpanded polystyrene | $108.9^{\mathrm{a}}$ | $116.0^{\mathrm{b}}$ | $124.0^{\mathrm{c}}$ | $126.2^{\mathrm{c}}$ | $118.8^{\mathrm{bc}}$ |
| River sand | $111.6^{\mathrm{a}}$ | $117.2^{\mathrm{b}}$ | $123.8^{\mathrm{c}}$ | $132.6^{\mathrm{d}}$ | $121.3^{\mathrm{c}}$ |
| Sawdust | $117.5^{\mathrm{b}}$ | $112.3^{\mathrm{a}}$ | $119.5^{\mathrm{b}}$ | $115.8^{\mathrm{ab}}$ | $116.3^{\mathrm{b}}$ |
| Main effect-level (SE=1.86) | $112.8^{\mathrm{a}}$ | $114.8^{\mathrm{b}}$ | $119.5^{\mathrm{c}}$ | $122.0^{\mathrm{d}}$ |  |
| Calculated DFI | 113.0 | 118.0 | 124.0 | 130.0 |  |

Table 4.5: $\quad$ The coefficient of the regression equations, of the form $y=a+b x$ where $x$ is the level of diluent in the feed, showing the relationship between the level of diluent in the feed and the feed intake of the laying hen.

| Diluent | $\mathrm{a}(\mathrm{SE}=2.19)$ | $\mathrm{b}(\mathrm{SE}=0.234)$ |
| :--- | :---: | :---: |
| Wheat bran | 0.19 | 110.9 |
| Potter's clay | 0.40 | 114.7 |
| Unexpanded polystyrene | 1.19 | 109.9 |
| River sand | 1.39 | 110.8 |
| Sawdust | 0.04 | 115.9 |

Table 4.4 shows that when comparing the feed intake of birds on the various feeding treatments with those calculated as the desired feed intake (DFI) only those hens given feed diluted with river sand were able to consume sufficient of the feed to meet their nutrient requirements at all inclusion rates of the diluent. Hens given feeds diluted with
potter's clay and polystyrene were able to consume sufficient of the feed at a dilution rate of $50 \mathrm{~g} / \mathrm{kg}$ but, at $100 \mathrm{~g} / \mathrm{kg}$ the feed diluted with potters clay became constraining and desired feed intake was not achieved (Table 4.4). However, in only one treatment (sawdust at a dilution of $150 \mathrm{~g} / \mathrm{kg}$ ) was egg output significantly reduced, i.e. feed intake was sufficient in all other cases to sustain egg output over the six-week period and intake continue to increase as the level of dilution increased (Figure 4.5). Mean egg output for each of the dietary treatments is recorded in Table 4.6.

Table 4.6: $\quad$ The calculated mean egg output ( $g / 100$ birds) for hens over the total six week period, not including the data from the adaptation week. Means with different superscripts differ significantly ( $P<0.05$ ).

| Diluent | Dilution rate (g/kg) |  |  |  | Mean |
| :--- | :---: | :---: | :---: | :---: | :---: |
|  | 0 | 50 | 100 | 150 |  |
| Wheat bran | 57.50 | 56.78 | 55.08 | 58.24 | 56.85 |
| Potter's clay | 57.36 | 59.15 | 54.84 | 58.32 | 57.44 |
| Unexpanded polystyrene | 57.13 | 57.97 | 57.43 | 59.26 | 57.99 |
| River sand | 56.64 | 60.56 | 57.42 | 59.17 | 58.46 |
| Sawdust | $58.64^{\mathrm{a}}$ | $57.83^{\mathrm{a}}$ | $58.56^{\mathrm{a}}$ | $51.64^{\mathrm{b}}$ | 56.64 |
| Mean | 57.53 | 58.44 | 26.69 | 57.28 |  |



Figure 4.5: $\quad$ The linear regression relationship between feed intake ( $g / b i r d . d$ ) and the rate of inclusion (\%) of the diluent in the feed, where B, C, P, S and SD refer to the wheat bran, potter's clay, unexpanded polystyrene, river sand and sawdust treatments, respectively. The calculated means are represented as $\quad \dot{\Delta}=$ wheat bran, $\mathbf{\Lambda}=$ sowdust,$*=$ potter's clay, $\times=$ river sand, and ${ }^{*}=$ unexpanded polystyrene

The egg output (EO) of the hens was calculated from the rate of lay (ROL) and the egg weight $(E W) ; E O=R O L \times E W$. Table 4.6 reports the $E O$ in $g / 100$ birds. No significant difference was shown between the EW and the ROL for all diluents and levels of inclusion, except the $150 \mathrm{~g} / \mathrm{kg}$ sawdust-diluted diet, which had a significantly lower egg weight $(\mathrm{SE}=0.949)$ and rate of lay $(\mathrm{SE}=1.142)$ than the lower inclusion levels. The egg weight and rate of lay was not analysed because of the lack of effect of the treatments on both parameters. The recorded data is available in the Appendix.

Feed intake was recorded weekly and is represented graphically for each of the treatments in Figure 4.6. Mean feed intake over all diluents increased substantially during the second week of the trial, i.e. immediately after the diluted feeds were introduced. Feed intake then decreased for the following three weeks and then increased once more. This suggests that the hens did not become acclimatised to the bulky feeds, which would have been evident
had the intakes increased steadily throughout the six-week period. The hen's intake should have increased rapidly to compensate for the dilution of nutrients, but the rate of intake would slow as the hen's capacity for intake increased to adapt to the 'bulkier' feed. The hens should have also increased their overall intake of feed. This may have been observed had the trial run for longer (Kyriazakis and Emmans, 1994).


Figure 4.6: $\quad$ Relationship between feed intake in $g / b i r d d$ and time in weeks in Trial 2. The calculated means are represented as $\dot{\star}=$ wheat bran, $\mathbf{\Delta}=$ sawdust,$\stackrel{\text { potter's cloy, }}{ } \times=$ river sand, and $*=$ unexpanded polystyrene

Egg production was not decreased significantly by the dietary treatments; therefore, the data collected in this trial cannot be used to determine the maximum gut capacity of the hens, except in the case of the sawdust at $150 \mathrm{~g} / \mathrm{kg}$ dilution where egg production was constrained. Feed intake increased for all diluents and levels from the average feed intake of $101.7 \mathrm{~g} / \mathrm{bird} \mathrm{d}$ in the adaptation week. The levels of inclusion were dropped from those used in Chapter 3 to determine the point at which the intake would stop increasing and either even out or decrease.

It is now evident that the inclusion levels of the diluents in this trial were not sufficient to constrain the feed intake other than the sawdust-diluted feed at an inclusion rate of 150 $\mathrm{g} / \mathrm{kg}$. The feeds in Trial 1 did constrain feed intake and the equation derived would, therefore, predict the constrained feed intake. This line was added to Figure 4.4. Feed intake is predicted to be constrained above this line as can be seen from Figure 4.4 and very few of the observations made in this trial lie above the line $y=46.3+83.5 x$.


Figure 4.7: A graphic representation of the relationship between 1/WHC of a feed and the scaled feed intake in $g / b i r d . d$. The observed values are shown as points: $\square=B,=C, \Delta=S D, x=S$ and ${ }^{*}=P$, where $B, C$, SD, $S$ and $P$ refer to wheat bran, potter's clay, sawdust, river sand and unexpanded polystyrene, respectively. The feint line represents the equation $y=46.3+83.5 x$.

### 4.4. Processing of birds

The weights of the gastric content of the different portions of the gastrointestinal tract (GIT) are given in Table 4.7, and the empty weights of the same components of the gastro intestinal tract are given in Table 4.8. The GIT was removed and weighed full (Table 4.7). It was then divided and cut into the different components and weighed, the contents weight was calculated by difference of the empty and full weights of the components (Table 4.7). The contents were then removed and the components washed and then weighed again (Table 4.8).

Table 4.7: $\quad$ Table showing the mean weight $(\mathrm{g})$ of material contained in the entire gastrointestinal tract (GIT), and the distribution of the contents throughout the different components of the GIT.

|  | Devel <br> Diluent | Full weight (g) | Weight of contents (g) |  |  |  |
| :--- | ---: | :---: | :---: | :---: | :---: | :---: |
|  |  | GIT | Gizzard | Small <br> intestine | Colon | Caecum |
| Basal |  | 155.8 | 13.9 | 30.4 | 5.1 | 7.6 |
| Wheat |  | 159.3 | 15.1 | 35.2 | 2.1 | 7.1 |
| bran | 100 | 164.0 | 9.3 | 48.6 | 2.3 | 11.4 |
|  | 150 | 152.0 | 12.8 | 38.4 | 2.5 | 5.9 |
| Potter's | 50 | 148.4 | 12.4 | 36.6 | 3.7 | 5.7 |
| clay | 100 | 143.5 | 11.6 | 33.7 | 2.4 | 8.4 |
|  | 150 | 177.8 | 17.5 | 40.8 | 1.6 | 8.6 |
| Unexpanded | 50 | 135.6 | 10.2 | 27.3 | 2.3 | 8.0 |
| polystyrene | 100 | 144.7 | 13.9 | 28.1 | 1.0 | 7.3 |
|  | 150 | 132.2 | 10.2 | 31.2 | 0.9 | 4.6 |
| River sand | 50 | 143.9 | 24.6 | 29.6 | 2.0 | 5.7 |
|  | 100 | 150.2 | 23.0 | 25.3 | 2.1 | 8.9. |
|  | 150 | 152.4 | 36.8 | 37.1 | 1.0 | 7.4 |
| Sawdust | 50 | 150.1 | 28.7 | 29.8 | 5.2 | 7.2 |
|  | 100 | 157.1 | 22.0 | 33.0 | 2.2 | 7.6 |
|  | 150 | 141.3 | 13.7 | 26.2 | 2.2 | 5.7 |

The weights of the gizzard and small intestine contents of the river sand-diluted feeds were significantly higher than all the other feeds, but the weights of the colon and caecum contents were similar to the other feeds. The gut contents of the $150 \mathrm{~g} / \mathrm{kg}$ sawdust-diluted feed were the lowest of all the observations, and the observations of the sawdust-diluted feeds decreased from the $50 \mathrm{~g} / \mathrm{kg}$ level to the $150 \mathrm{~g} / \mathrm{kg}$ level for all observations. The type of diluent had an effect on the weight of the contents of the gastrointestinal tract. The sand, sawdust, bran and clay-diluted feeds all had heavier gastrointestinal tracts, and contents, than the polystyrene-diluted feeds, but this could be due to the weight of the digesta within the full tract and the size of the birds.

Table 4.8: $\quad$ Table of means of empty weights measured in $g$ and lengths measured in cm of the components of the digestive tract

| Diluent | Level | Empty Weights (g) |  |  |  | Lengths (cm) |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | Gizzard | Small intestine | Colon | Caecum | Small intestine | Colon | Саесит |
| Basal |  | 41.6 | 41.9 | 4.2 | 11.2 | 158.0 | 8.7 | 35.3 |
| Wheat bran | 5 | 40.0 | 41.0 | 3.2 | 10.1 | 164.0 | 8.7 | 40.3 |
|  | 10 | $40.4$ | 35.9 | 4.0 | 12.0 | 152.7 | 8.7 | 34.7 |
|  | 15 | 41.0 | 37.6 | 3.4 | 10.5 | 153.3 | 7.7 | 37.0 |
| Potter's clay | 5 | 37.5 | 40.5 | 2.9 | 8.9 | 156.0 | 7.5 | 35.7 |
|  | 10 | $32.4$ | 38.2 | 4.1 | 7.3 | 141.7 | 8.2 | 35.3 |
|  |  | 38.1 | 38.1 |  |  | 149.3 | 7.7 | 34.3 |
| Unexpanded polystyrene | 5 | 35.9 | 39.1 | 3.6 | 9.2 | 149.3 | 8.7 | 39.3 |
|  | 10 | $43.0$ | 37.6 | 4.2 | 9.6 | 148.0 | 9.3 | 36.7 |
|  |  | 38.3 | 33.9 | 3.3 | 9.3 | 152.7 | 8.2 | 32.3 |
| River sand | 5 | 37.7 | 40.5 | 4.1 | 9.2 | 143.3 | 7.8 | 32.7 |
|  | 10 | 41.6 | 39.3 | 4.6 | 10.1 | 146.7 | 8.7 | 36.3 |
|  | 15 | 37.3 | 37.0 | 3.2 | 11.9 | 155.3 | 7.0 | 37.0 |
| Sawdust | 5 | 40.6 | 39.5 | 4.7 | 9.4 | 153.3 | 10.0 | 38.3 |
|  | 10 | 41.6 | 41.4 | 3.1 | 9.4 | 146.7 | 7.8 | 38.0 |
|  | 15 | 43.0 | 36.0 | 4.9 | 9.7 | 142.7 | 9.0 | 35.0 |

Statistical analysis of the processing data showed no significant effects of the feed on the changes to the GIT and was not investigated further. Feed bulk had no effect on the empty weights or the lengths of the components of the gastrointestinal tract. There was no change caused by the feed on these components. This could be because the feeds were not very limiting on the voluntary feed intake of the hens. Had the same process been exercised in Trial 1 a different pattern may have resulted, due to the higher inclusion rate of the diluents. The diluent inclusion rates in Trials 1 were more limiting on the feed intake than the diluent inclusion levels used in Trial 2. These results do not agree with the results reported by Kyriazakis and Emmans (1994), who found that, in pigs, the progressive increase of wheat-bran in the feed resulted in a significant $(P<0.05)$ increase in the weights
of the stomach and large intestine, but a significant decrease $(P<0.05)$ in the weight of the small intestine. This discrepancy could be due to the effect that the gizzard has on the digesta, which is not present in the pig. The grinding of the feed could cause this discrepancy, allowing for a faster flow rate of the digesta.

### 4.5. Conclusions

The feed intakes in this trial increased as the level of the diluent in the feed increased, but there was no significant decrease in production, other than in one case, meaning that the hens consumed sufficient nutrients to maintain their level of production except where the food was diluted with 150 g sawdust $/ \mathrm{kg}$ feed (circled in Figure 4.4).

When the feed intake during the trial was compared over time in weeks no significant adaptation to the feeds was evident because the feeds were not constraining. In fact, feed intake decreased after the initial rise, possibly due to high temperatures experienced at that time. However, temperature was not recorded.

As with the first trial, the crude fibre, dry density and wet density proved to be poor measures for the prediction feed intake, bearing no relation to the amount of feed consumed by the hens. The water-holding capacity of the feeds, however, appeared to afford some measure of predictability in that the equation derived in Trial 1 to predict constrained feed intake, demonstrated that few of the feeds in Trial 2 would have constrained feed intake.

It is clear that the water-holding capacity of the feed is not an infallible means of determining when the bulkiness of a feed will be such that the desired feed intake cannot be achieved, but it does appear to give a reasonable estimate of the constraining effect of a feed. In order to test the accuracy of the estimated equation (Equation 34) derived in the previous chapter, data from the two trials conducted here, and from a previous trial at this University (Williams, 1993) could profitably be combined. This will be done in the following chapter of this thesis.

## CHAPTER 5

## General Discussion and Conclusions

### 5.1. SUMMARY

The aim of this study was to determine a suitable method of measuring and predicting feed bulk, such that this could be used to predict when a laying hen would be unable to consume sufficient of a given feed to meet her nutrient requirements. Two trials were conducted using a number of diluents, with each being included over a range of concentrations, and voluntary feed intake of hens housed individually was measured. In both trials, it was concluded that the water holding capacity of the feed was the best measure of feed bulk for the desired purpose.

In the first trial, five diluents were used, namely, cellulose, plasterer' sand, sunflower husks, sawdust and vermiculite, and these were included at 100,250 and $500 \mathrm{~g} / \mathrm{kg}$ into a commercial layer feed which was used as the basal feed. The trial was divided into three phases of 21 days each. After each phase, either the diluent fed was changed or the inclusion level of the diluent was changed. It was observed that as the water-holding capacity of the feed increased, so the feed intake decreased. At the greatest dilution of the basal feed, there was a substantial reduction in voluntary feed intake. Intakes on the intermediate foods were greater than those on the basal foods. The constraining effect of feed bulk was expressed as decreased production by the hens. A significant difference in feed intake was observed when the diluents fed to the hen were changed. The scaled feed intake of the hens was fitted to the reciprocal of the water-holding capacity (WHC) to give a linear relationship represented by:

$$
\begin{equation*}
\mathrm{SFI}(\mathrm{~g} / \mathrm{kg} \mathrm{BW})=46.3( \pm 12.2)+83.5( \pm 60.8) .1 / \mathrm{WHC} \tag{33}
\end{equation*}
$$

This regression was the best fit, representing the maximum amount of feed that the laying hen could consume when the constraint is measured as the reciprocal of WHC. However, Tsaras et al. (1998) have argued that such a regression should pass through the origin (no
constant term), so a second regression line was fitted to the data, this time with no constant term, and the relationship in this case was;

$$
\begin{equation*}
\mathrm{SFI}(\mathrm{~g} / \mathrm{kg} \mathrm{BW})=272.4( \pm 8.9) .1 / \mathrm{WHC} \tag{34}
\end{equation*}
$$

The equivalent regression coefficient obtained by Tsaras et al. (1998), for pigs, was 234.6, this value being lower than the above coefficient obtained with laying hens. Of prime importance when fitting such a regression to the data is to exclude any points where the feed intake is patently not constraining, so of all the treatments applied in the first trial, only those with a WHC value greater than 4 g water/g dry feed appeared to constrain feed intake, as measured by a reduced rate of egg output. If only these data points are included in the regression analysis above the equation becomes:

$$
\begin{equation*}
\mathrm{SFI}(\mathrm{~g} / \mathrm{kg} \mathrm{BW})=313.6( \pm 8.9) \cdot 1 / \mathrm{WHC} \tag{36}
\end{equation*}
$$

The objective of the second trial was to identify physical characteristics of the feed that best describe the bulkiness of the feed, and to determine the extent to which, and the rate at which, the laying hen can adapt to feeds that are high in bulk. Once again five diluents were used, namely, wheat bran, potter's clay, unexpanded polystyrene, river sand and sawdust, but in this trial the dilution rates were lower than the first trial, being 50, 100 and $150 \mathrm{~g} / \mathrm{kg}$. The trial was not divided into phases and the hens were fed the same feed for six weeks. The voluntary feed intake of the hens in Trial 2 was sufficient on all but one treatment to maintain production, the exception being the treatment in which 150 g of sawdust was used per kg feed. The equation derived from Trial 1 (Equation 36) was fitted to the data from Trial 2 and the trend showed few of the treatments to be constraining.

Feeds shown to be constraining from Trials 1 and 2, along with data from Williams (1993), were combined, and the results of this are shown in Figure 5.1, to obtain a more accurate assessment of the relationship between scaled feed intake and 1/WHC than could be obtained from just one trial. Although the best fitting regression through these data includes a constant term the intercept was not significantly different from zero, so the
regression of the SFI on $1 / \mathrm{WHC}$ was recalculated with the intercept suppressed. The line is represented by the equation:

$$
\begin{equation*}
\text { SFI }(\mathrm{g} / \mathrm{kg} \mathrm{BW})=301.4 .1 / \mathrm{WHC} \tag{37}
\end{equation*}
$$

This regression represents the upper limit to feed intake when a hen is offered a bulky feed. As an example, if a 2 kg hen were offered a feed with a water-holding capacity of 6.667 $(1 / \mathrm{WHC}=0.15)$ her intake on that feed would be $90 \mathrm{~g} / \mathrm{d}(=2 \times 45 \mathrm{~g} / \mathrm{kg} \mathrm{BW})$.

Standard commercial feeds have a water-holding capacity of around 4 g water $/ \mathrm{g}$ dry feed $(1 / \mathrm{WHC}=0.25)$, so feeds with values for $1 / \mathrm{WHC}$ greater than 0.25 are unlikely to constrain feed intake due to their bulkiness, except in highly unusual circumstances. Consequently, the graph presented in Figure 5.1, was drawn to include only feeds, from Trial 1, Trial 2 and Williams (1993), with values for $1 / \mathrm{WHC}$ less than 0.25 .


Figure 5.1: $\quad$ The relationship between scaled feed intake (SFI, $g / \mathrm{kg}$ BW) and the reciprocal of waterholding capacity for data collected in Trials 1 and 2, and Williams (1993); including values for 1/WHC of 0.25 and less.

The discrepancy that exists between the equations derived here and that from Trial 1 could be due to a difference in the methodology used to determine the water-holding capacity. Robertson and Eastwood (1981) reported that the methods of drying fibre before
determining the water-holding capacity of a material affect its structure and that the changes can affect the water-holding capacity values. Tsaras et al. (1998) reported a discrepancy in their results when using freeze-drying and oven-drying, and the values determined by Williams (1993) were not freeze-dried after centrifugation, but oven dried, and all values determined in this thesis were from freeze-dried samples. This would have caused a difference in results.

### 5.2. Calculation of bulk constraint

A feed is formulated according to the estimated feed intake of the hen. The feed intake is an average of a population, meaning that some hens may eat more and others less depending on their body weight and potential egg output. When predicting voluntary feed intake of a hen when presented with a given feed, it is relatively simple to predict what the feed intake would need to be (desired) in order for the hen to meet her nutrient requirements, but it is as important, but more difficult, to determine whether the hen would be able to consume the desired amount of food. For this purpose it is necessary to determine whether the gut capacity of the hen would be capable of accommodating the food bulk. The equation given above will give a reasonable prediction of how much of the given food she would be capable of consuming and, if this is less than the desired feed intake, feed intake will be constrained, and her performance would be adjusted downward accordingly.

If a given feed were diluted with a diluent with a water-holding capacity of 10 g water $/ \mathrm{g}$ dry feed $(1 / \mathrm{WHC}=0.1)$ then the constrained feed intake could be calculated from the proportions of the basal feed and diluent added together, i.e.:

If the basal feed has a calculated $1 / \mathrm{WHC}$ of 0.26 and the desired feed intake (DFI) of the basal feed is $110 \mathrm{~g} /$ bird d , this can be converted to a scaled feed intake (SFI) of $58 \mathrm{~g} / \mathrm{kg}$ body weight (BW) for a 1.9 kg bird. If the basal feed is diluted with a diluent at the rate of $100 \mathrm{~g} / \mathrm{kg}$, then the scaled constrained feed intake (CFI) can be calculated as;

$$
\mathrm{CFI}(\mathrm{~g} / \text { bird.d })(10 \% \text { dilution })=(0.9 \times 0.26)+(0.1 \times 0.1)
$$

By including a value for WHC in a feed composition matrix, the value for a mixed feed would be calculated automatically. Table 5.1 presents some hypothetical values to illustrate this theory, which are presented in Figure 5.2. The values were chosen to represent a range of WHC values. The lowest value, $1 / \mathrm{WHC}_{1}$ was chosen to represent a feed with a very high WHC, i.e. cellulose.

Table 5.1: Calculation of the constrained feed intake (CFI) of a 1.9 kg laying hen from the effect of bulk, expressed as the inverse of WHC, on the desired feed intake (DFI) over a range of hypothetical bulk values.

| Dilution <br> Rate | DFI | Scaled <br> DFI | $1 / \mathrm{WHC}_{1}$ <br> $=0.07$ | $\mathrm{CFI}_{1}$ | $1 / \mathrm{WHC}_{2}$ <br> $=0.1$ | $\mathrm{CFI}_{2}$ | $1 / \mathrm{WHC}_{3}$ <br> $=0.2$ | $\mathrm{CFI}_{3}$ | $1 / \mathrm{WHC}_{4}$ <br> $=0.3$ | $\mathrm{CFI}_{4}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 0 | 110 | 58 | 0.26 | 78.4 | 0.26 | 78.4 | 0.26 | 78.4 | 0.26 | 78.4 |
| 5 | 116 | 61 | 0.25 | 75.5 | 0.25 | 76.0 | 0.26 | 77.5 | 0.26 | 79.0 |
| 10 | 121 | 64 | 0.24 | 72.6 | 0.24 | 73.5 | 0.25 | 76.6 | 0.26 | 79.6 |
| 15 | 127 | 67 | 0.23 | 69.8 | 0.24 | 71.3 | 0.25 | 75.7 | 0.27 | 80.2 |
| 25 | 138 | 72 | 0.21 | 64.1 | 0.22 | 66.3 | 0.25 | 73.8 | 0.27 | 81.4 |
| 50 | 165 | 87 | 0.17 | 49.7 | 0.18 | 54.3 | 0.23 | 69.3 | 0.28 | 84.4 |

The raw materials used to formulate the basal feed used in Trial 2 were individually analysed to determine their WHC's. These results were then added into the WinFeed formulation programme (EFG Software) matrix and the feed was formulated according to the same specifications as in Trial 2. However, the calculated WHC of the feed formulated was not the same as the measured WHC, being 2.97 g water $/ \mathrm{g}$ dry feed and 3.8 g water $/ \mathrm{g}$ dry feed, respectively. The difference could be due to the fact that the error in measurement would be greater when a number of measurements were added together. Also the sample taken and measured may not have been from the same batch of raw materials that was used during the mixing of the feed and could have slightly different properties. However, the formulation shows that the WHC is additive.


Figure 5.2: Graphic representation of the effect of diluting a feed with four inert fillers with WHC's of 3.3,5, 10 and 14.3 ( $g$ water/g dry feed) respectively on constrained (CFI), desired (DFI) and actual feed intake (AFI, represented by the feint dotted lines); where CFI, represents a feed with a WHC of $14, \mathrm{CFI}_{2}$ of $10, \mathrm{CFI}_{3}$ of 5 and $\mathrm{CFI}_{4}$ of 3.3 g water/g dry feed

The actual feed intake (AFI) is then the lesser of the DFI and the CFI. This represents the actual pattern of consumption of the hen when constrained by the bulk of the feed and is represented in Figure 5.2 as the feint dotted lines. As was expected, the AFI of the hen decreases as the water-holding capacity of the feed increases.

The equation developed in Figure 5.2 does not predict the desired feed intake of the hen. The desired feed intake is predicted by determining the limiting nutrient requirement of the hen and dividing this by the content of this nutrient in the feed on offer. If the constrained feed intake, calculated by means of the equation, is greater than the desired feed intake, then, in this case, the actual feed intake is the DFI. Where the CFI is less than the DFI the actual feed intake will be insufficient to meet the requirements of the hen for betential egg output, and this will necessarily suffer as a result.

It is understood that the equation developed is not perfect, as there are points on either side of the regression line, meaning that in some cases the hens were capable of consuming more than was predicted by the equation, and other times less. This represents the individual variation in gut capacity, or the way in which feed intake was scaled. It might be more accurate to scale the feed intake to the lean tissue content of the hen to give a more accurate result. An inaccuracy may also be present in the measurement of the WHC, which is evident in the variation between the calculated and measured WHC of the basal feed in Trial 2, as well as the variation in the calculated WHC for the feeds in the previous chapter.

### 5.3. Conclusions

The prediction of the effect of feed bulk on the voluntary feed intake of the hen is an important aspect for accurately predicting the feed intake of the hen and formulating a 'perfect' diet. The results obtained in the two trials conducted for this thesis conform well to previous work in this Discipline, but the variation in constrained intakes was not accurately predicted by the WHC of the feed, although this measure of bulkiness was considerably better than any of the other measures applied. Until such time as a better measure of feed bulk is obtained, the equation developed in this thesis may be used to give a reasonable idea of when feed bulk will constrain feed intake in laying hens.

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APPENDICES

Appendix A: Trial 1 body weight data

| PEN | TREAT. | INITIAL | WEEK 3 | BW change | BW change/day | WEEK 6 | BW change | BW change/day | WEEK 9 | BW change | BW change/day |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 1 | 9 | 1637 | 1567 | -70 | -3.3 | 1619 | 52 | 2.5 | 1667 | 48 | 2.3 |
| 2 | 6 | 1943 | 1730 | -213 | -10.1 | 1206 | -524 | -25.0 | 1791 | 585 | 27.9 |
| 3 | 11 | 1860 | 1698 | -162 | -7.7 | 1609 | -89 | -4.2 | 1601 | -8 | -0.4 |
| 4 | 10 | 1760 | 1705 | -55 | -2.6 | 1337 | -368 | -17.5 | 1666 | 329 | 15.7 |
| 5 | 18 | 1865 | 1819 | -46 | -2.2 | 1579 | -240 | -11.4 | 1959 | 380 | 18.1 |
| 6 | 2 | 1792 | 1750 | -42 | -2.0 | 1406 | -344 | -16.4 | 1649 | 243 | 11.6 |
| 7 | 14 | 1817 | 1803 | -14 | -0.7 | 1715 | -88 | -4.2 | 1736 | 21 | 1.0 |
| 8 | 10 | 1925 | 1887 | -38 | -1.8 | 1561 | -326 | -15.5 | 1912 | 351 | 16.7 |
| 9 | 20 | 1908 | 1695 | -213 | -10.1 | 1890 | 195 | 9.3 | 1659 | -231 | -11.0 |
| 10 | 19 | 1886 | 1677 | -209 | -10.0 | 1736 | 59 | 2.8 | 1808 | 72 | 3.4 |
| 11 | 16 | 1858 | 1708 | -150 | -7.1 | 1780 | 72 | 3.4 | 1183 | -597 | -28.4 |
| 12 | 12 | 1525 | 1183 | -342 | -16.3 | 1466 | 283 | 13.5 | 1163 | -303 | -14.4 |
| 13 | 17 | 1892 | 1839 | -53 | -2.5 | 1886 | 47 | 2.2 | 1834 | -52 | -2.5 |
| 14 | 11 | 1954 | 1637 | -317 | -15.1 | 1622 | -15 | -0.7 | 1694 | 72 | 3.4 |
| 15 | 4 | 2053 | 1505 | -548 | -26.1 | 1845 | 340 | 16.2 | 1391 | -454 | -21.6 |
| 16 | 1 | 1851 | 1713 | -138 | -6.6 | 1808 | 95 | 4.5 | 1818 | 10 | 0.5 |
| 17 | 13 | 1645 | 1629 | -16 | -0.8 | 1610 | -19 | -0.9 | 1616 | 6 | 0.3 |
| 18 | 20 | 1724 | 1457 | -267 | -12.7 | 1684 | 227 | 10.8 | 1425 | -259 | -12.3 |
| 19 | 18 | 2065 | 1755 | -310 | -14.8 | 1445 | -310 | -14.8 | 1655 | 210 | 10.0 |
| 20 | 12 | 1990 | 1427 | -563 | -26.8 | 1791 | 364 | 17.3 | 1291 | -500 | -23.8 |
| 21 | 15 | 1874 | 1740 | -134 | -6.4 | 1730 | -10 | -0.5 | 1313 | -417 | -19.9 |
| 22 | 5 | 1591 | 1531 | -60 | -2.9 | 1589 | 58 | 2.8 | 1611 | 22 | 1.0 |
| 23 | 7 | 2002 | 1580 | -422 | -20.1 | 1255 | -325 | -15.5 | 1700 | 445 | 21.2 |
| 24 | 17 | 1963 | 1911 | -52 | -2.5 | 1876 | -35 | -1.7 | 1770 | -106 | -5.0 |
| 25 | 9 | 1929 | 1918 | -11 | -0.5 | 1943 | 25 | 1.2 | 1979 | 36 | 1.7 |
| 26 | 4 | 1538 | 1160 | -378 | -18.0 | 1413 | 253 | 12.0 | 1070 | -343 | -16.3 |
| 27 | 3 | 1834 | 1862 | 28 | 1.3 | 1760 | -102 | -4.9 | 1712 | -48 | -2.3 |
| 28 | 2 | 1864 | 1868 | 4 | 0.2 | 1469 | -399 | -19.0 | 1825 | 356 | 17.0 |


| PEN | TREAT. | INITIAL | WEEK 3 | BW change | BW change/day | WEEK 6 | BW change | BW change/day | WEEK 9 | BW change | BW change/day |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 29 | 6 | 2019 | 1939 | -80 | -3.8 | 1356 | -583 | -27.8 | 1892 | 536 | 25.5 |
| 30 | 16 | 1805 | 1631 | -174 | -8.3 | 1794 | 163 | 7.8 | 1277 | -517 | -24.6 |
| 31 | 3 | 1822 | 1655 | -167 | -8.0 | 1688 | 33 | 1.6 | 1647 | -41 | -2.0 |
| 32 | 1 | 1703 | 1695 | -8 | -0.4 | 1697 | 2 | 0.1 | 1725 | 28 | 1.3 |
| 33 | 19 | 1967 | 1702 | -265 | -12.6 | 1684 | -18 | -0.9 | 1772 | 88 | 4.2 |
| 34 | 5 | 2014 | 1933 | -81 | -3.9 | 2001 | 68 | 3.2 | 2020 | 19 | 0.9 |
| 35 | 8 | 2159 | 1556 | -603 | -28.7 | 2051 | 495 | 23.6 | 2058 | 7 | 0.3 |
| 36 | 8 | 2006 | 1389 | -617 | -29.4 | 1830 | 441 | 21.0 | 1806 | -24 | -1.1 |
| 37 | 13 | 1972 | 1911 | -61 | -2.9 | 1965 | 54 | 2.6 | 1997 | 32 | 1.5 |
| 38 | 7 | 1840 | 1425 | -415 | -19.8 | 1337 | -88 | -4.2 | 1856 | 519 | 24.7 |
| 39 | 15 | 1936 | 1947 | 11 | 0.5 | 1864 | -83 | -4.0 | 1455 | -409 | -19.5 |
| 40 | 14 | 1791 | 1690 | -101 | -4.8 | 1662 | -28 | -1.3 | 1803 | 141 | 6.7 |
| 41 | 10 | 1819 | 1681 | -138 | -6.6 | 1418 | -263 | -12.5 | 1687 | 269 | 12.8 |
| 42 | 15 | 1835 | 1752 | -83 | -4.0 | 1773 | 21 | 1.0 | 1351 | -422 | -20.1 |
| 43 | 3 | 1778 | 1784 | 6 | 0.3 | 1680 | -104 | -5.0 | 1651 | -29 | -1.4 |
| 44 | 3 | 2150 | 1835 | -315 | -15.0 | 1777 | -58 | -2.8 | 1819 | 42 | 2.0 |
| 45 | 11 | 1980 | 1668 | -312 | -14.9 | 1625 | -43 | -2.0 | 1692 | 67 | 3.2 |
| 46 | 17 | 1956 | 1850 | -106 | -5.0 | 1900 | 50 | 2.4 | 1844 | -56 | -2.7 |
| 47 | 16 | 2005 | 1898 | -107 | -5.1 | 1941 | 43 | 2.0 | 1489 | -452 | -21.5 |
| 48 | 20 | 1861 | 1406 | -455 | -21.7 | 1670 | 264 | 12.6 | 1480 | -190 | -9.0 |
| 49 | 7 | 1555 | 1114 | -441 | -21.0 | 926 | -188 | -9.0 | 1542 | 616 | 29.3 |
| 50 | 9 | 1605 | 1637 | 32 | 1.5 | 1672 | 35 | 1.7 | 1648 | -24 | -1.1 |
| 51 | 4 | 1678 | 1394 | -284 | -13.5 | 1611 | 217 | 10.3 | 1284 | -327 | -15.6 |
| 52 | 4 | 1973 | 1562 | -411 | -19.6 | 1870 | 308 | 14.7 | 1489 | -381 | -18.1 |
| 53 | 10 | 1665 | 1473 | -192 | -9.1 | 1278 | -195 | -9.3 | 1316 | 38 | 1.8 |
| 54 | 6 | 1657 | 1422 | -235 | -11.2 | 867 | -555 | -26.4 | 1509 | 642 | 30.6 |
| 55 | 19 | 1862 | 1576 | -286 | -13.6 | 1658 | 82 | 3.9 | 1763 | 105 | 5.0 |
| 56 | 20 | 1903 | 1595 | -308 | -14.7 | 1814 | 219 | 10.4 | 1563 | -251 | -12.0 |
| 57 | 15 | 1732 | 1589 | -143 | -6.8 | 1744 | 155 | 7.4 |  | -1744 | -83.0 |
| 58 | 11 | 1935 | 1781 | -154 | -7.3 | 1601 | -180 | -8.6 | 1566 | -35 | -1.7 |


| PEN | TREAT. | INITIAL | WEEK 3 | BW change | BW change/day | WEEK 6 | BW change | BW change/day | WEEK 9 | BW change | BW change/day |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 59 | 16 | 2215 | 1945 | -270 | -12.9 | 1942 | -3 | -0.1 | 1382 | -560 | -26.7 |
| 60 | 8 | 1696 | 1038 | -658 | -31.3 | 1353 | 315 | 15.0 | 1712 | 359 | 17.1 |
| 61 | 13 | 1441 | 1448 | 7 | 0.3 | 1478 | 30 | 1.4 | 1485 | 7 | 0.3 |
| 62 | 18 | 1905 | 1810 | -95 | -4.5 | 1499 | -311 | -14.8 | 1829 | 330 | 15.7 |
| 63 | 18 | 1819 | 1707 | -112 | -5.3 | 1365 | -342 | -16.3 | 1739 | 374 | 17.8 |
| 64 | 14 | 1785 | 1651 | -134 | -6.4 | 1662 | 11 | 0.5 | 1550 | -112 | -5.3 |
| 65 | 1 | 1684 | 1724 | 40 | 1.9 | 1731 | 7 | 0.3 | 1880 | 149 | 7.1 |
| 66 | 6 | 1942 | 1764 | -178 | -8.5 | 1305 | -459 | -21.9 | 1783 | 478 | 22.8 |
| 67 | 17 | 2448 | 2300 | -148 | -7.0 | 2260 | -40 | -1.9 | 2261 | 1 | 0.0 |
| 68 | 5 | 2103 | 2159 | 56 | 2.7 | 2121 | -38 | -1.8 | 2209 | 88 | 4.2 |
| 69 | 14 | 1532 | 1530 | -2 | -0.1 | 1529 | -1 | 0.0 | 1496 | -33 | -1.6 |
| 70 | 1 | 1726 | 1644 | -82 | -3.9 | 1655 | 11 | 0.5 | 1651 | -4 | -0.2 |
| 71 | 2 | 1868 | 1787 | -81 | -3.9 | 1636 | -151 | -7.2 | 1743 | 107 | 5.1 |
| 72 | 5 | 1899 | 1892 | -7 | -0.3 | 1914 | 22 | 1.0 | 1931 | 17 | 0.8 |
| 73 | 12 | 1862 | 1420 | -442 | -21.0 | 1742 | 322 | 15.3 | 1377 | -365 | -17.4 |
| 74 | 2 | 2010 | 2029 | 19 | 0.9 | 1641 | -388 | -18.5 | 2003 | 362 | 17.2 |
| 75 | 8 | 1916 | 1448 | -468 | -22.3 | 1650 | 202 | 9.6 | 1931 | 281 | 13.4 |
| 76 | 9 | 1885 | 1935 | 50 | 2.4 | 1971 | 36 | 1.7 | 2020 | 49 | 2.3 |
| 77 | 12 | 1648 | 1248 | -400 | -19.0 | 1515 | 267 | 12.7 | 1240 | -275 | -13.1 |
| 78 | 19 | 1955 | 1673 | -282 | -13.4 | 1578 | -95 | -4.5 | 1612 | 34 | 1.6 |
| 79 | 13 | 1634 | 1548 | -86 | -4.1 | 1563 | 15 | 0.7 | 1670 | 107 | 5.1 |
| 80 | 7 | 1843 | 1571 | -272 | -13.0 | 1344 | -227 | -10.8 | 1810 | 466 | 22.2 |
| 81 | 3 | 1922 | 1764 | -158 | -7.5 | 1777 | 13 | 0.6 | 1633 | -144 | -6.9 |
| 82 | 5 | 1768 | 1789 | 21 | 1.0 | 1770 | -19 | -0.9 | 1840 | 70 | 3.3 |
| 83 | 1 | 2031 | 1963 | -68 | -3.2 | 1990 | 27 | 1.3 | 2067 | 77 | 3.7 |
| 84 | 6 | 1767 | 1626 | -141 | -6.7 | 1782 | 156 | 7.4 | 1689 | -93 | -4.4 |
| 85 | 15 | 1787 | 1736 | -51 | -2.4 | 1728 | -8 | -0.4 | 1314 | -414 | -19.7 |
| 86 | 17 | 1805 | 1741 | -64 | -3.0 | 1679 | -62 | -3.0 | 1616 | -63 | -3.0 |
| 87 | 20 | 1423 | 1139 | -284 | -13.5 | 1346 | 207 | 9.9 | 1199 | -147 | -7.0 |
| 88 | 14 | 1881 | 1840 | -41 | -2.0 | 1600 | -240 | -11.4 | 1787 | 187 | 8.9 |


| PEN | TREAT. | INITIAL | WEEK 3 | BW change | BW change/day | WEEK 6 | BW change | BW change/day | WEEK 9 | BW change | BW change/day |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 89 | 8 | 1634 | 1073 | -561 | -26.7 | 1398 | 325 | 15.5 | 1615 | 217 | 10.3 |
| 90 | 5 | 1675 | 1568 | -107 | -5.1 | 1674 | 106 | 5.0 | 1694 | 20 | 1.0 |
| 91 | 12 | 1774 | 1164 | -610 | -29.0 | 1583 | 419 | 20.0 | 1038 | -545 | -26.0 |
| 92 | 4 | 1919 | 1564 | -355 | -16.9 | 1772 | 208 | 9.9 | 1486 | -286 | -13.6 |
| 93 | 3 | 1807 | 1593 | -214 | -10.2 | 1636 | 43 | 2.0 | 1578 | -58 | -2.8 |
| 94 | 14 | 1697 | 1652 | -45 | -2.1 | 1556 | -96 | -4.6 | 1562 | 6 | 0.3 |
| 95 | 7 | 1706 | 1363 | -343 | -16.3 | 1074 | -289 | -13.8 | 1730 | 656 | 31.2 |
| 96 | 10 | 1747 | 1587 | -160 | -7.6 | 1204 | -383 | -18.2 | 1672 | 468 | 22.3 |
| 97 | 2 | 1930 | 1862 | -68 | -3.2 | 1742 | -120 | -5.7 | 1954 | 212 | 10.1 |
| 98 | 16 | 1802 | 1629 | -173 | -8.2 | 1745 | 116 | 5.5 | 1197 | -548 | -26.1 |
| 99 | 9 | 2001 | 1944 | -57 | -2.7 | 2054 | 110 | 5.2 | 2038 | -16 | -0.8 |
| 100 | 16 | 1886 | 1474 | -412 | -19.6 | 1741 | 267 | 12.7 | 1261 | -480 | -22.9 |
| 101 | 1 | 1794 | 1773 | -21 | -1.0 | 1765 | -8 | -0.4 | 1815 | 50 | 2.4 |
| 102 | 19 | 1745 | 1543 | -202 | -9.6 | 1513 | -30 | -1.4 | 1516 | 3 | 0.1 |
| 103 | 6 | 1793 | 1781 | -12 | -0.6 | 1157 | -624 | -29.7 | 1806 | 649 | 30.9 |
| 104 | 9 | 1709 | 1707 | -2 | -0.1 | 1647 | -60 | -2.9 | 1728 | 81 | 3.9 |
| 105 | 20 | 2017 | 1706 | -311 | -14.8 | 1872 | 166 | 7.9 | 1623 | -249 | -11.9 |
| 106 | 13 | 2012 | 1921 | -91 | -4.3 | 1995 | 74 | 3.5 | 1984 | -11 | -0.5 |
| 107 | 8 | 1926 | 1317 | -609 | -29.0 | 1842 | 525 | 25.0 | 1836 | -6 | -0.3 |
| 108 | 18 | 1765 | 1600 | -165 | -7.9 | 1398 | -202 | -9.6 | 1604 | 206 | 9.8 |
| 109 | 4 | 2221 | 1837 | -384 | -18.3 | 2033 | 196 | 9.3 | 1867 | -166 | -7.9 |
| 110 | 13 | 2350 | 2164 | -186 | -8.9 | 2258 | 94 | 4.5 | 2293 | 35 | 1.7 |
| 111 | 7 | 1827 | 1484 | -343 | -16.3 | 1251 | -233 | -11.1 | 1783 | 532 | 25.3 |
| 112 | 17 | 1650 | 1412 | -238 | -11.3 | 1233 | -179 | -8.5 | 717 | -516 | -24.6 |
| 113 | 15 | 2072 | 1840 | -232 | -11.0 | 2018 | 178 | 8.5 | 1623 | -395 | -18.8 |
| 114 | 11 | 1816 | 1555 | -261 | -12.4 | 1442 | -113 | -5.4 | 1405 | -37 | -1.8 |
| 115 | 2 | 1694 | 1562 | -132 | -6.3 | 1405 | -157 | -7.5 | 1571 | 166 | 7.9 |
| 116 | 11 | 1869 | 1432 | -437 | -20.8 | 1345 | -87 | -4.1 | 1141 | -204 | -9.7 |
| 117 | 10 | 2071 | 1930 | -141 | -6.7 | 1588 | -342 | -16.3 | 1771 | 183 | 8.7 |
| 118 | 19 | 1872 | 1555 | -317 | -15.1 | 1650 | 95 | 4.5 | 1677 | 27 | 1.3 |


| PEN | TREAT. | INITIAL | WEEK 3 | BW change | BW change/day | WEEK 6 | BW change | BW change/day | WEEK 9 | BW change | BW change/day |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 119 | 12 | 2003 | 1514 | -489 | -23.3 | 1859 | 345 | 16.4 | 1546 | -313 | -14.9 |
| 120 | 18 | 1972 | 1868 | -104 | -5.0 | 1522 | -346 | -16.5 | 1923 | 401 | 19.1 |
| 121 | 5 | 2325 | 2182 | -143 | -6.8 | 2142 | -40 | -1.9 | 2123 | -19 | -0.9 |
| 122 | 2 | 1830 | 1576 | -254 | -12.1 | 1352 | -224 | -10.7 | 1618 | 266 | 12.7 |
| 123 | 19 | 1864 | 1478 | -386 | -18.4 | 1431 | -47 | -2.2 | 1472 | 41 | 2.0 |
| 124 | 17 | 1892 | 1686 | -206 | -9.8 | 1652 | -34 | -1.6 | 1690 | 38 | 1.8 |
| 125 | 9 | 1932 | 1907 | -25 | -1.2 | 1919 | 12 | 0.6 | 1939 | 20 | 1.0 |
| 126 | 10 | 2010 | 1794 | -216 | -10.3 | 1448 | -346 | -16.5 | 1712 | 264 | 12.6 |
| 127 | 11 | 1886 | 1570 | -316 | -15.0 | 1629 | 59 | 2.8 | 1570 | -59 | -2.8 |
| 128 | 1 | 1653 | 1619 | -34 | -1.6 | 1652 | 33 | 1.6 | 1602 | -50 | -2.4 |
| 129 | 8 | 1777 | 1230 | -547 | -26.0 | 1636 | 406 | 19.3 | 1712 | 76 | 3.6 |
| 130 | 20 | 1639 | 1318 | -321 | -15.3 | 1592 | 274 | 13.0 | 1350 | -242 | -11.5 |
| 131 | 11 | 1942 | 1681 | -261 | -12.4 | 1675 | -6 | -0.3 | 1695 | 20 | 1.0 |
| 132 | 20 | 1917 | 1555 | -362 | -17.2 | 1739 | 184 | 8.8 | 1614 | -125 | -6.0 |
| 133 | 7 | 1925 | 1471 | -454 | -21.6 | 1331 | -140 | -6.7 | 1920 | 589 | 28.0 |
| 134 | 18 | 2001 | 1851 | -150 | -7.1 | 1502 | -349 | -16.6 | 1970 | 468 | 22.3 |
| 135 | 7 | 2164 | 1576 | -588 | -28.0 | 1365 | -211 | -10.0 | 2097 | 732 | 34.9 |
| 136 | 3 | 2083 | 1749 | -334 | -15.9 | 1719 | -30 | -1.4 | 1728 | 9 | 0.4 |
| 137 | 14 | 2034 | 1941 | -93 | -4.4 | 1771 | -170 | -8.1 | 1809 | 38 | 1.8 |
| 138 | 12 | 2032 | 1589 | -443 | -21.1 | 1812 | 223 | 10.6 | 1505 | -307 | -14.6 |
| 139 | 8 | 2033 | 1442 | -591 | -28.1 | 1886 | 444 | 21.1 | 1841 | -45 | -2.1 |
| 140 | 16 | 2001 | 1780 | -221 | -10.5 | 1909 | 129 | 6.1 | 1392 | -517 | -24.6 |
| 141 | 2 | 1989 | 1962 | -27 | -1.3 | 1438 | -524 | -25.0 | 1737 | 299 | 14.2 |
| 142 | 9 | 2310 | 2168 | -142 | -6.8 | 2252 | 84 | 4.0 | 2298 | 46 | 2.2 |
| 143 | 18 | 2069 | 1897 | -172 | -8.2 | 1550 | -347 | -16.5 | 1963 | 413 | 19.7 |
| 144 | 5 | 1957 | 1783 | -174 | -8.3 | 1751 | -32 | -1.5 | 1757 | 6 | 0.3 |
| 145 | 15 | 1844 | 1664 | -180 | -8.6 | 1697 | 33 | 1.6 | 1359 | -338 | -16.1 |
| 146 | 17 | 2009 | 2000 | -9 | -0.4 | 1964 | -36 | -1.7 | 1956 | -8 | -0.4 |
| 147 | 13 | 1825 | 1746 | -79 | -3.8 | 1835 | 89 | 4.2 | 1787 | -48 | -2.3 |
| 148 | 12 | 1962 | 1443 | -519 | -24.7 | 1890 | 447 | 21.3 | 1473 | -417 | -19.9 |


| PEN | TREAT. | INITIAL | WEEK 3 | BW change | BW change/day | WEEK 6 | BW change | BW change/day | WEEK 9 | BW change | BW change/day |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 149 | 3 | 1836 | 1571 | -265 | -12.6 | 1542 | -29 | -1.4 | 1476 | -66 | -3.1 |
| 150 | 13 | 1734 | 1680 | -54 | -2.6 | 1642 | -38 | -1.8 | 1618 | -24 | -1.1 |
| 151 | 15 | 1911 | 1785 | -126 | -6.0 | 1810 | 25 | 1.2 | 1456 | -354 | -16.9 |
| 152 | 4 | 2071 | 1780 | -291 | -13.9 | 1849 | 69 | 3.3 | 1517 | -332 | -15.8 |
| 153 | 1 | 2424 | 2323 | -101 | -4.8 | 2372 | 49 | 2.3 | 2401 | 29 | 1.4 |
| 154 | 14 | 2010 | 1936 | -74 | -3.5 | 1897 | -39 | -1.9 | 1842 | -55 | -2.6 |
| 155 | 16 | 2083 | 1877 | -206 | -9.8 | 1907 | 30 | 1.4 | 1407 | -500 | -23.8 |
| 156 | 4 | 1861 | 1381 | -480 | -22.9 | 1641 | 260 | 12.4 | 1297 | -344 | -16.4 |
| 157 | 19 | 1683 | 1410 | -273 | -13.0 | 1429 | 19 | 0.9 | 1442 | 13 | 0.6 |
| 158 | 6 | 1734 | 1562 | -172 | -8.2 | 1127 | -435 | -20.7 | 1659 | 532 | 25.3 |
| 159 | 6 | 2390 | 1932 | -458 | -21.8 | 1405 | -527 | -25.1 | 1905 | 500 | 23.8 |
| 160 | 10 | 1871 | 1732 | -139 | -6.6 | 1347 | -385 | -18.3 | 1874 | 527 | 25.1 |

## Appendix B: Trial 1 feed intake data






| PEN | TREAT. | DILUENT | LEVEL | FEEDIN | FEED OUT | IntakE1 | INTAKEIDAY | FEEDIN | FEED OUT | INTAKE2 | Intakeiday | FEEOM | FEED OUT | INTAKE3 | InTAKEDAY | AVE FIIDAY |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 64 | 14 | 4 | 50 | 2004.9 | 725.1 | 1279.8 | 182.8 | 1700.9 | 787.0 | 913.9 | 130.6 | 2042.8 | 629.5 | 1413.3 | 201.9 | 171.8 |  |  |
| 65 |  | 1 SF |  | 1606.1 | 849.9 | 756.2 | 108.0 | 1778.3 | 746.6 | 1031.7 | 147.4 | 1775.1 | 1108.0 | 669.1 | 95.6 | 117. | 1731 | 67.6 |
| 68 |  |  | 50 | 1122.8 | 945.1 | 177.7 | 25.4 | 1333.8 | 1043.4 | 290.4 | 41.5 | 1458.4 | 1155.2 | 303.2 | 43.3 | 36.7 | 1305 |  |
| 67 | 17 | - SD | 0 | 1154.6 | 240.0 | 914.6 | 130.7 | 1262.8 | 462.7 | 800,1 | 114.3 | 1447.7 | 408.5 | 1039.2 | 148.5 | 131.1 | 2250 | 58.0 |
| 68 |  | 5 |  | 1586.7 | 593.1 | 993.6 | 141.9 | 1606.9 | 661.5 | 945.4 | 135.1 | 1794.4 | 743.6 | 1050.8 | 150.1 | 142.4 | 2121 |  |
| 69 | 14 |  | 50 | 1934.3 | 766.1 | 1168.2 | 166.9 | 1883.7 | 547.8 | 1135.9 | 162.3 | 1882.9 | 534.8 | 1328.1 | 189.7 | 173.0 | 1529 |  |
| 70 |  | 1 SF |  | 1586.5 | 794.3 | 792.2 | 113.2 | 1685.7 | 871.6 | 814.1 | 116.3 | 1704.6 | 895.4 | 809.4 | 115.6 | 115.0 | 1655 | 69.5 |
| 71 |  | SF | 50 | 1065.1 | 306.2 | 758.9 | 108.4 | 1128.7 |  | 807.5 | 115.4 | 1161.3 | 178.0 | 983.3 | 140.5 | 121.4 |  | 74.2 |
| 72 | - 5 | 5 |  | 1811.9 | 693.2 | 918.7 | 131.2 | 1722.2 | 852.6 | 869.6 | 124.2 | 1824.4 | 913.0 | 911.4 | 130.2 | 128.6 | 1914 | 67.2 |
| 73 | 12 | - V | 10 | 1432.3 | 428.6 | 1003.7 | 143.4 | 1381.4 | 561.0 | 820.4 | 117.2 | 1548.4 | 592.1 | 956.3 | 136.6 | 132.4 |  | 76.0 |
| 74 |  | $2-\mathrm{SF}$ | 50 | 1023.7 | 429.6 | 594.1 | 84,9 | 1136.5 | 325.3 | 811.2 | 115.9 | 1073.7 | 251.1 | 822.6 | 177.5 | 106.1 |  |  |
| 75 | , | 8 c | 10 | 1549.9 | 753.4 | 796.5 | 113.8 | 1865.9 | 1151.6 | 514.3 | 73.5 | 1757.0 | 1014.0 | 743.0 | 108.1 | 97.8 | 1650 | 59.3 |
| 76 |  | 9 V |  | 1620.4 | 636.6 | 983.8 | 140.5 | 1689.9 | 928.6 | 761.3 | 108.8 | 1798.2 | 814.0 | 984.2 | 140.6 | 130.0 |  | 65.9 |
| 77 | 12 | - V | 10 | 1387.9 | -582.1 | 805.8 | 115.1 | 1507.5 | 629.6 | 877.9 | 125.4 | 1532.6 | 653.9 | 878.7 | 125.5 | 122.0 | 1515 | 80.5 |
| 78 | 19 | - so | 25 | 1255.1 | - 248.2 | 1006.9 | 143.8 | 1274.2 | 350.2 | 924.0 | 132.0 | 1229.2 | 308.1 | 921.1 | 131.6 | 135.8 | 1578 | 86.1 |
|  |  |  |  | 1552.7 | 698.2 | 854.5 | 122.1 | 1712.2 | 954.1 | 758.1 | 108.3 | 1825.0 | 1041.4 | 783.6 | 111.9 | 114.1 |  | 73.0 |
| 80 | 7 | 7 | 25 | 1210.6 | 832.1 | 378.5 | 54.1 | 1538.0 | 1163.8 | 374.2 | 53.5 | 1690.1 | 1322.0 | 368.1 | 52.6 | 53.4 | -1344 | 39.7 |
| 81 | 3 | 3 SF | 25 | 1240.6 | 326.2 | 914.4 | 130.6 | 1253.7 | 447.4 | 806.3 | 115.2 | 1306.8 | 331.2 | 975.6 | 139.4 | 128.4 | 1777 | 72.3 |
| 82 | 5 | 5 | 0 | 1606.2 | -696.4 | 909.8 | 130.0 | 1714.8 | 877.6 | 837.2 | 119.6 | 1824.5 | 950.9 | 873.6 | 124.8 | 124.8 |  | 70.5 |
| 83 |  | 1 SF | - 0 | 1628.9 | -694.9 | 934.0 | 133.4 | 1814.2 | 833.3 | 980.9 | 140.1 | 1790.5 | 796.9 | 993.6 | 141.9 | 138.5 | 1990 | 69.6 |
|  |  |  | 50 | 1159.0 | 1104.1 | 54.9 | 7.8 | 1542.2 | 1261.2 | 281.0 | 40.1 | 1329.5 | 1089.7 | 239.8 | 34.3 | 27.4 | 1782 |  |
| 85 | 15 | 5 | 25 | 1461.4 | - 487.2 | 974.2 | 139.2 | 1521.0 | 655.6 | 865.4 | 123.6 | 1788.7 | 769.3 | 1019.4 | 145.6 | 136.1 | 1728 |  |
| 86 | 17 | - sp |  | 1127.5 | 434.8 | 692.7 | 99.0 | 1364.8 | 663.1 | 701.7 | 100.2 | 1404.2 | 641.2 | 763.0 | 109.0 | 102.7 | 1679 | 61.2 |
|  |  |  |  | 1337.8 | 571.9 | 765.9 | 109.4 | 1424.0 | 791.9 | 632.1 | 90.3 | 1615.6 | 985.0 | 650.6 | 92.9 | 97.6 | 1346 |  |
| 88 | 14 | 1 - ${ }^{4}$ | 50 | 2068.1 | 804.7 | 1263.4 | 180.5 | 1692.7 | 524.6 | 1168.1 | 166.9 | 1992.4 | 890.4 | 1102.0 | 1574. | 168.3 | 1600 | 105.2 |
| 89 |  | - c | 10 | 1502.6 | 1034.9 | 467.7 | 66.8 | 1502.0 | 816.2 | 685.6 | 98.0 | 1709.9 | 1096.6 | 813.3 | 87.6 | 84.1 |  |  |
| 90 | - 5 | 5 - C | 0 | 1508.3 | 581.8 | 926.5 | 132.4 | 1545.3 | 668.2 | 877.1 | 125.3 | 1714.5 | 858.0 | 856.5 | 122.4 | 126.7 | 1874 |  |
| 91 | 12 | - V | 10 | 1491.1 | 490.0 | 1001.1 | 143.0 | 1315.0 | 274.9 | 1040.1 | 148.6 | 1441.6 | 525.9 | 915.7 | 130.8 | 140.8 | 1583 | 88.9 |
| 92 | , | 4 - SF |  | 1322.7 | 385.0 | 937.7 | 134.0 | 1361.9 | 428.0 | 935.9 | 133.7 | 1534.4 | 579.1 | 955.3 | 136.5 | 134.7 | 1772 |  |
| 93 |  | 3 SF | 25 | 1192.7 | 175.7 | 1017.0 | 145.3 | 1321.6 | 302.3 | 1019.3 | 145.6 | 1307.3 | 311.5 | 995.8 | 142.3 | 144.4 | 1836 | 88.3 |
| 94 | 14 | \| | 50 | 1852.3 | 460.2 | 1392.1 | 198.9 | 1884.0 | 382.4 | 1501.6 | 214.5 | 1941.8 | 472.4 | 1469.4 | 209.9 | 2078 | 1556 |  |
| 95 |  |  | 25 | 1174.7 | 979.1 | 195.6 | 27.9 | 1696.7 | 1483.4 | 213.3 | 30.5 | 1483.4 | 1366.0 | 117.4 |  |  |  |  |
| 96 | 10 | - V | 50 | 942.3 | 574.0 | 368.3 | 52.6 | 1111.3 | 764.9 | 348.4 | 49.5 | 1114.6 | 817.3 | 297.3 | 42.5 | 48.2 | 1204 | 40.0 |
|  |  | SF | 50 | 1063.2 | -369.8 | 693.4 | 99.1 | 1028.2 | 146.6 | 881,6 | 125.9 | 1134.9 | 218.4 | 918.5 | 131.2 | 118.7 |  | 68.2 |
| 98 | 16 | - s | 10 | 1335.4 | -288.3 | 1047.1 | 149.6 | 1482.6 | 494.7 | 987.9 | 141.1 | 1545.1 | 612.8 | 932.3 | 133.2 | 141.3 | 1745 | 81.0 |
| 99 |  | $9-\mathrm{V}$ |  | 1493.4 | 532.8 | 960.6 | 137.2 | 1678.6 | 1000.7 | 677.9 | 96.8 | 1835.7 | 918.6 | 917.1 | 131.0 | 121.7 | 2054 | 59.2 |
| 100 | 16 | 6 | 10 | 1302.7 | 422.5 | 880.2 | 125.7 | 1600.4 | 916.1 | 684.3 | 97.8 | 1545.4 | 630.1 | 916.3 | 130.8 | 118.1 | 1741 |  |
| 109 |  | 1 SF |  | 1672.0 | 797.5 | 874.5 | 124,9 | 1645.2 | 770.6 | 874.6 | 124.9 | 1709.3 | 884.5 | 824.8 | 117.8 | 122.6 | 1765 | 69,4 |
| 102 | 19 | - SD | 25 | 1270.4 | 525.7 | 744.7 | 106.4 | 1277.5 | 526.3 | 751.2 | 107.3 | 1305.3 | 528.4 | 776.9 | 111.0 | 108.2 | 1513 |  |
| 103 | 6 | 6 | 50 | 1171.9 | 1124.9 | 47.0 | 6.7 | 1468.5 | 1181.6 | 286.9 | 41.0 | 1499.7 | 1308.8 | 192.9 | 27.6 | 25.1 | 1157 | 21.7 |
| 104 | - 9 | - V |  | 1451.8 | 932.9 | 518.9 | 74.1 | 1664.5 | 1057.0 | 607.5 | 86.8 | 1687.7 | 921.9 | 765.8 | 109.4 | 90.1 | 1647 | 64.7 |
| 105 |  | - SD | 10 | 1325.7 | 502.9 | 822.8 | 117.5 | 1446.8 | 517.1 | 929.7 | 132.8 | 1512.8 | 585.3 | 927.5 | 132.5 | 127.6 | 1872 | 68.2 |
| 106 | 13 | 3 | 0 | 1400.7 | 5937 | 807.0 | 115.3 | 1631.0 | 847.6 | 783.2 | 111.9 | 1715.3 | 890.4 | 824.9 | 117.8 | 115.0 | 1895 | 57.6 |
| 107 |  |  | 10 | 1433.4 | 705.1 | 728.3 | 104.0 | 1621.6 | 858.4 | 763.2 | 109.0 | 1748.7 | 823.6 | 925.1 | 132.2 | 115.1 | ${ }^{1842}$ | 82.5 |
| 108 | 18 | S SD | 50 | 937.9 | 332.3 | 605.6 | 86.6 | 1009.0 | 408,9 | 600.1 | 85.7 | 1068.0 | 431.0 | 637.0 | 91.0 | 87.7 | 1398 | 62.8 |
| 109 | 4 | 4 SF | 10 | 1365.0 | 189.4 | 1175.6 | 167.9 | 1568.7 | 505.5 | 1063.2 | 151.9 | 1479.6 | 520.1 | 959.5 | 137.1 | 152.3 | 2033 | 74.9 |
| 110 | 13 | 3 |  | 1478.5 | 574.7 | 903.8 | 129.1 | 1742.4 | 849.6 | 892.9 | 127.6 | 1739.4 | 817.3 | 922.1 | 131.7 | 129.5 | 2258 | 57.3 |
| 111 | 7 | 7 | 25 | ${ }^{1356.8}$ | - 971.6 | 385.2 | 55.0 | 1548.1 | 1207.3 | 340.8 | 48.7 | 1544.5 | 1360.2 | 184.3 | 26.3 | 43.3 | 1251 | 34.7 |
| 112 | 17 | 7 SD |  | 1076.6 | 874.9 | 201.7 | 28.8 | 1421.2 | 1260.9 | 160.3 | 22.9 | 1260.9 | 908.0 | 354.9 | 50.7 | 34.1 | 1233 | 27.7 |
| 113 | 15 | 5 | 25 | 1582.5 | 385.2 | 1197.3 | 171.0 | 1574.8 | 469.5 | 1105.3 | 157.9 | 1645.4 | 579.5 | 1065.9 | 152.3 | 160.4 | 2018 | 79.5 |
| 114 | 11 | 1 v | 25 | 1079.6 | 235.6 | 844.0 | 120.6 | 1147.2 | 305.6 | 841.6 | 120.2 | 1248.6 | 391.7 | 856.9 | 122.4 | 121.1 | 1442 | 84.0 |
| 115 |  | SF | 50 | 1079.0 | 532.9 | 546.1 | 78.0 | 1073.5 | 437.7 | 635.8 | 90.8 | 1184.1 | 314.9 | 869.2 | 124.2 | 97.7 | 1405 | 69.5 |
| 116 | 11 | 1 v | 25 | 1101.6 | 702.5 | 399.1 | 57.0 | 1281.9 | 863.0 | 418.9 | 59.8 | 1327.8 | 946.1 | 381.7 | 54.5 | 57.1 | 1345 | 42.5 |
| 117 | 10 | V | 50 | 993.0 | 508.5 | 486.5 | 69.5 | 1000.5 | ${ }^{375.3}$ | 625.2 | 89.3 | 1123.2 | 653.0 | 470.2 | 67.2 | 75.3 | 1588 | 47.4 |
| 118 | 19 | - SD | 25 | 1154.4 | 319.7 | 834.7 | 119.2 | 1279.3 | 365.1 | 913.2 | 130.5 | 1241.9 | 278.1 | ${ }^{963.8}$ | 137.7 | 129.1 | 1650 | 78.3 |
| 119 | 12 | - | 10 | 1386.0 | 397,0 | 989.0 | 141.3 | 1485.2 | 575.8 | 909.4 | 129.9 | 1486.1 | 449.4 | 1036.7 | 148.1 | 139.8 | 1859 | 75.2 |
| 120 | 18 | - SD | 50 | 940.6 | 855.6 | 85.0 | 12.1 | 1077.3 | 514.2 | 563.1 | 80.4 | 1053.9 | 189.6 | 864.3 | 123.5 | 72.0 | 1522 | 47.3 |
| $\frac{121}{122}$ | - 5 | 5 | 0 | 1535.0 | 587.7 | 947.3 | 135.3 | 1621.1 | 732.4 | 888.7 | 127.0 | 1784.1 | 841.6 | 942.5 | 134.6 | 132.3 | 2142 | $\frac{61.8}{65.1}$ |
| ${ }^{122}$ |  | S SF | $\stackrel{50}{25}$ | 1097.4 | 460.8 | $\begin{array}{r}636.6 \\ \hline 767\end{array}$ | 90.9 1096 | 1113.0 1293 | 554.7 | 518.3 | 74.0 | 1096.5 | 403.6 | 692.9 | 99.0 | - $\quad 188.0$ | $\frac{1352}{1431}$ | $\frac{65.1}{775}$ |
| $\underline{123}$ | 19 | - SD | $\frac{25}{0}$ | 1263.0 1090.3 | ${ }^{495.9}$ | 767.1 | 109.6 113.6 | 1293.5 | 546.3 | 747.2 | 106.7 111.6 | 1307.1 1467.7 | 492.7 538.5 | $\begin{array}{r}814.4 \\ \hline 929.2\end{array}$ | 116.3 132.7 | - 110.9 | 1431 | 77.5 |
| 125 |  |  |  | 1499.8 | 6359 | 863.9 | 123.4 | 1707.4 | 860.2 | 847.2 | 121.0 | 1800.9 | 893.8 | 907.1 | 129.6 | 124.7 | 1919 | 65.0 |
| 126 | 10 |  | 50 | 921.8 | 537.6 | 384.2 | 54.9 | 1098.5 | 574.2 | 524.3 | 74.9 | 1140.4 | 528.6 | 611.8 | 87.4 | 72.4 | 1448 | 50.0 |


| PEN | treat. | DILUENT | LEVEL | FEEDIN | FEED OUT | INTAKE1 | Intakeiday | FEEDIN | FEED OUT | INTAKE2 | INTAKEIDAY | FEED IN | FEED OUT | INTAKE3 | INTAKEIDAY | AVE FIUDAY | BW |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 127 | 11 |  | 25 | 1101.9 | 228.0 |  | 124.8 | 1144.2 | 275.5 | 868.7 | 124.1 | 1225.4 | 279.3 | 946.1 |  | 128.0 |  |  |
| 128 | 1 | SF | 仡 | 1618.1 | 840.8 | 777.3 | 111.0 | 1577.4 | 904.6 | 672.9 | 96.1 | 1765.1 | 1100.7 | 654.4 | 94.9 | 100.7 | 1652 | 61.0 |
| 129 | 8 | c | - 10 | 1401.0 | 412.7 | 988.3 | 141.2 | 1582.3 | 846.9 | 735.4 | 105.1 | 1638.4 | 751.9 | 888.5 | 128.6 | 124.3 | 1636 |  |
| 130 | 20 | SD |  | 1362.2 | 412.8 | 949.4 | 135.6 | 1540.8 | 678.1 | 862.7 | 123.2 | 1522.7 | 598.6 | ${ }_{924.1}$ | 132.0 | 130.3 | 1592 | 81.8 |
| 131 | 11 | $v$ | - 25 | 1093.0 | 184.7 | 908.3 | 129.8 | 1138.4 | 277.1 | 861.3 | 123.0 | 1268.5 | 329.7 | 938.8 | 134.1 | 129.0 | 1675 | 77.0 |
| 132 | 20 | SO | 10 | 1270.9 | 355.5 | 915.4 | 130.8 | 1579.4 |  | 887.8 | 124.0 | 1556.5 | 635.4 |  | 131.4 | 128.7 | 1739 | 74.0 |
| 133 |  |  | 25 | 1430,4 | 886.5 | 533.9 | 76.3 | 1588.0 | 1180.0 | 409.0 | 58.4 | 1512.2 | 1239.4 | 272.8 | 39.0 | 57,9 | 1331 | 43.6 |
| 134 | 18 | SD | 50 | 900.3 | 305.7 | 594.6 | 84.9 | 1008.9 | 356.8 | 850.1 | 92.9 | 972.7 | 384.5 | 688.2 | 84.0 | 87.3 | 1502 |  |
| 135 |  |  | 25 | 1244.9 | 906.9 | 338.0 | 48.3 | 1561.7 | 1342.8 | 218.9 | 31.3 | 1342.8 | 1251.8 | 91.0 | 13.0 | 30.9 | 1365 |  |
| 136 | 3 | SF | 25 | 1207.2 | 303.2 | 904.0 | 129.1 | 1332.8 | 362.7 | 970.1 | 138.6 | 1408.3 | 403.7 | 1004.6 | 143.5 | 137.1 | 1719 | 79.7 |
| 137 | 14 | - s | 50 | 1943.0 | 344.3 | 1598.7 | 228.4 | 1886.6 | 584.0 | 1272.5 | 181.8 | 2034.2 | 471.6 | 1562.6 | 223.2 | 211.1 |  |  |
| 138 | 12 |  | 10 | 1379.4 | 454,1 | 926.3 | 132.2 | 1365.4 | 444.2 | 921.2 | 131.6 | 1487.0 | 521.2 | 965.8 | 138.0 |  |  |  |
| 139 | 8 | - c | 10 | 1285.1 | 698.3 | 586.8 | 83.8 | 1755.6 | 950.7 | 804.9 | 115.0 | 1708.6 | 939.6 | 769.1 | 109.9 | 102.9 | 1886 | 54.6 |
| 140 | 16 |  | 10 | 1287.6 | 309.1 | 978.5 | 139.8 | 1434.4 | 519.7 | 914.7 | 130.7 | 1552.5 | 637.8 | 914.7 | 130.7 | 133.7 | 1809 |  |
| 141 |  | SF | 50 | 1086.1 | 591.6 | 494.5 | 70.6 | 10940 | 524.4 | 569.6 | 81.4 | 1149.4 | 441.6 | 707.8 | 101.1 | 84.4 | 1438 |  |
| 142 |  |  |  | 1531.2 | 675.7 | 855.5 | 122.2 | 1523.9 | 672.8 | 851.1 | 121.6 | 1694.1 | 809.5 | 884.6 | 126.4 | 123.4 | 2252 | 54.8 |
| 143 | 18 | SD | 50 | 848.6 | 543.6 | 305.2 | 43.6 | 1056.1 | 579.8 | 476.3 | 68.0 | 1048.9 | 574.2 | 474.7 | 67.8 | 59.8 | 1550 | 38.6 |
| 144 |  |  |  | 1589.1 | 710.7 | 878.4 | 125.5 | 1889.2 | 908.4 | 782.8 | 109.0 | 1784.1 | 876.2 | 807.9 | 129.7 | 121.4 | 1751 | 69.3 |
| 145 | 15 |  | 25 | 1435.4 | 447.0 | 988.4 | 141.2 | 1527.8 | 573.3 | 954.5 | 136.4 | 1632.4 | 707.5 | 924.9 | 132.1 | 136.6 | 1697 | 80.5 |
| 146 | 17 | SD |  | 1167.5 | 240.6 | 926.9 | 132.4 | 1337.1 | 425.2 | 911.9 | 130.3 | 1343.7 | 450.6 | 893.1 | 127.6 | 130.1 | 1984 |  |
| 147 | 13 |  | - 0 | 1478.7 | 527.8 | 950.9 | 135.8 | 1491.2 | 594.5 | 896.7 | 128.1 | 1667.5 | 730.9 | 936.6 | 133.8 | 132.6 | 1835 | 72.3 |
| 148 | 12 | , | 10 | 1442.0 | 282.1 | 1159.9 | 185.7 | 1496.4 | 554.5 | 941.9 | 134.6 | 1467.1 | 391.7 | 1075.4 | 153.6 | 151.3 | 1890 |  |
| 149 |  | SF | 25 | 1173.7 | 177.2 | 998.5 | 142.4 | 1224.6 | 235.5 | 989.1 | 141.3 | 1257.7 | 227.6 | 1030.1 | 147.2 | 143.6 | 1542 | 93.1 |
| 150 | 13 | - ${ }^{\text {s }}$ | , | 1358.6 | 810.8 | 747.8 | 108.8 | 1718.6 | 1190.7 | 527,9 | 75.4 | 1752.3 | 990.0 | 762.3 | 108.9 | 97.0 | 1642 | 69.1 |
| 151 | 15 |  | 25 | 1471.3 | 310.4 | 1160.9 | 165.8 | 1483.9 | 421.5 | 1072.4 | 153.2 | 1571.9 | 454.8 | 1117. | 159.6 | 159.5 | 1810 |  |
| 152 | 4 | SF | 10 | 1478.9 | 391.6 | 1087.3 | 155.3 | 1402.2 | 458.4 | 943.8 | 134.8 | 1536.7 | 551.8 | 984.9 | 140.7 | 143.6 | 1849 | 77.7 |
| 153 | 1 | SF | 0 | 1587.4 | 646.4 | 941.0 | 134.4 | 1890.2 | 768.7 | 921.5 | 131.6 | 1784.0 | 798.0 | 966.0 | 138.0 | 134.7 | 2372 | 56.8 |
| 154 | 14 |  | 50 | 1988.7 | 704.6 | 1294.1 | 184.9 | 1970,8 | 663.9 | 1306.9 | 186.7 | 1964.9 | 429.0 | 1535.9 | 219.4 | 197.0 | 1897 |  |
| 155 | 16 | - | 10 | 1301.5 | 256.6 | 1044.9 | 149.3 | 1506.0 | 598.3 | 907.7 | 129.7 | 1550.5 | 499.0 | 1051.5 | 150.2 | 143.1 | 1907 | 75.0 |
| ${ }^{156}$ | 4 | SF | 10 | 1339.4 | 439.9 | 899.5 | 128.5 | 1451.7 | 676.1 | 775.6 | 110.8 | 1571.3 | 849.2 | 922.1 | 131.7 | 123.7 | 1641 | 75.4 |
| 157 | 19 | - SD | 25 | - 1148.8 | 358.4 | 790.4 | 112.9 | 1327.3 | - 536.0 | 791.3 | 113.0 | 1279.1 | 212.7 | 1066.4 | 152.3 | 128.1 | 1429 | 88.2 |
| 158 | 6 | - | 50 | 1097.6 | 1083.7 | 33.9 | 4.8 | 1378.5 | 1130.2 | 248.3 | 35.5 | 1438.4 | 1161.4 | 277.0 | 39.6 | 26.6 | 1127 | 23.6 |
| $\begin{array}{r}159 \\ \hline 160 \\ \hline\end{array}$ |  |  | 50 | $\begin{array}{r}1150.4 \\ \hline 919.2\end{array}$ | $\frac{.09 .4}{706.4}$ | $\frac{\frac{152.0}{152.0}}{213.22}$ | 21.7 30.5 | $\frac{1385.9}{9918}$ | $\frac{1112.4}{642.8}$ | 273.5 |  | 11358.8 | - $\begin{array}{r}1115.5 \\ 668.1\end{array}$ | $\frac{243.3}{477.2}$ | $\frac{34.8}{68.2}$ | 31.89 |  | $\frac{22.7}{36.7}$ |



| PEN | TREAT. | DILUENT | LEVEL | FEED IN | FEED OUT | INTAKE1 | INTAKEIDAY | FEEDIN T | FEEDOUT | INTAKE2 |  | FEEDIN | FEED OUT | INTAKE3 | INTAKEIDAY | AVE FUDAY | BW 19 | SFI |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  | 10 | 1810.3 | 831.8 | 978.5 | 139.6 | 1929.7 | 913.4 | 1016.3 | 145.2 | 1470.5 | 490.8 | 978.7 | 140.0 | 141.6 | 1783 | 79.4 |
| 67 | 17 | SF | . 0 | 1194.9 | 415.9 | 779.0 | 111.3 | 1430.7 | 598.8 | 833.9 | 119.1 | 1456.7 | 405.4 | 1051.3 | 150.2 | 126.9 | 2261 |  |
| 68 | 5 | 8 | - 0 | 1606.2 | 793.1 | 813.1 | 118.2 | 1790.2 | 720.9 | 1069.3 | 152.8 | 1684.2 | 584.6 | 1099.8 | 157.1 | 142.0 | 2209 | 64.3 |
| 69 | 14 | c | 10 | 1258.4 | 6577 | 800.7 | 85. ${ }^{\text {B }}$ | 1342.6 | 710.7 | 632.1 | 60,3 | 1175.9 | 474.3 | 701.6 | 100.2 | 92.1 | 1496 | 61.6 |
| 70 |  | sD | - 0 | 1500.4 | 725.9 | 783.5 | 111.9 | 1769.1 | 4471.5 | 297.6 | 42.5 | 1441, ${ }^{\text {a }}$ | 827.2 | 614.6 | 87.8 | 80.7 | 1651 | 48.9 |
| 71 |  | SD | 10 | 1654.8 | 667.0 | 986.8 | 141.0 | 1471.5 | 587.6 | 883.6 | 126.2 | 1809.2 | 442.7 | 1366.5 | 195.2 | 154.1 | 1743 |  |
| 72 | 5 | 5 | - 0 | 1807.9 | 628.5 | 979.4 | 139.9 | 1701.6 | 803.3 | 898.2 | 128.3 | 1147 | 1053 | 94 | 13.4 | 93.9 | 1931 | 48.6 |
| 73 | 12 |  | 50 | 980.5 | 480.7 | 499,8 | 71.4 | 1105.0 | 430.9 | 874.1 | 86.3 | 1561.7 | 378 | 1163.7 | 169.1 | 112.3 | 1377 |  |
| 74 | 2 | SD | 10 | 1484.2 | 533.3 | 050,9 | 135.8 | 1843.0 | 542.7 | 1100.3 | 157.2 | 1893.8 | 498.4 | 1395.4 | 198.3 | 164.1 | 2003 | 81.9 |
| 75 |  |  | 50. | 2403.2 | 841.8 | 1561.4 | 223.1 | 2425.7 | 1032.3 | 1383.4 | 198.1 | 2398.2 | 980.2 | 1418 | 202.6 | 208.2 | 1931 |  |
| 76 | 9 | V | - | 1630.1 | 535.2 | 1094.9 | 156.4 | 1885,5 | 771.6 | 914.0 | 130.6 | 1101 | 709.4 | 391.6 | 53.9 | 114.3 | 2020 | 58.6 |
| 77 | 12 | v | 50 | 950.6 | 414.5 | 536.1 | 78.6 | 1157.8 |  | 587.8 | 85.4 | 1312.8 | 378.4 | 933.5 | 133.4 |  |  |  |
| 78 | 19 | SF | 25 | 1324.1 | 371.4 | 952.7 | 136.1 | 1416.2 | 665 | 850.2 | 121.5 | 1308.4 | 313.5 | 992.9 | 141.8 | 133.1 | 1612 | 82.6 |
| 79 | 13 |  |  | 1481.4 | 680.1 | 781.3 | 111.6 | 1753.8 | 956.5 | 797.3 | 113.9 | 1747.9 | ${ }^{999.6}$ | 748.3 | 106.9 | 110.8 | 1670 |  |
| 80 | 7 | s | 25 | 1930.1 | 537.9 | 1392.2 | 198.9 | 1934.6 | 872.9 | 1281.6 | 180.2 | 2023.9 | 723.7 | 1300.2 | 185.7 | 188.3 | 1810 | 104.0 |
| 81 | 3 | SD | 25 | 1184.7 | 404.2 | 780.5 | 111.5 | 1246.1 | 490.7 | 755.4 | 107.9 | 1184.8 | 235.6 | 949.2 | 135.6 | 118.3 | 1833 | 72.5 |
| 82 | 5 | - s | - | 1700, ${ }^{\text {a }}$ | 645.8 | 1055.0 | 150.7 | 1810.8 | 924 | 888.8 | 128.7 | 1660.9 | 864.9 | 796 | 113.7 | 130.4 | 1840 | 70.9 |
| 83 | 1 | So | - 0 | 1363.5 | 398.4 | 985.1 | 137.9 | 1652.1 | 705.4 | 948.7 | 135.2 | 1880.7 | 647.6 | 1033.1 | 147.6 | 140.2 | 2067 | 67.8 |
| 84 | 6 |  | 10 | 1800.5 | 921 | 879.5 | 125.6 | 1802.3 | 986.2 | 836.1 | 118.4 | 1838.4 | 608.2 | 1230.2 | 175.7 | 140.3 | 1689 | 83.1 |
| 85 | 15 | c | 25 | 1244.0 | 1089.5 | 154.5 | 221 | 1089.5 | 936.3 | 163.2 | 21.9 | 1185.2 | 10392 | 146 | 20.8 | 21.6 | 1314 | 16.4 |
| 86 | 17 | SF |  | 1307.5 | 511.2 | 796,3 | 113.8 | 1376.6 | 806.4 | 570.2 | 81.5 | 1325.6 | 598 | 727.6 | 103.8 | 99.7 | 1616 | 61.7 |
| 87 | 20 | SF | 60 | 1023.4 | 518.1 | 505.3 | 72.2 | 1108.3 | 708.6 | 397.7 | 58.8 | 1077.2 | 394 | 683.2 | 97.6 | 75.5 | 1198 | 83.0 |
| 88 | 14 |  | 10 | 1248.5 | 677.1 | 571.4 | 81.6 | 1433.1 | 746.5 | 688.6 | 88.1 | 1287.9 | 448.9 | 819 | 117.0 | 98.9 | 1787 | 55.3 |
| 89 | . | S | 50 | 2286.1 | 1070.8 | 1195.3 | 170.8 | 2629.4 | 1207, ${ }^{\text {a }}$ | 1421.6 | 203.1 | 1740 | 710.9 | 1029.1 | 147.0 | 173.6 | 1615 |  |
| 90 |  | S |  | 1596.7 | 683.0 | 912.8 | 130.4 | 1776.6 | 817.1 | 859.5 | 122.8 | 1854.5 | 806.6 | 847.9 | 121, 1 | 124.8 | 1684 | 73.7 |
| 91 | 12 | v | 50 | ${ }^{938.3}$ | 791 | 147.3 | 21.0 | 1229.2 | 1017.4 | 211.8 | 30.3 | 1122 | 880.8 | 231.2 | 33.0 | 28,1 | 1038 | 27.1 |
| 92 |  | SD | 50 | 998.7 | 332.6 | 566.1 | 80.9 | 958.8 | 448.3 | 510.5 | 72.9 | 986 | 183.1 | 812.9 | 116.1 | 80.0 | 1488 | 60.5 |
| 93 | 3 | so | 25 | 1194,0 | 260.6 | 833.4 | 133.3 | 1247.6 | 484.2 | 783.6 | 111.9 | 1181.5 | 202.1 | 979.4 | 139.9 | 128.4 | 1578 |  |
| 94 | 14 | c | 10 | 1298.4 | 734.6 | 663.8 | 80.5 | 1325.6 | 716.7 | 608.9 | 87.0 | 1243.6 | 429.3 | 814.3 | 116.3 | 94.6 | 1562 | 60.6 |
| 95 |  | , | 25 | 2080.8 | 838.8 | 1242.0 | 177.4 | 2293.7 | 1052.4 | 1241.3 | 177.3 | 2190.9 | 753.2 | 1437.7 | 205.4 | 188.7 | 1730 |  |
| 96 | 10 | v | 10 | 2027.9 | 780.5 | 1247.4 | 178.2 | 1580.0 | 730.3 | 849.7 | 121.4 | 1516.6 | 498.8 | 1017.8 | 145.4 | 148.3 | 1872 | 88.7 |
| 97 | 2 | SD | 10 | 1505.5 | 635.9 | 869.6 | 124.2 | 1513.6 | 641.8 | 871.8 | 124.5 | 1438,2 | 471 | 967.2 | 138.2 | 129.0 | 1954 |  |
| 98 | 16 | c | 50 | 1159.9 | 875.25 | 184.7 | 26.4 | 1087.7 | 877.43 | 210.3 | 30.0 | 1059.9 | 873.34 | 186.56 | 28.7 | 27.7 | 1197 | 23.1 |
| 99 | 9 | V |  | 1645.4 | 647.7 | 987.7 | 142.5 | 1719.2 | 765.7 | 953.5 | 136.2 | 1884.4 | 889.7 | 994.7 | 142.1 | 140.3 |  |  |
| 100 | 16 | c | 50 | 1081.2 | 922.84 | 158.4 | 22.6 | 1087.0 | 894.69 | 192.3 | 27.5 | 1047.1 | 891.34 | 155.76 | 22.3 | 24.1 | 1261 | 19.1 |
| 101 | , | SD | 0 | 1549.1 | 599 | 950.1 | 135.7 | 1699,0 | 914.9 | 784.1 | 112.0 | 1676.8 | 819.4 | 857.4 | 122.5 | 123.4 | 1815 |  |
| 102 | 19 | SF | 25 | 1278.1 | 393.6 | 884.3 | 126.3 | 1276.3 | 567.5 | 700.8 | 101.3 | 1344.2 | 447.8 | 896.4 | 128.1 | 118.5 | 1516 | 78.2 |
| 103 | 6 | S | 10 | 1814.0 | 702.9 | 1111.1 | 158.7 | 2009.3 | 757.7 | 1251.6 | 178.8 | 2120.8 | 756.6 | 13842 | 194.9 | 177.5 |  | 88.3 |
| 104 | 9 | , |  | 1657.7 | 801.1 | 856.6 | 122.4 | 1663.8 | 885.1 | 778.7 | 191.2 | 1599.2 | ${ }^{838.6}$ | 780.6 | 100.7 | 114.1 | 1728 | 66.0 |
| 105 | 20 | SF | 50 | 1032.5 | 452.2 | 580.3 | 82.9 | 1083.4 | 413.1 | 650.3 | 92.8 | -1084.6 | 175.8 | 908.8 | 129.8 | 101.9 | 1823 | 62.8 |
| 106 | 13 |  |  | 1483.1 | 681.6 | 801.5 | 114.5 | 1623.7 | 828.9 | 794.8 | 113.5 | 1720.9 | 841.7 | 879.2 | 125.6 | 117.9 | 1984 | 59.4 |
| 107 | 8 | 5 | 50 | 2467.1 | 734.3 | 1732.8 | 247.5 | 2604.7 | 607.9 | 1698.8 | 242.4 | 2459.6 | 835.5 | 1624.1 | 232.0 | 240.7 | 1836 |  |
| 108 | 18 | SF | 10 | 1587.9 | 781.8 | 786.1 | 112.3 | 1627.1 | 784.5 | 842.6 | 120.4 | 1412.4 | 508.8 | 903.6 | 129.1 | 120.6 | 1604 | 75.2 |
| 109 | 4 | SD | 50 | 920.2 | 811.5 | 108.7 | 15.5 | 1056.1 | 430.9 | 625.2 | 89.3 | 1670.4 | 244.6 | 1425.8 | 203.7 | 102.8 | 1887 | 55.1 |
| 110 | 13 | c |  | 1372.5 | 429.3 | 943.2 | 134.7 | 1514.9 | 628.4 | 88.5 | 128.9 | 1670.8 | 734.5 | 936.3 | 133.8 | 131.8 | 2293 | 57.5 |
| 111 | 7 | 5 | 25 | 2130.5 | 732.7 | 1387.8 | 199.7 | +945.1 | 722.4 | 1222.7 | 174.7 | 22393 | 638.2 | 1601.1 | 228.7 | 201.0 | 1783 |  |
| 112 | 17 | SF | 0 | 1274.9 | 1001.8 | 273.1 | 39.0 | 1529.8 | 1257.7 | 272.1 | 38.9 | 1257.7 | 1134.7 | 123 | 17.6 | 31.8 | 717 | 44.4 |
| 113 | 15 | C | 25 | 1182.2 | 1062.5 | 119.7 | 17.1 | 1375.0 | 1191.7 | 183.3 | 26.2 | 1191.7 | 1057 | 134.7 | 19.2 | 20.8 | 1623 | 12.8 |
| 114 | 11 | v | 25 | 1660.4 | 565.5 | 1094.9 | 156.4 | 1261.3 | 509.1 | 752.2 | 107.5 | 1223.1 | 267.8 | 955.3 | 136.5 | 133.4 | 1405 | 05.0 |
| 115 | 2 | SD | 10 | 1610,8 | 863.2 | 747.6 | 106.8 | 1608.5 | 805.3 | 803.2 | 114.7 | 1650.9 | 742.5 | 908.4 | 129.8 | 117.1 | 1571 | 74.5 |
| 116 | 11 | v | 25 | 1044.1 | 985.2 | 58.9 | 8.4 | 1347.4 | 1025.7 | 321.7 | 46.0 | 1363.1 | 809.8 | 553.3 | 79.0 | 44.5 | 1141 | 39.0 |
| 117 | 10 | v | 10 | 1757.7 | 847.7 | 810.0 | 130.0 | 1684.5 | 858.7 | 805.8 | 115.1 | 1460 | 551.6 | ${ }^{908.4}$ | 129.8 | 125.0 | 1771 | 70.8 |
| 119 | 19 | SF | 25 | 1153.4 | 350.5 | 802.9 | 114.7 | 1357,0 | 519.8 | 837.2 | 119.6 | 1412.6 | 313.6 | 1098.9 | 157.0 | 130.4 | 1877 | 77.8 |
| 119 | 12 | v | 60 | 952.7 | 439.7 | 513.0 | 73.3 | 117.7 | 550.3 | 627.4 | 89.6 | 1070.7 | 248 | 822.7 | 117.5 | 93.6 | 1546 | 60.5 |
| 120 | 18 | SF | 10 | 1518.6 | 441 | 1075.6 | 153.7 | 1682.4 | 725.8 | 950.6 | 136.7 | 1476.7 | 465.5 | 1011.2 | 144.5 | 144.9 | 1923 | 75.4 |
| 121 | 5 | S | 0 | 1821.4 | 630.3 | 991.1 | 141.6 | 1723.8 | 906.8 | 617.0 | 116.7 | 1670.2 | 780.1 | 890.1 | 127.2 | 128.5 | 2123 | 60.5 |
| 122 |  | SD | 10 | 1524.6 | 698.6 | 628.0 | 148.0 | 1524.7 | 842.8 | 681.8 | 97.4 | 1497.6 | 618.9 | 978.6 | 139.8 | 118.4 | 1618 | 73.2 |
| 123 | 19 | SF | 25 | 1202.9 | 702.3 | 500.6 | 71.5 | 1458.6 | 704.8 | 753.8 | 107.7 | 1427.8 | 572.2 | 855.6 | 122.2 | 100.5 | 1472 | 68.3 |
| 124 | 17. | sf | 0 | 1272.4 | 407 | 865,4 | 123.6 | 1489.3 | 662.1 | 827.2 | 118.2 | 1991.7 | 887.3 | 1104.4 | 157.8 | 133.2 | 1690 | 78.8 |
| 125 | 9 | v | - | 1841.4 | 625 | 1016.4 | 145.2 | 1778.1 | B76.4 | 801.7 | 128.8 | 1700.3 | 899.6 | 850.7 | 121.5 | 131.8 | 1939 | 68.0 |
| 126 | 10 | v | 10 | 1995.9 | 822.9 | 1073.0 | 163.3 | 1590.5 | 647.8 | 942.7 | 134.7 | 1434.9 | 588.2 | 848.7 | 121.2 | 136.4 | 1712 | 79.7 |
| 127 | 11 |  | 25 | 1582.3 | 441.9 | 1140.4 | 182.9 | 1172.8 | 382.3 | 790.6 | 112.9 | 1206.2 | 202.2 | 1004 | 143.4 | 139.8 | 1570 | 89.0 |
| 128 |  | SD |  | 1530.3 | 971.3 | 559.0 | 70.9 | 1837.6 | 1192.7 | 644,9 | 82.1 | 1701.2 | 1021.8 | 679.4 | 07.1 | 89.7 | 1602 | 56.0 |
| 129 | 8 | $s$ | 50 | 2185.5 | 964 | 1221.5 | 174.5 | 2406.9 | 1057.1 | 1349.8 | 192.8 | 2634 | $\therefore 1010.4$ | 1623.6 | 231.9 | 199.8 | 1742 | 116.7 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 51.2 |


| PEN | TREAT. | DILUENT | LEVEL | FEEDIN | FEED OUT | INTAKE1 | INTAKEDAY | FEEDIN | FEED OUT | INTAKE2 | INTAKEDAY | FEEDIN | FEED OUT | INTAKE3 | INTAKEDAY | AVE FVDAY |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 131 | 11 | v | 25 | 1709.0 | 471.5 | 1237.5 | 178.8 | 1317.3 | 418.7 | 900.6 | 128.7 | 1230.9 | 211.4 | 1098.7 | 145.5 | 150.3 | 1695 | 88.7 |
| 132 | 20 | SF | 50 | 1007,7 | 524.4 | 483.3 | 68.0 | 1047.4 | 428.6 | 818.8 | 88.4 | 1788.1 | 474.8 | 1313.3 | 187.6 | 115.0 | 1614 | 71.3 |
| 133 | 7 | S. | 25 | 2080.2 | 827.5 | 1252.7 | 179.0 | 2144.3 | 745.7 | 1398.6 | 198.8 | 2065,9 | 726.3 | 1339.6 | 191.4 | 190.0 | 1920 | 89.0 |
| 134 | 18 | SF | 10 | 1593.7 | 871.1 | 722.6 | 103.2 | 1597.6 | 740.5 | 857.1 | 122.4 | 2088.2 | 860.8 | 1227.3 | 175.3 | 133.7 | 1970 | 67.8 |
| 135 |  | - ${ }^{\text {s }}$ | 25 | 1963.6 | 711.3 | 12523 | 178.9 | 1985.6 | 645.8 | 1319.8 | 188.5 | 2110 | 400.8 | 1700.2 | 242.9 | 203.4 | 2097 | 97.0 |
| 136 | 3 | sp | 25 | 1251.6 | 844.3 | 607.3 | 88.6 | 1341.2 | 661 | 680.2 | 97.2 | 1801.2 | 632.9 | 1268.3 | 181.2 | 121.7 | 1728 | 70.4 |
| 137 | 14 | c | 10 | 1262.7 | 600.3 | 656.4 | 93.8 | 1435.4 | 640.1 | 795.3 | 113.6 | 1234.7 | 322.6 | 912.1 | 130.3 | 112.6 | 1809 | 62.2 |
| 138 | 12 | V | 50 | 954.8 | 467.7 | 487.1 | 69.6 | 1125.3 | 482.4 | 642.9 | 81.8 | 1131.3 | 231.4 | 899.9 | 128.6 | 96.7 | 1505 | 64.2 |
| 139 |  | 5 | 50 | 2451.8 | 1170.3 | 1281.5 | 183.1 | 2721.1 | 1092.4 | 1828.7 | 2327 | 2627.4 | 1050.9 | 1576.5 | 225.2 | 213.7 | 1841 | 116.1 |
| 140 | 16 | c | 50 | 1160.7 | 1020.1 | 140.6 | 20.1 | 1170.7 | 1050.35 | 120.4 | 17.2 | 888.7 | 857.31 | 131.38 | 18.8 | 18.7 | 1392 | 13.4 |
| 141 |  | SD | 10 | 1458.5 | 456.1 | 1002.4 | 143.2 | 1523.7 | 825.5 | 888.2 | 128.3 | 1365.5 | 327.4 | 1038.1 | 148.3 | 139.9 | 1737 | 80.6 |
| 142 |  |  |  | 1486.6 | 536.6 | 950.0 | 135.7 | 1613.9 | 753.5 | 860.4 | 122.9 | 1687.1 | 880.5 | 906.6 | 129.5 | 129.4 | 2298 |  |
| 143 | 18 | SF | 10 | 1548.8 | 678.9 | 869.9 | 124.3 | 1613.3 | 663.5 | 949.8 | 135.7 | 1519.3 | 871.6 | 947,7 | 135.4 | 131.8 | 1963 | 67.1 |
| 144 |  |  |  | 1668.4 | 897.1 | 772.3 | 110.3 | 1783.6 | 1438.5 | 345.1 | 49.3 | 1438.5 | 666.8 | 71.7 | 110.2 | 90.0 | 1767 |  |
| 145 | 15 | c | 25 | 1315.6 | 1097,4 | 218.2 | 31.2 | 1313.9 | 1116.9 | 197.0 | 28.1 | 1362.4 | 1100,5 | 162.9 | 23.3 | 27.5 | 1359 | 20.3 |
| 146 | 17 | SF | 0 | 1312.9 | 413.5 | 899.4 | 128.5 | 1445.9 | 610 | 835.9 | 119.4 | 1353.6 | 337.7 | 1015.9 | 145.1 | 131.0 | 1956 | 67.0 |
| 147 | 13 |  |  | 1396.4 | 883.7 | 512.7 | 73.2 | 1813.4 | 780.4 | 823.0 | 117.6 | 1577.3 | 710.8 | 866.5 | 123.6 | 104.9 | 1787 |  |
| 148 | 12 | v | 50 | 932.4 | 375.4 | 557.0 | 78.6 | 10572 | 4522 | 605.0 | 89.4 | 1090.9 | 373.2 | 717.7 | 102.5 | 89.5 | 1473 | 60.8 |
| 149 |  | sD | 23 | 1198.9 | 213.5 | 985.4 | 140.8 | 1205.8 | 534.5 | 671.3 | 85.9 | 1193.1 | 232.2 | 960.9 | 137.3 | 124.6 |  |  |
| 150 | 13 | c | 0 | 1434.8 | 622.6 | 812.2 | 116.0 | 1704.6 | 918.7 | 785.9 | 112.3 | 1636.9 | 891 | 745.9 | 100.6 | 111.6 | 1618 | 69.0 |
| 151 | 15 |  | 25 | 1138.7 | 956.8 | 181.9 | 26.0 | 1312.3 | 1039.6 | 272.5 | 38.9 | 1257.8 | 1049.8 | 208 | 29.7 | 31.5 | 1456 | 21.7 |
| 152 | 4 | so | 50 | 924.6 | 388.6 | 538.0 | 78.9 | 958.9 | 518.9 | 442.0 | 63.1 | 1007,2 | 210,0 | 798.3 | 113.8 | 84.6 | 1517 | 55.8 |
| 153 |  | S0 |  | 1519.9 | 501,9 | 1018.0 | 145.4 | 1648.7 | 698.7 | 950.0 | 135.7 | 1598.8 | 832.8 | 266 | 138.0 | 138.7 | 2401 | 58.2 |
| 154 | 14 | c | 10 | 1301.0 | 845.9 | 655.1 | 93.6 | 1253.6 | 555.9 | 697.7 | 88.7 | 1286.5 | 619.2 | 647.3 | 92.5 | 95.2. | 1842 | 51.7 |
| 155 | 16 |  | 50 | 1115.5 | 873.07 | 142.4 | 20.3 | 1082.2 | 977.13 | 105.1 | 15.0 | 1043.8 | ${ }^{938.37}$ | 105.43 | 15.1 | 16.6 | 1407 | 11.9 |
| 156 | 4 | 80 | 50 | 956.1 | 587.7 | 370.4 | 52.9 | 1025.4 | 595.4 | 430.0 | 61.4 | 1021.8 | 441.1 | 580.7 | 83.0 | 65.8 | 1297 | 50.7 |
| 157 | 19 | SF | 25 | 1154.8 | 297.9 | 856.9 | 422.4 | 1291.2 | 553.2 | 738.0 | 105.4 | 1409.5 | 353.2 | 1058.3 | 150.9 | 128.2 | 1442 | 87.6 |
| 158 | 6 | S | 10 | 1868.2 | 729.8 | 1136.4 | 162.3 | 1753.3 | 687.5 | 1085.8 | 152.3 | 1852.2 | 522.6 | 1129.4 | 161.3 | 158.6 | 1659 | 95,6 |
| 159 | , | S | 10 | 1783.6 | 779,2 | 1004.4 | 143.5 | 1649.2 | 780.3 | 858.9 | 122.7 | 1688 | 349.3 | 1338.7 | 191.2 | 152.5 | 1905 | 80.0 |
| 160 |  |  |  | 1730.4 | 536.1 | 1194.3 | 170.6 | 1634.6 | 710.3 | 924.3 | 132.0 | 1509.4 | 447.5 | 1061.9 | 151.7 | 151.5 |  | 80.8 |

## Appendix C: Trial 1 egg production data

Phase 1:

| PEN | TREAT | DILUENT | LEVEL | FREQ1 | FREQ2 | FREQ3 | ROL | AVE EW | AVE EO |
| ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: |
| 2 | 6 | C | 10 | 5 | 5 | 3 | 61.9 | 51.4 | 31.8 |
| 22 | 5 | C | 0 | 6 | 5 | 5 | 76.2 | 55.5 | 42.3 |
| 23 | 7 | C | 25 | 3 | 1 | 0 | 19.0 | 36.2 | 6.9 |
| 29 | 6 | C | 10 | 5 | 6 | 6 | 81.0 | 42.4 | 34.3 |
| 34 | 5 | C | 0 | 6 | 6 | 6 | 85.7 | 55.3 | 47.4 |
| 35 | 8 | C | 50 | 2 | 0 | 0 | 9.5 | 15.0 | 1.4 |
| 36 | 8 | C | 50 | 2 | 0 | 0 | 9.5 | 18.8 | 1.8 |
| 38 | 7 | C | 25 | 2 | 0 | 0 | 9.5 | 20.3 | 1.9 |
| 49 | 7 | C | 25 | 3 | 2 | 0 | 23.8 | 18.7 | 4.5 |
| 54 | 6 | C | 10 | 3 | 3 | 1 | 33.3 | 34.8 | 11.6 |
| 60 | 8 | C | 50 | 3 | 0 | 0 | 14.3 | 18.2 | 2.6 |
| 66 | 6 | C | 10 | 6 | 4 | 6 | 76.2 | 59.6 | 45.4 |
| 68 | 5 | C | 0 | 5 | 4 | 5 | 66.7 | 65.3 | 43.5 |
| 72 | 5 | C | 0 | 5 | 6 | 6 | 81.0 | 59.4 | 48.1 |
| 75 | 8 | C | 50 | 3 | 0 | 0 | 14.3 | 10.1 | 1.4 |
| 80 | 7 | C | 25 | 2 | 0 | 0 | 9.5 | 9.4 | 0.9 |
| 82 | 5 | C | 0 | 5 | 5 | 5 | 71.4 | 71.0 | 50.7 |
| 84 | 6 | C | 10 | 5 | 3 | 0 | 38.1 | 40.9 | 15.6 |
| 89 | 8 | C | 50 | 4 | 0 | 0 | 19.0 | 11.8 | 2.3 |
| 90 | 5 | C | 0 | 5 | 6 | 7 | 85.7 | 58.3 | 49.9 |
| 95 | 7 | C | 25 | 3 | 0 | 0 | 14.3 | 18.6 | 2.7 |
| 103 | 6 | C | 10 | 5 | 6 | 5 | 76.2 | 59.7 | 45.5 |
| 107 | 8 | C | 50 | 3 | 0 | 0 | 14.3 | 10.4 | 1.5 |
| 111 | 7 | C | 25 | 3 | 1 | 0 | 19.0 | 13.6 | 2.6 |
| 121 | 5 | C | 0 | 5 | 4 | 5 | 66.7 | 65.5 | 43.7 |
| 129 | 8 | C | 50 | 4 | 0 | 1 | 23.8 | 19.1 | 4.6 |
| 133 | 7 | C | 25 | 4 | 0 | 0 | 19.0 | 19.6 | 3.7 |
| 135 | 7 | C | 25 | 4 | 1 | 0 | 23.8 | 21.3 | 5.1 |
| 139 | 8 | C | 50 | 3 | 0 | 0 | 14.3 | 19.8 | 2.8 |
| 144 | 5 | C | 0 | 6 | 5 | 4 | 71.4 | 56.7 | 40.5 |
| 158 | 6 | C | 10 | 5 | 0 | 0 | 23.8 | 18.4 | 4.4 |
| 159 | 6 | C | 10 | 4 | 4 | 4 | 57.1 | 42.0 | 24.0 |
| 11 | 16 | S | 50 | 5 | 6 | 5 | 76.2 | 61.0 | 46.5 |
| 17 | 13 | S | 0 | 5 | 5 | 5 | 71.4 | 57.4 | 41.0 |
| 21 | 15 | S | 25 | 6 | 6 | 6 | 85.7 | 59.0 | 50.5 |
| 30 | 16 | S | 50 | 4 | 6 | 3 | 61.9 | 58.2 | 36.0 |
| 37 | 13 | S | 0 | 5 | 6 | 6 | 81.0 | 62.8 | 50.8 |
| 39 | 15 | S | 25 | 6 | 6 | 5 | 81.0 | 67.0 | 54.2 |
| 40 | 14 | S | 10 | 5 | 6 | 6 | 81.0 | 50.5 | 40.9 |
| 42 | 15 | S | 25 | 6 | 5 | 4 | 71.4 | 64.2 | 45.8 |
| 47 | 16 | S | 50 | 4 | 5 | 4 | 61.9 | 65.7 | 40.6 |
| 57 | 15 | S | 25 | 6 | 5 | 6 | 81.0 | 61.0 | 49.3 |
| 59 | 16 | S | 50 | 4 | 0 | 5 | 42.9 | 38.3 | 16.4 |
| 61 | 13 | S | 0 | 6 | 6 | 6 | 85.7 | 49.1 | 42.1 |
| 64 | 14 | S | 10 | 6 | 6 | 6 | 85.7 | 60.9 | 52.2 |
| 69 | 14 | S | 10 | 4 | 6 | 5 | 71.4 | 56.4 | 40.3 |
|  |  |  |  |  |  |  |  |  |  |


| PEN | TREAT | DILUENT | LEVEL | FREQ1 | FREQ2 | FREQ3 | ROL | AVE EW | AVE EO |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 79 | 13 | S | 0 | 5 | 6 | 5 | 76.2 | 64.8 | 49.4 |
| 85 | 15 | S | 25 | 6 | 6 | 5 | 81.0 | 60.2 | 48.7 |
| 88 | 14 | S | 10 | 6 | 7 | 6 | 90.5 | 51.8 | 46.9 |
| 94 | 14 | S | 10 | 6 | 7 | 7 | 95.2 | 63.0 | 60.0 |
| 98 | 16 | S | 50 | 6 | 7 | 6 | 90.5 | 54.6 | 49.4 |
| 100 | 16 | S | 50 | 5 | 4 | 3 | 57.1 | 51.0 | 29.2 |
| 106 | 13 | S | 0 | 5 | 7 | 6 | 85.7 | 59.8 | 51.3 |
| 110 | 13 | S | 0 | 5 | 6 | 7 | 85.7 | 55.3 | 47.4 |
| 113 | 15 | S | 25 | 6 | 6 | 6 | 85.7 | 62.4 | 53.5 |
| 137 | 14 | S | 10 | 6 | 6 | 6 | 85.7 | 65.4 | 56.1 |
| 140 | 16 | S | 50 | 5 | 5 | 5 | 71.4 | 54.1 | 38.6 |
| 145 | 15 | S | 25 | 5 | 4 | 3 | 57.1 | 67.4 | 38.5 |
| 147 | 13 | S | 0 | 6 | 5 | 7 | 85.7 | 63.3 | 54.2 |
| 150 | 13 | S | 0 | 6 | 6 | 4 | 76.2 | 60.5 | 46.1 |
| 151 | 15 | S | 25 | 6 | 5 | 5 | 76.2 | 52.1 | 39.7 |
| 154 | 14 | S | 10 | 5 | 7 | 6 | 85.7 | 62.6 | 53.6 |
| 155 | 16 | S | 50 | 5 | 6 | 4 | 71.4 | 49.2 | 35.1 |
| 5 | 18 | SD | 10 | 6 | 4 | 5 | 71.4 | 62.8 | 44.8 |
| 9 | 20 | SD | 50 | 5 | 0 | 0 | 23.8 | 18.7 | 4.5 |
| 10 | 19 | SD | 25 | 4 | 3 | 2 | 42.9 | 66.8 | 28.6 |
| 13 | 17 | SD | 0 | 5 | 6 | 5 | 76.2 | 62.3 | 47.5 |
| 18 | 20 | SD | 50 | 4 | 1 | 0 | 23.8 | 18.2 | 4.3 |
| 19 | 18 | SD | 10 | 5 | 4 | 0 | 42.9 | 36.1 | 15.5 |
| 24 | 17 | SD | 0 | 4 | 7 | 5 | 76.2 | 62.1 | 47.3 |
| 33 | 19 | SD | 25 | 5 | 6 | 6 | 81.0 | 61.7 | 50.0 |
| 46 | 17 | SD | 0 | 6 | 4 | 5 | 71.4 | 55.3 | 39.5 |
| 48 | 20 | SD | 50 | 3 | 0 | 3 | 28.6 | 27.5 | 7.8 |
| 55 | 19 | SD | 25 | 4 | 4 | 2 | 47.6 | 62.1 | 29.6 |
| 56 | 20 | SD | 50 | 3 | 0 | 0 | 14.3 | 19.5 | 2.8 |
| 62 | 18 | SD | 10 | 6 | 6 | 5 | 81.0 | 57.8 | 46.8 |
| 63 | 18 | SD | 10 | 6 | 6 | 6 | 85.7 | 63.7 | 54.6 |
| 67 | 17 | SD | 0 | 6 | 6 | 6 | 85.7 | 60.4 | 51.8 |
| 78 | 19 | SD | 25 | 6 | 6 | 5 | 81.0 | 57.7 | 46.7 |
| 86 | 17 | SD | 0 | 5 | 5 | 3 | 61.9 | 59.4 | 36.8 |
| 87 | 20 | SD | 50 | 2 | 0 | 0 | 9.5 | 16.7 | 1.6 |
| 102 | 19 | SD | 25 | 6 | 4 | 5 | 71.4 | 61.7 | 44.1 |
| 105 | 20 | SD | 50 | 3 | 0 | 0 | 14.3 | 6.1 | 0.9 |
| 108 | 18 | SD | 10 | 6 | 6 | 6 | 85.7 | 58.2 | 49.9 |
| 112 | 17 | SD | 0 | 4 | 2 | 0 | 28.6 | 35.7 | 10.2 |
| 118 | 19 | SD | 25 | 6 | 5 | 4 | 71.4 | 51.0 | 36.5 |
| 120 | 18 | SD | 10 | 5 | 7 | 6 | 85.7 | 59.3 | 50.8 |
| 123 | 19 | SD | 25 | 4 | 5 | 5 | 66.7 | 57.1 | 38.1 |
| 124 | 17 | SD | 0 | 5 | 6 | 5 | 76.2 | 63.1 | 48.1 |
| 130 | 20 | SD | 50 | 3 | 0 | 1 | 19.0 | 38.4 | 7.3 |
| 132 | 20 | SD | 50 | 4 | 4 | 1 | 42.9 | 41.0 | 17.6 |
| 134 | 18 | SD | 10 | 5 | 6 | 7 | 85.7 | 53.1 | 45.5 |
| 143 | 18 | SD | 10 | 5 | 6 | 6 | 81.0 | 56.3 | 45.6 |
| 146 | 17 | SD | 0 | 6 | 5 | 4 | 71.4 | 64.3 | 45.9 |
| 157 | 19 | SD | 25 | 5 | 6 | 5 | 76.2 | 50.6 | 38.5 |


| PEN | TREAT | DILUENT | LEVEL | FREQ1 | FREQ2 | FREQ3 | ROL | AVE EW | AVE EO |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 6 | 2 | SF | 10 | 6 | 6 | 6 | 85.7 | 65.2 | 55.9 |
| 15 | 4 | SF | 50 | 3 | 0 | 1 | 19.0 | 40.3 | 7.7 |
| 16 | 1 | SF | 0 | 6 | 7 | 7 | 95.2 | 57.5 | 54.7 |
| 26 | 4 | SF | 50 | 1 | 0 | 0 | 4.8 | 18.9 | 0.9 |
| 27 | 3 | SF | 25 | 3 | 4 | 4 | 52.4 | 57.6 | 30.2 |
| 28 | 2 | SF | 10 | 5 | 3 | 6 | 66.7 | 44.0 | 29.4 |
| 31 | 3 | SF | 25 | 4 | 6 | 6 | 76.2 | 64.0 | 48.7 |
| 32 | 1 | SF | 0 | 5 | 6 | 7 | 85.7 | 60.9 | 52.2 |
| 43 | 3 | SF | 25 | 5 | 3 | 0 | 38.1 | 44.7 | 17.0 |
| 44 | 3 | SF | 25 | 6 | 5 | 6 | 81.0 | 41.4 | 33.5 |
| 51 | 4 | SF | 50 | 2 | 0 | 0 | 9.5 | 19.3 | 1.8 |
| 52 | 4 | SF | 50 | 4 | 2 | 0 | 28.6 | 20.1 | 5.7 |
| 65 | 1 | SF | 0 | 6 | 6 | 6 | 85.7 | 52.4 | 44.9 |
| 70 | 1 | SF | 0 | 5 | 4 | 5 | 66.7 | 55.0 | 36.7 |
| 71 | 2 | SF | 10 | 6 | 4 | 6 | 76.2 | 67.8 | 51.7 |
| 74 | 2 | SF | 10 | 4 | 6 | 5 | 71.4 | 61.2 | 43.7 |
| 81 | 3 | SF | 25 | 5 | 5 | 5 | 71.4 | 60.8 | 43.4 |
| 83 | 1 | SF | 0 | 4 | 5 | 5 | 66.7 | 65.3 | 43.5 |
| 92 | 4 | SF | 50 | 5 | 3 | 1 | 42.9 | 21.2 | 9.1 |
| 93 | 3 | SF | 25 | 5 | 5 | 6 | 76.2 | 58.4 | 44.5 |
| 97 | 2 | SF | 10 | 3 | 1 | 5 | 42.9 | 24.1 | 10.3 |
| 101 | 1 | SF | 0 | 6 | 7 | 7 | 95.2 | 64.1 | 61.1 |
| 109 | 4 | SF | 50 | 5 | 5 | 4 | 66.7 | 49.0 | 32.7 |
| 115 | 2 | SF | 10 | 5 | 5 | 6 | 76.2 | 64.1 | 48.9 |
| 122 | 2 | SF | 10 | 5 | 3 | 6 | 66.7 | 58.0 | 38.6 |
| 128 | 1 | SF | 0 | 5 | 5 | 5 | 71.4 | 51.1 | 36.5 |
| 136 | 3 | SF | 25 | 5 | 3 | 7 | 71.4 | 66.7 | 47.6 |
| 141 | 2 | SF | 10 | 6 | 6 | 7 | 90.5 | 55.7 | 50.4 |
| 149 | 3 | SF | 25 | 6 | 5 | 6 | 81.0 | 46.7 | 37.8 |
| 152 | 4 | SF | 50 | 2 | 1 | 3 | 28.6 | 42.7 | 12.2 |
| 153 | 1 | SF | 0 | 5 | 6 | 7 | 85.7 | 67.3 | 57.7 |
| 156 | 4 | SF | 50 | 3 | 0 | 0 | 14.3 | 16.5 | 2.4 |
| 1 | 9 | V | 0 | 5 | 5 | 6 | 76.2 | 59.7 | 45.5 |
| 3 | 11 | V | 25 | 5 | 5 | 4 | 66.7 | 55.8 | 37.2 |
| 4 | 10 | V | 10 | 6 | 5 | 6 | 81.0 | 60.1 | 48.6 |
| 8 | 10 | V | 10 | 6 | 5 | 7 | 85.7 | 60.2 | 51.6 |
| 12 | 12 | V | 50 | 4 | 0 | 0 | 19.0 | 15.0 | 2.9 |
| 14 | 11 | V | 25 | 6 | 6 | 6 | 85.7 | 53.7 | 46.0 |
| 20 | 12 | $V$ | 50 | 3 | 0 | 1 | 19.0 | 39.0 | 7.4 |
| 25 | 9 | V | 0 | 3 | 4 | 3 | 47.6 | 68.7 | 32.7 |
| 41 | 10 | V | 10 | 5 | 5 | 5 | 71.4 | 61.3 | 43.8 |
| 45 | 11 | V | 25 | 5 | 6 | 4 | 71.4 | 66.7 | 47.6 |
| 50 | 9 | V | 0 | 5 | 7 | 6 | 85.7 | 58.7 | 50.3 |
| 53 | 10 | V | 10 | 5 | 6 | 6 | 81.0 | 56.1 | 45.4 |
| 58 | 11 | V | 25 | 5 | 7 | 2 | 66.7 | 65.7 | 43.8 |
| 73 | 12 | V | 50 | 3 | 0 | 0 | 14.3 | 19.7 | 2.8 |
| 76 | 9 | V | 0 | 6 | 7 | 5 | 85.7 | 46.6 | 39.9 |
| 77 | 12 | V | 50 | 3 | 0 | 0 | 14.3 | 18.1 | 2.6 |
| 91 | 12 | V | 50 | 3 | 1 | 0 | 19.0 | 19.9 | 3.8 |


| PEN | TREAT | DILUENT | LEVEL | FREQ1 | FREQ2 | FREQ3 | ROL | AVE EW | AVE EO |
| ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: |
| 96 | 10 | V | 10 | 6 | 5 | 4 | 71.4 | 54.1 | 38.6 |
| 99 | 9 | V | 0 | 6 | 6 | 6 | 85.7 | 66.2 | 56.8 |
| 104 | 9 | V | 0 | 5 | 7 | 6 | 85.7 | 52.4 | 44.9 |
| 114 | 11 | V | 25 | 6 | 5 | 6 | 81.0 | 55.9 | 45.2 |
| 116 | 11 | V | 25 | 2 | 0 | 0 | 9.5 | 21.4 | 2.0 |
| 117 | 10 | V | 10 | 4 | 6 | 5 | 71.4 | 63.8 | 45.6 |
| 119 | 12 | V | 50 | 4 | 1 | 0 | 23.8 | 18.6 | 4.4 |
| 125 | 9 | V | 0 | 6 | 7 | 7 | 95.2 | 58.4 | 55.6 |
| 126 | 10 | V | 10 | 6 | 5 | 6 | 81.0 | 57.4 | 46.5 |
| 127 | 11 | V | 25 | 5 | 5 | 5 | 71.4 | 58.6 | 41.9 |
| 131 | 11 | V | 25 | 4 | 6 | 5 | 71.4 | 66.6 | 47.6 |
| 138 | 12 | V | 50 | 4 | 0 | 0 | 19.0 | 19.9 | 3.8 |
| 142 | 9 | V | 0 | 5 | 6 | 7 | 85.7 | 59.0 | 50.6 |
| 148 | 12 | V | 50 | 4 | 2 | 3 | 42.9 | 17.7 | 7.6 |
| 160 | 10 | V | 10 | 6 | 6 | 7 | 90.5 | 59.4 | 53.8 |

Phase 2:

| PEN | TREAT | DILUENT | LEVEL | FREQ4 | FREQ5 | FREQ6 | ROL | AVE EW | AVE EO |
| ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: |
| 2 | 6 | C | 50 | 2 | 0 | 0 | 9.5 | 42.9 | 4.1 |
| 22 | 5 | C | 0 | 5 | 6 | 6 | 81.0 | 58.2 | 47.1 |
| 23 | 7 | C | 25 | 0 | 0 | 0 | 0.0 | 44.9 | 0.0 |
| 29 | 6 | C | 50 | 3 | 0 | 0 | 14.3 | 22.0 | 3.1 |
| 34 | 5 | C | 0 | 6 | 7 | 7 | 95.2 | 55.8 | 53.1 |
| 35 | 8 | C | 10 | 1 | 0 | 3 | 19.0 | 38.0 | 7.2 |
| 36 | 8 | C | 10 | 0 | 0 | 6 | 28.6 | 39.9 | 11.4 |
| 38 | 7 | C | 25 | 0 | 0 | 0 | 0.0 | 37.6 | 0.0 |
| 49 | 7 | C | 25 | 0 | 0 | 0 | 0.0 | 0.0 | 0.0 |
| 54 | 6 | C | 50 | 2 | 0 | 0 | 9.5 | 0.0 | 0.0 |
| 60 | 8 | C | 10 | 0 | 1 | 0 | 4.8 | 0.0 | 0.0 |
| 66 | 6 | C | 50 | 3 | 0 | 0 | 14.3 | 48.5 | 6.9 |
| 68 | 5 | C | 0 | 5 | 5 | 6 | 76.2 | 59.3 | 45.2 |
| 72 | 5 | C | 0 | 6 | 6 | 5 | 81.0 | 58.8 | 47.6 |
| 75 | 8 | C | 10 | 0 | 0 | 0 | 0.0 | 19.2 | 0.0 |
| 80 | 7 | C | 25 | 0 | 0 | 0 | 0.0 | 20.0 | 0.0 |
| 82 | 5 | C | 0 | 6 | 5 | 5 | 76.2 | 61.3 | 46.7 |
| 84 | 6 | C | 50 | 0 | 0 | 0 | 0.0 | 50.3 | 0.0 |
| 89 | 8 | C | 10 | 0 | 1 | 0 | 4.8 | 17.4 | 0.8 |
| 90 | 5 | C | 0 | 6 | 7 | 7 | 95.2 | 39.9 | 38.0 |
| 95 | 7 | C | 25 | 0 | 0 | 0 | 0.0 | 20.4 | 0.0 |
| 103 | 6 | C | 50 | 1 | 0 | 0 | 4.8 | 41.3 | 2.0 |
| 107 | 8 | C | 10 | 0 | 0 | 0 | 0.0 | 38.3 | 0.0 |
| 111 | 7 | C | 25 | 0 | 0 | 0 | 0.0 | 44.9 | 0.0 |
| 121 | 5 | C | 0 | 7 | 4 | 7 | 85.7 | 60.7 | 52.0 |
| 129 | 8 | C | 10 | 0 | 0 | 2 | 9.5 | 44.6 | 4.2 |
| 133 | 7 | C | 25 | 0 | 0 | 1 | 4.8 | 41.1 | 2.0 |
| 135 | 7 | C | 25 | 0 | 1 | 0 | 4.8 | 66.2 | 3.2 |
| 139 | 8 | C | 10 | 0 | 0 | 1 | 4.8 | 18.8 | 0.9 |
| 144 | 5 | C | 0 | 7 | 7 | 7 | 100.0 | 36.5 | 36.5 |
|  |  |  |  |  |  |  |  |  | 0 |


| PEN | TREAT | DILUENT | LEVEL | FREQ4 | FREQ5 | FREQ6 | ROL | AVE EW | AVE EO |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 158 | 6 | C | 50 | 2 | 0 | 0 | 9.5 | 18.0 | 1.7 |
| 159 | 6 | C | 50 | 2 | 0 | 0 | 9.5 | 22.5 | 2.1 |
| 7 | 14 | S | 50 | 5 | 6 | 7 | 85.7 | 48.6 | 41.6 |
| 11 | 16 | S | 10 | 5 | 6 | 6 | 81.0 | 41.8 | 33.9 |
| 17 | 13 | S | 0 | 6 | 6 | 5 | 81.0 | 58.1 | 47.1 |
| 21 | 15 | S | 25 | 7 | 6 | 6 | 90.5 | 60.6 | 54.9 |
| 30 | 16 | S | 10 | 4 | 6 | 6 | 76.2 | 61.4 | 46.8 |
| 37 | 13 | S | 0 | 5 | 6 | 6 | 81.0 | 65.1 | 52.7 |
| 39 | 15 | S | 25 | 7 | 6 | 7 | 95.2 | 65.3 | 62.2 |
| 40 | 14 | S | 50 | 6 | 7 | 6 | 90.5 | 57.1 | 51.7 |
| 42 | 15 | S | 25 | 4 | 6 | 6 | 76.2 | 38.4 | 29.3 |
| 47 | 16 | S | 10 | 4 | 5 | 5 | 66.7 | 41.5 | 27.7 |
| 57 | 15 | S | 25 | 5 | 6 | 6 | 81.0 | 21.2 | 17.1 |
| 59 | 16 | S | 10 | 6 | 7 | 6 | 90.5 | 19.9 | 18.0 |
| 61 | 13 | S | 0 | 6 | 6 | 6 | 85.7 | 17.7 | 15.2 |
| 64 | 14 | S | 50 | 6 | 5 | 6 | 81.0 | 19.1 | 15.4 |
| 69 | 14 | S | 50 | 6 | 6 | 5 | 81.0 | 43.0 | 34.8 |
| 79 | 13 | S | 0 | 6 | 6 | 6 | 85.7 | 40.7 | 34.9 |
| 85 | 15 | S | 25 | 6 | 7 | 6 | 90.5 | 60.5 | 54.7 |
| 88 | 14 | S | 50 | 7 | 6 | 5 | 85.7 | 57.2 | 49.0 |
| 94 | 14 | S | 50 | 7 | 7 | 7 | 100.0 | 41.1 | 41.1 |
| 98 | 16 | S | 10 | 7 | 7 | 7 | 100.0 | 61.1 | 61.1 |
| 100 | 16 | S | 10 | 1 | 0 | 5 | 28.6 | 42.4 | 12.1 |
| 106 | 13 | S | 0 | 5 | 6 | 7 | 85.7 | 60.8 | 52.1 |
| 110 | 13 | S | 0 | 7 | 6 | 7 | 95.2 | 56.6 | 53.9 |
| 113 | 15 | S | 25 | 6 | 6 | 6 | 85.7 | 59.5 | 51.0 |
| 137 | 14 | S | 50 | 7 | 7 | 5 | 90.5 | 43.0 | 38.9 |
| 140 | 16 | S | 10 | 6 | 5 | 6 | 81.0 | 33.9 | 27.4 |
| 145 | 15 | S | 25 | 4 | 6 | 5 | 71.4 | 43.1 | 30.8 |
| 147 | 13 | S | 0 | 7 | 7 | 6 | 95.2 | 40.4 | 38.4 |
| 150 | 13 | S | 0 | 5 | 5 | 4 | 66.7 | 42.5 | 28.3 |
| 151 | 15 | S | 25 | 6 | 7 | 7 | 95.2 | 62.1 | 59.1 |
| 154 | 14 | S | 50 | 6 | 6 | 6 | 85.7 | 40.3 | 34.6 |
| 155 | 16 | S | 10 | 6 | 7 | 6 | 90.5 | 56.4 | 51.0 |
| 5 | 18 | SD | 50 | 4 | 0 | 0 | 19.0 | 47.8 | 9.1 |
| 9 | 20 | SD | 10 | 0 | 4 | 6 | 47.6 | 59.4 | 28.3 |
| 10 | 19 | SD | 25 | 1 | 4 | 4 | 42.9 | 37.4 | 16.0 |
| 13 | 17 | SD | 0 | 7 | 6 | 5 | 85.7 | 30.9 | 26.5 |
| 18 | 20 | SD | 10 | 0 | 2 | 6 | 38.1 | 43.2 | 16.5 |
| 19 | 18 | SD | 50 | 1 | 3 | 1 | 23.8 | 65.2 | 15.5 |
| 24 | 17 | SD | 0 | 7 | 6 | 6 | 90.5 | 58.1 | 52.6 |
| 33 | 19 | SD | 25 | 6 | 6 | 6 | 85.7 | 58.0 | 49.7 |
| 46 | 17 | SD | 0 | 4 | 5 | 6 | 71.4 | 41.6 | 29.7 |
| 48 | 20 | SD | 10 | 4 | 6 | 6 | 76.2 | 40.3 | 30.7 |
| 55 | 19 | SD | 25 | 2 | 3 | 2 | 33.3 | 19.1 | 6.4 |
| 56 | 20 | SD | 10 | 0 | 4 | 6 | 47.6 | 22.1 | 10.5 |
| 62 | 18 | SD | 50 | 4 | 1 | 0 | 23.8 | 0.0 | 0.0 |
| 63 | 18 | SD | 50 | 4 | 0 | 0 | 19.0 | 0.0 | 0.0 |
| 67 | 17 | SD | 0 | 6 | 6 | 5 | 81.0 | 58.8 | 47.6 |


| PEN | TREAT | DILUENT | LEVEL | FREQ4 | FREQ5 | FREQ6 | ROL | AVE EW | AVE EO |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 78 | 19 | SD | 25 | 6 | 7 | 6 | 90.5 | 41.4 | 37.5 |
| 86 | 17 | SD | 0 | 5 | 6 | 6 | 81.0 | 21.5 | 17.4 |
| 87 | 20 | SD | 10 | 0 | 0 | 5 | 23.8 | 38.7 | 9.2 |
| 102 | 19 | SD | 25 | 6 | 6 | 5 | 81.0 | 49.6 | 40.1 |
| 105 | 20 | SD | 10 | 0 | 3 | 7 | 47.6 | 39.9 | 19.0 |
| 108 | 18 | SD | 50 | 3 | 0 | 0 | 14.3 | 37.2 | 5.3 |
| 112 | 17 | SD | 0 | 0 | 0 | 0 | 0.0 | 40.9 | 0.0 |
| 118 | 19 | SD | 25 | 1 | 5 | 5 | 52.4 | 60.9 | 31.9 |
| 120 | 18 | SD | 50 | 4 | 0 | 0 | 19.0 | 40.4 | 7.7 |
| 123 | 19 | SD | 25 | 4 | 5 | 5 | 66.7 | 66.3 | 44.2 |
| 124 | 17 | SD | 0 | 6 | 6 | 6 | 85.7 | 61.0 | 52.3 |
| 130 | 20 | SD | 10 | 0 | 5 | 6 | 52.4 | 61.6 | 32.3 |
| 132 | 20 | SD | 10 | 0 | 6 | 6 | 57.1 | 60.4 | 34.5 |
| 134 | 18 | SD | 50 | 4 | 4 | 0 | 38.1 | 18.6 | 7.1 |
| 143 | 18 | SD | 50 | 5 | 0 | 0 | 23.8 | 17.4 | 4.2 |
| 146 | 17 | SD | 0 | 6 | 6 | 5 | 81.0 | 55.3 | 44.8 |
| 157 | 19 | SD | 25 | 5 | 5 | 5 | 71.4 | 35.6 | 25.4 |
| 6 | 2 | SF | 50 | 3 | 1 | 4 | 38.1 | 48.6 | 18.5 |
| 15 | 4 | SF | 10 | 0 | 0 | 5 | 23.8 | 20.4 | 4.9 |
| 16 | 1 | SF | 0 | 7 | 7 | 6 | 95.2 | 55.7 | 53.0 |
| 26 | 4 | SF | 10 | 0 | 2 | 5 | 33.3 | 17.5 | 5.8 |
| 27 | 3 | SF | 25 | 6 | 6 | 6 | 85.7 | 40.5 | 34.7 |
| 28 | 2 | SF | 50 | 3 | 3 | 0 | 28.6 | 37.6 | 10.7 |
| 31 | 3 | SF | 25 | 6 | 6 | 6 | 85.7 | 66.2 | 56.7 |
| 32 | 1 | SF | 0 | 6 | 6 | 7 | 90.5 | 39.8 | 36.0 |
| 43 | 3 | SF | 25 | 5 | 6 | 5 | 76.2 | 41.0 | 31.2 |
| 44 | 3 | SF | 25 | 6 | 7 | 6 | 90.5 | 41.7 | 37.8 |
| 51 | 4 | SF | 10 | 0 | 4 | 7 | 52.4 | 19.8 | 10.4 |
| 52 | 4 | SF | 10 | 0 | 4 | 6 | 47.6 | 19.9 | 9.5 |
| 65 | 1 | SF | 0 | 6 | 7 | 3 | 76.2 | 60.0 | 45.7 |
| 70 | 1 | SF | 0 | 6 | 5 | 6 | 81.0 | 55.3 | 44.8 |
| 71 | 2 | SF | 50 | 6 | 1 | 0 | 33.3 | 63.4 | 21.1 |
| 74 | 2 | SF | 50 | 5 | 2 | 2 | 42.9 | 39.2 | 16.8 |
| 81 | 3 | SF | 25 | 6 | 5 | 6 | 81.0 | 59.2 | 47.9 |
| 83 | 1 | SF | 0 | 7 | 6 | 7 | 95.2 | 67.6 | 64.3 |
| 92 | 4 | SF | 10 | 0 | 4 | 6 | 47.6 | 31.7 | 15.1 |
| 93 | 3 | SF | 25 | 6 | 6 | 6 | 85.7 | 39.6 | 33.9 |
| 97 | 2 | SF | 50 | 5 | 0 | 0 | 23.8 | 38.9 | 9.3 |
| 101 | 1 | SF | 0 | 7 | 6 | 7 | 95.2 | 56.4 | 53.8 |
| 109 | 4 | SF | 10 | 5 | 6 | 7 | 85.7 | 57.3 | 49.1 |
| 115 | 2 | SF | 50 | 5 | 2 | 0 | 33.3 | 69.1 | 23.0 |
| 122 | 2 | SF | 50 | 4 | 2 | 2 | 38.1 | 57.4 | 21.9 |
| 128 | 1 | SF | 0 | 7 | 6 | 6 | 90.5 | 55.2 | 49.9 |
| 136 | 3 | SF | 25 | 5 | 5 | 6 | 76.2 | 66.5 | 50.7 |
| 141 | 2 | SF | 50 | 5 | 4 | 4 | 61.9 | 17.9 | 11.1 |
| 149 | 3 | SF | 25 | 6 | 7 | 7 | 95.2 | 54.6 | 52.0 |
| 152 | 4 | SF | 10 | 5 | 6 | 6 | 81.0 | 60.8 | 49.2 |
| 153 | 1 | SF | 0 | 7 | 6 | 7 | 95.2 | 43.0 | 41.0 |
| 156 | 4 | SF | 10 | 0 | 4 | 7 | 52.4 | 39.8 | 20.8 |


| PEN | TREAT | DILUENT | LEVEL | FREQ4 | FREQ5 | FREQ6 | ROL | AVE EW | AVE EO |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 1 | 9 | V | 0 | 6 | 6 | 6 | 85.7 | 58.2 | 49.9 |
| 3 | 11 | V | 25 | 4 | 5 | 5 | 66.7 | 59.3 | 39.5 |
| 4 | 10 | V | 50 | 4 | 0 | 0 | 19.0 | 38.3 | 7.3 |
| 8 | 10 | V | 50 | 4 | 0 | 0 | 19.0 | 44.0 | 8.4 |
| 12 | 12 | V | 10 | 0 | 3 | 5 | 38.1 | 39.3 | 15.0 |
| 14 | 11 | V | 25 | 4 | 3 | 5 | 57.1 | 39.3 | 22.5 |
| 20 | 12 | V | 10 | 0 | 0 | 4 | 19.0 | 41.3 | 7.9 |
| 25 | 9 | V | 0 | 3 | 3 | 1 | 33.3 | 34.0 | 11.3 |
| 41 | 10 | V | 50 | 5 | 0 | 0 | 23.8 | 16.0 | 3.8 |
| 45 | 11 | V | 25 | 5 | 5 | 5 | 71.4 | 23.7 | 16.9 |
| 50 | 9 | V | 0 | 6 | 6 | 5 | 81.0 | 19.6 | 15.9 |
| 53 | 10 | $V$ | 50 | 4 | 1 | 0 | 23.8 | 16.9 | 4.0 |
| 58 | 11 | V | 25 | 6 | 4 | 4 | 66.7 | 44.5 | 29.7 |
| 73 | 12 | V | 10 | 1 | 1 | 5 | 33.3 | 19.5 | 6.5 |
| 76 | 9 | V | 0 | 7 | 6 | 6 | 90.5 | 37.3 | 33.8 |
| 77 | 12 | V | 10 | 0 | 2 | 7 | 42.9 | 17.6 | 7.6 |
| 91 | 12 | V | 10 | 0 | 1 | 6 | 33.3 | 42.3 | 14.1 |
| 96 | 10 | V | 50 | 4 | 0 | 0 | 19.0 | 20.8 | 4.0 |
| 99 | 9 | V | 0 | 7 | 4 | 2 | 61.9 | 54.7 | 33.9 |
| 104 | 9 | V | 0 | 6 | 5 | 4 | 71.4 | 61.5 | 44.0 |
| 114 | 11 | V | 25 | 5 | 6 | 6 | 81.0 | 56.8 | 46.0 |
| 116 | 11 | V | 25 | 0 | 0 | 0 | 0.0 | 42.6 | 0.0 |
| 117 | 10 | V | 50 | 4 | 1 | 0 | 23.8 | 42.2 | 10.0 |
| 119 | 12 | V | 10 | 0 | 2 | 6 | 38.1 | 46.1 | 17.6 |
| 125 | 9 | V | 0 | 6 | 6 | 7 | 90.5 | 55.2 | 49.9 |
| 126 | 10 | V | 50 | 4 | 0 | 2 | 28.6 | 17.6 | 5.0 |
| 127 | 11 | V | 25 | 4 | 3 | 5 | 57.1 | 53.6 | 30.6 |
| 131 | 11 | V | 25 | 4 | 5 | 5 | 66.7 | 66.9 | 44.6 |
| 138 | 12 | V | 10 | 0 | 4 | 6 | 47.6 | 16.6 | 7.9 |
| 142 | 9 | V | 0 | 6 | 6 | 6 | 85.7 | 38.7 | 33.1 |
| 148 | 12 | V | 10 | 0 | 5 | 6 | 52.4 | 57.6 | 30.2 |
| 160 | 10 | V | 50 | 3 | 0 | 0 | 14.3 | 20.9 | 3.0 |

Phase 3:

| PEN | TREAT | DILUENT | LEVEL | FREQ7 | FREQ8 | FREQ9 | ROL | AVE EW | AVE EO |
| ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: |
| 7 | 14 | C | 10 | 5 | 6 | 4 | 71.4 | 58.1 | 41.5 |
| 11 | 16 | C | 50 | 4 | 0 | 0 | 19.0 | 20.2 | 3.8 |
| 17 | 13 | C | 0 | 6 | 7 | 5 | 85.7 | 58.7 | 50.3 |
| 21 | 15 | C | 25 | 5 | 0 | 0 | 23.8 | 18.1 | 4.3 |
| 30 | 16 | C | 50 | 4 | 0 | 0 | 19.0 | 19.2 | 3.6 |
| 37 | 13 | C | 0 | 6 | 6 | 6 | 85.7 | 59.6 | 51.1 |
| 39 | 15 | C | 25 | 4 | 0 | 0 | 19.0 | 20.7 | 3.9 |
| 40 | 14 | C | 10 | 5 | 5 | 6 | 76.2 | 55.1 | 42.0 |
| 42 | 15 | C | 25 | 3 | 0 | 0 | 14.3 | 20.6 | 2.9 |
| 47 | 16 | C | 50 | 3 | 0 | 0 | 14.3 | 22.4 | 3.2 |
| 57 | 15 | C | 25 | 5 | 0 | 0 | 23.8 | 19.3 | 4.6 |
| 59 | 16 | C | 50 | 5 | 0 | 0 | 23.8 | 19.8 | 4.7 |
| 61 | 13 | C | 0 | 7 | 6 | 6 | 90.5 | 50.9 | 46.0 |


| PEN | TREAT | DILUENT | LEVEL | FREQ | FREQ8 | FREQ9 | ROL | AVE EW | AVE EO |
| ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: |
| 64 | 14 | C | 10 | 6 | 6 | 4 | 76.2 | 59.7 | 45.5 |
| 69 | 14 | C | 10 | 5 | 4 | 4 | 61.9 | 57.7 | 35.7 |
| 79 | 13 | C | 0 | 5 | 4 | 5 | 66.7 | 65.0 | 43.3 |
| 85 | 15 | C | 25 | 5 | 0 | 0 | 23.8 | 20.5 | 4.9 |
| 88 | 14 | C | 10 | 5 | 4 | 0 | 42.9 | 36.9 | 15.8 |
| 94 | 14 | C | 10 | 5 | 5 | 5 | 71.4 | 57.6 | 41.2 |
| 98 | 16 | C | 50 | 6 | 0 | 0 | 28.6 | 17.6 | 5.0 |
| 100 | 16 | C | 50 | 5 | 0 | 0 | 23.8 | 18.8 | 4.5 |
| 106 | 13 | C | 0 | 6 | 6 | 6 | 85.7 | 61.3 | 52.6 |
| 110 | 13 | C | 0 | 7 | 7 | 7 | 100.0 | 54.5 | 54.5 |
| 113 | 15 | C | 25 | 5 | 0 | 1 | 28.6 | 39.5 | 11.3 |
| 137 | 14 | C | 10 | 7 | 4 | 6 | 81.0 | 67.7 | 54.8 |
| 140 | 16 | C | 50 | 5 | 0 | 0 | 23.8 | 19.3 | 4.6 |
| 145 | 15 | C | 25 | 3 | 0 | 0 | 14.3 | 21.9 | 3.1 |
| 147 | 13 | C | 0 | 7 | 5 | 6 | 85.7 | 64.8 | 55.5 |
| 150 | 13 | C | 0 | 6 | 5 | 7 | 85.7 | 64.9 | 55.6 |
| 151 | 15 | C | 25 | 4 | 0 | 0 | 19.0 | 19.0 | 3.6 |
| 154 | 14 | C | 10 | 6 | 5 | 2 | 61.9 | 61.2 | 37.9 |
| 155 | 16 | C | 50 | 3 | 0 | 0 | 14.3 | 19.2 | 2.7 |
| 2 | 6 | S | 10 | 0 | 0 | 4 | 19.0 | 15.5 | 3.0 |
| 22 | 5 | S | 0 | 6 | 6 | 6 | 85.7 | 54.3 | 46.6 |
| 23 | 7 | S | 25 | 0 | 0 | 0 | 0.0 | 0.0 | 0.0 |
| 29 | 6 | S | 10 | 1 | 2 | 7 | 47.6 | 17.2 | 8.2 |
| 34 | 5 | S | 0 | 6 | 7 | 7 | 95.2 | 55.1 | 52.4 |
| 35 | 8 | S | 50 | 6 | 7 | 6 | 90.5 | 57.6 | 52.1 |
| 36 | 8 | S | 50 | 5 | 6 | 6 | 81.0 | 58.5 | 47.4 |
| 38 | 7 | S | 25 | 0 | 0 | 6 | 28.6 | 19.1 | 5.5 |
| 49 | 7 | S | 25 | 0 | 0 | 5 | 23.8 | 17.2 | 4.1 |
| 54 | 6 | S | 10 | 0 | 0 | 4 | 19.0 | 17.8 | 3.4 |
| 60 | 8 | S | 50 | 0 | 0 | 0 | 0.0 | 0.0 | 0.0 |
| 66 | 6 | S | 10 | 0 | 0 | 6 | 28.6 | 19.1 | 5.5 |
| 68 | 5 | S | 0 | 5 | 5 | 6 | 76.2 | 64.9 | 49.4 |
| 72 | 5 | S | 0 | 6 | 6 | 5 | 81.0 | 59.4 | 48.1 |
| 75 | 8 | S | 50 | 0 | 0 | 0 | 0.0 | 0.0 | 0.0 |
| 80 | 7 | S | 25 | 0 | 0 | 6 | 28.6 | 18.9 | 5.4 |
| 82 | 5 | S | 0 | 6 | 5 | 7 | 85.7 | 74.7 | 64.0 |
| 84 | 6 | S | 10 | 0 | 0 | 1 | 4.8 | 0.0 | 0.0 |
| 89 | 8 | S | 50 | 0 | 0 | 0 | 0.0 | 0.0 | 0.0 |
| 90 | 5 | S | 0 | 5 | 7 | 6 | 85.7 | 58.0 | 49.7 |
| 95 | 7 | S | 25 | 1 | 0 | 0 | 4.8 | 19.6 | 0.9 |
| 103 | 6 | S | 10 | 0 | 0 | 2 | 9.5 | 0.0 | 0.0 |
| 107 | 8 | S | 50 | 3 | 6 | 6 | 71.4 | 60.3 | 43.0 |
| 111 | 7 | S | 25 | 0 | 2 | 7 | 42.9 | 18.9 | 8.1 |
| 121 | 5 | S | 0 | 6 | 5 | 6 | 81.0 | 67.1 | 54.3 |
| 129 | 8 | S | 50 | 4 | 6 | 6 | 76.2 | 57.6 | 43.9 |
| 133 | 7 | S | 25 | 0 | 0 | 2 | 9.5 | 0.0 | 0.0 |
| 135 | 7 | S | 25 | 0 | 0 | 0 | 0.0 | 0.0 | 0.0 |
| 139 | 8 | S | 50 | 5 | 6 | 5 | 76.2 | 60.2 | 45.9 |
|  | 5 | S | 0 | 7 | 3 | 0 | 47.6 | 31.3 | 14.9 |
| 10 |  |  |  |  |  |  |  |  |  |


| PEN | TREAT | DILUENT | LEVEL | FREQ7 | FREQ8 | FREQ9 | ROL | AVE EW | AVE EO |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 158 | 6 | S | 10 | 0 | 1 | 6 | 33.3 | 18.9 | 6.3 |
| 159 | 6 | S | 10 | 0 | 1 | 7 | 38.1 | 20.4 | 7.8 |
| 6 | 2 | SD | 10 | 3 | 7 | 5 | 71.4 | 62.8 | 44.9 |
| 15 | 4 | SD | 50 | 4 | 0 | 0 | 19.0 | 20.4 | 3.9 |
| 16 | 1 | SD | 0 | 7 | 7 | 7 | 100.0 | 57.6 | 57.6 |
| 26 | 4 | SD | 50 | 3 | 0 | 0 | 14.3 | 18.0 | 2.6 |
| 27 | 3 | SD | 25 | 6 | 5 | 4 | 71.4 | 59.7 | 42.6 |
| 28 | 2 | SD | 10 | 0 | 3 | 6 | 42.9 | 16.6 | 7.1 |
| 31 | 3 | SD | 25 | 6 | 6 | 6 | 85.7 | 59.2 | 50.7 |
| 32 | 1 | SD | 0 | 6 | 7 | 6 | 90.5 | 62.6 | 56.6 |
| 43 | 3 | SD | 25 | 6 | 5 | 1 | 57.1 | 66.2 | 37.8 |
| 44 | 3 | SD | 25 | 6 | 5 | 4 | 71.4 | 61.9 | 44.2 |
| 51 | 4 | SD | 50 | 3 | 0 | 0 | 14.3 | 18.2 | 2.6 |
| 52 | 4 | SD | 50 | 4 | 0 | 0 | 19.0 | 20.6 | 3.9 |
| 65 | 1 | SD | 0 | 0 | 3 | 5 | 38.1 | 38.4 | 14.6 |
| 70 | 1 | SD | 0 | 6 | 4 | 0 | 47.6 | 35.5 | 16.9 |
| 71 | 2 | SD | 10 | 4 | 5 | 6 | 71.4 | 67.6 | 48.3 |
| 74 | 2 | SD | 10 | 2 | 5 | 4 | 52.4 | 61.3 | 32.1 |
| 81 | 3 | SD | 25 | 5 | 5 | 6 | 76.2 | 58.5 | 44.5 |
| 83 | 1 | SD | 0 | 7 | 5 | 6 | 85.7 | 66.3 | 56.8 |
| 92 | 4 | SD | 50 | 4 | 1 | 0 | 23.8 | 43.6 | 10.4 |
| 93 | 3 | SD | 25 | 5 | 5 | 5 | 71.4 | 58.2 | 41.6 |
| 97 | 2 | SD | 10 | 0 | 5 | 7 | 57.1 | 37.7 | 21.6 |
| 101 | 1 | SD | 0 | 6 | 6 | 5 | 81.0 | 63.7 | 51.6 |
| 109 | 4 | SD | 50 | 4 | 1 | 0 | 23.8 | 36.4 | 8.7 |
| 115 | 2 | SD | 10 | 1 | 5 | 4 | 47.6 | 45.7 | 21.8 |
| 122 | 2 | SD | 10 | 3 | 5 | 5 | 61.9 | 56.8 | 35.2 |
| 128 | 1 | SD | 0 | 5 | 4 | 5 | 66.7 | 50.5 | 33.6 |
| 136 | 3 | SD | 25 | 6 | 1 | 3 | 47.6 | 60.6 | 28.8 |
| 141 | 2 | SD | 10 | 4 | 6 | 6 | 76.2 | 58.4 | 44.5 |
| 149 | 3 | SD | 25 | 7 | 7 | 1 | 71.4 | 54.2 | 38.7 |
| 152 | 4 | SD | 50 | 4 | 0 | 0 | 19.0 | 19.8 | 3.8 |
| 153 | 1 | SD | 0 | 6 | 6 | 7 | 90.5 | 69.4 | 62.7 |
| 156 | 4 | SD | 50 | 5 | 1 | 0 | 28.6 | 35.2 | 10.0 |
| 5 | 18 | SF | 10 | 0 | 0 | 2 | 9.5 | 0.0 | 0.0 |
| 9 | 20 | SF | 50 |  | 0 | 0 | 19.0 | 21.8 | 4.2 |
| 10 | 19 | SF | 25 | 2 | 3 | 5 | 47.6 | 60.3 | 28.7 |
| 13 | 17 | SF | 0 | 7 | 5 | 7 | 90.5 | 63.1 | 57.1 |
| 18 | 20 | SF | 50 | 5 | 0 | 0 | 23.8 | 19.7 | 4.7 |
| 19 | 18 | SF | 10 | 0 | 0 | 0 | 0.0 | 0.0 | 0.0 |
| 24 | 17 | SF | 0 | 6 | 6 | 5 | 81.0 | 58.3 | 47.2 |
| 33 | 19 | SF | 25 | 6 | 7 | 3 | 76.2 | 61.2 | 46.7 |
| 46 | 17 | SF | 0 | 5 | 5 | 6 | 76.2 | 63.7 | 48.6 |
| 48 | 20 | SF | 50 | 5 | 1 | 0 | 28.6 | 37.3 | 10.7 |
| 55 | 19 | SF | 25 | 4 | 2 | 3 | 42.9 | 45.9 | 19.7 |
| 56 | 20 | SF | 50 | 5 | 0 | 0 | 23.8 | 19.9 | 4.7 |
| 62 | 18 | SF | 10 | 0 | 4 | 6 | 47.6 | 37.0 | 17.6 |
| 63 | 18 | SF | 10 | 0 | 0 | 4 | 19.0 | 18.9 | 3.6 |
| 67 | 17 | SF | 0 | 6 | 5 | 6 | 81.0 | 59.1 | 47.9 |


| PEN | TREAT | DILUENT | LEVEL | FREQ7 | FREQ8 | FREQ9 | ROL | AVE EW | AVE EO |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 78 | 19 | SF | 25 | 5 | 6 | 6 | 81.0 | 56.4 | 45.6 |
| 86 | 17 | SF | 0 | 5 | 5 | 5 | 71.4 | 64.1 | 45.8 |
| 87 | 20 | SF | 50 | 2 | 1 | 0 | 14.3 | 37.8 | 5.4 |
| 102 | 19 | SF | 25 | 5 | 5 | 3 | 61.9 | 62.6 | 38.7 |
| 105 | 20 | SF | 50 | 3 | 0 | 0 | 14.3 | 19.6 | 2.8 |
| 108 | 18 | SF | 10 | 1 | 5 | 5 | 52.4 | 44.3 | 23.2 |
| 112 | 17 | SF | 0 | 0 | 0 | 0 | 0.0 | 0.0 | 0.0 |
| 118 | 19 | SF | 25 | 4 | 4 | 6 | 66.7 | 59.3 | 39.5 |
| 120 | 18 | SF | 10 | 0 | 6 | 6 | 57.1 | 38.6 | 22.0 |
| 123 | 19 | SF | 25 | 5 | 1 | 4 | 47.6 | 57.6 | 27.4 |
| 124 | 17 | SF | 0 | 7 | 5 | 6 | 85.7 | 64.4 | 55.2 |
| 130 | 20 | SF | 50 | 5 | 0 | 0 | 23.8 | 21.2 | 5.0 |
| 132 | 20 | SF | 50 | 4 | 1 | 0 | 23.8 | 40.7 | 9.7 |
| 134 | 18 | SF | 10 | 0 | 0 | 5 | 23.8 | 17.6 | 4.2 |
| 143 | 18 | SF | 10 | 0 | 2 | 6 | 38.1 | 19.3 | 7.4 |
| 146 | 17 | SF | 0 | 6 | 5 | 6 | 81.0 | 67.0 | 54.2 |
| 157 | 19 | SF | 25 | 5 | 6 | 3 | 66.7 | 60.1 | 40.1 |
| 1 | 9 | V | 0 | 5 | 6 | 6 | 81.0 | 61.8 | 50.0 |
| 3 | 11 | V | 25 | 5 | 3 | 6 | 66.7 | 54.2 | 36.1 |
| 4 | 10 | V | 10 | 0 | 1 | 6 | 33.3 | 20.5 | 6.8 |
| 8 | 10 | V | 10 | 0 | 3 | 6 | 42.9 | 37.7 | 16.1 |
| 12 | 12 | V | 50 | 5 | 1 | 0 | 28.6 | 34.9 | 10.0 |
| 14 | 11 | V | 25 | 5 | 3 | 2 | 47.6 | 52.5 | 25.0 |
| 20 | 12 | V | 50 | 3 | 0 | 0 | 14.3 | 21.3 | 3.0 |
| 25 | 9 | V | 0 | 3 | 2 | 3 | 38.1 | 70.0 | 26.7 |
| 41 | 10 | V | 10 | 3 | 5 | 4 | 57.1 | 56.8 | 32.4 |
| 45 | 11 | V | 25 | 5 | 6 | 0 | 52.4 | 44.7 | 23.4 |
| 50 | 9 | V | 0 | 7 | 6 | 6 | 90.5 | 57.2 | 51.7 |
| 53 | 10 | V | 10 | 1 | 5 | 5 | 52.4 | 38.1 | 19.9 |
| 58 | 11 | V | 25 | 5 | 5 | 3 | 61.9 | 71.4 | 44.2 |
| 73 | 12 | V | 50 | 5 | 0 | 1 | 28.6 | 42.7 | 12.2 |
| 76 | 9 | V | 0 | 7 | 7 | 7 | 100.0 | 58.3 | 58.3 |
| 77 | 12 | V | 50 | 3 | 0 | 0 | 14.3 | 18.3 | 2.6 |
| 91 | 12 | V | 50 | 3 | 0 | 0 | 14.3 | 18.9 | 2.7 |
| 96 | 10 | V | 10 | 0 | 0 | 0 | 0.0 | 0.0 | 0.0 |
| 99 | 9 | V | 0 | 4 | 6 | 7 | 81.0 | 63.3 | 51.2 |
| 104 | 9 | V | 0 | 6 | 5 | 7 | 85.7 | 55.6 | 47.6 |
| 114 | 11 | V | 25 | 6 | 3 | 1 | 47.6 | 35.7 | 17.0 |
| 116 | 11 | V | 25 | 0 | 0 | 0 | 0.0 | 0.0 | 0.0 |
| 117 | 10 | V | 10 | 0 | 2 | 6 | 38.1 | 19.4 | 7.4 |
| 119 | 12 | V | 50 | 4 | 0 | 0 | 19.0 | 19.7 | 3.8 |
| 125 | 9 | V | 0 | 7 | 7 | 7 | 100.0 | 59.9 | 59.9 |
| 126 | 10 | V | 10 | 3 | 5 | 5 | 61.9 | 57.6 | 35.6 |
| 127 | 11 | V | 25 | 4 | 5 | 3 | 57.1 | 58.8 | 33.6 |
| 131 | 11 | V | 25 | 4 | 5 | 4 | 61.9 | 66.1 | 40.9 |
| 138 | 12 | V | 50 | 5 | 0 | 0 | 23.8 | 19.1 | 4.6 |
| 142 | 9 | V | 0 | 6 | 5 | 6 | 81.0 | 56.9 | 46.1 |
| 148 | 12 | V | 50 | 4 | 1 | 0 | 23.8 | 39.7 | 9.5 |
| 160 | 10 | V | 10 | 0 |  | 2 | 14.3 | 19.5 | 2.8 |

Appendix D: Trial 2 feed intake and body Weight data








[^0]:    ${ }^{1}$ Basal feed supplied by Nutrex (KZN) (Pty) LTD. P.O. Box 179, Umlaas Road, 3730.

[^1]:    ${ }^{1}$ Equation of the form $y=a x+c$

[^2]:    ${ }^{2}$ Polystyrene supplier: Isolite Polystyrene Manufacturers, Pinetown, KwaZulu-Natal

