MODELLING AND APPLICATION OF RESPONSE SURFACE METHODOLOGY FOR OPTIMIZATION OF WEIGHT GAIN OF EIGHT WEEKS OLD KENBRO SERVED WITH PUMPKIN (*Cucurbita pepo L*) SEEDS EXTRACT

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A Thesis Submitted to the Graduate School in Partial Fulfilment of the Requirements for the Award of the Degree of Masters of Science in Applied Statistics of Chuka University.

CHUKA UNIVERSITY

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DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and it has not been presented for an award of a Diploma or conferment of a Degree in any other University.

Signature.. Date...12/09/2019...

Charles Mugendi Kinyua
SM18/29042/16

Recommendation

This thesis has been submitted with our approval as the University Supervisors.

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DEDICATION

I dedicate this work to my dad Silveste Kinyua Ireri, my brother Kelvin Fundi Kinyua and my entire family. Your love, encouragement and financial support have enabled me to complete this Thesis. May Almighty God grant you abundant blessings and peace of mind.

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ABSTRACT

Extensive use of synthetic growth promoting antibiotics have become a threat to public health. Therefore, there is a need to search for bioactive phytochemicals that have positive effects on immune, growth and appetite status of the chicken. Such bioactive compounds can be obtained from plants. Pumpkin seeds have been found to be a valuable source of protein and bioactive compounds. Mathematical models have been used to study the effect of pumpkin extracts on the weight gain of chicken. However, most of these studies have focused on the levels of the application of pumpkin extract, rather than on optimising the levels of application on the weight gain of the chicken. Optimization is important since it provides information on the amount of pumpkin seed extracts that should be fed to the chicken without wastage. This study was conducted to evaluate the weight gained by the Kenbro chicken served with pumpkin (*Cucurbita pepo L*) seeds extract and examined the possible combination of the number of weeks and quantity of pumpkin seed extract that can result in maximum weight gain of the Kenbro chicken. The experiment was conducted using Random Complete Block Design at the poultry demonstration unit of Chuka University. Sixty- day old Kenbro chicks were randomly distributed to five treatments which contained 0, 50, 100, 150 and 200 ml of pumpkin seed extracts per litre of water. Each treatment was replicated three times with four chicken per replicate. The birds were fed with the same diets of finisher and starter feeds. The chickens were provided with enough feeds and water *ad libitum* in the morning at 8 am. The birds were weighed at the beginning of the experiment and thereafter at an interval of three days for eight weeks. Data was analysed using R statistical software. A Response surface model was fitted to the data and subjected to contour plots to characterize the nature of its turning point and to capture the combination of the number of weeks and quantity of pumpkin seed extract that brings maximum weight gain of the Kenbro chicken. The results showed that the average body weight gained was significant ($p < 0.05$). The birds served with 200 ml pumpkin seed extract for 4 weeks had the highest weight gain. The fitted Response Surface Model indicated that the number of weeks and quantity of pumpkin seed extract together with their interaction significantly ($p < 0.05$) determined the weight gain of Kenbro chickens. The study found that the quadratic model fitted using the data had an adjusted R-Squared value of 0.78. The optimal weight gain of 0.23 kg was achieved when the number of weeks was 3.18 with 192.40 ml of pumpkin seed extract. The number of weeks, pumpkin seed extract and their interaction play a key role in obtaining maximum weight gain of the Kenbro chicken. These factors should be put into consideration when developing a feeding system for Kenbro chickens. The study also guides the farmers on the optimization of Kenbro chicken production without incurring an extra cost in the input.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
BBD	Box Behnken Design
CCD	Central Composite Design
CRD	Complete Randomized Design
CRD	Chronic Respiratory Disease
DF	Degrees of Freedom
MOLD	Ministry of Livestock Domestic
NACOSTI	National Commission for Science, Technology and Innovation
NCD	Newcastle Disease
RCBD	Random Complete Block Design
RSM	Response Surface Methodology

CHAPTER ONE
INTRODUCTION

1.1 Background to the Study

Increasing productivity in poultry through improved chicken growth rate, feed utilization as well as the general well-being of the chicken has been in the frontline of research. For more than five decades, the use of growth hormones and growth-promoting antibiotics has been preferred by many livestock farmers (Ronquillo & Hernandez, 2017). The extensive and prolonged use of these synthetic growth-promoting antibiotics, included in subtherapeutic, in poultry feed has become a threat to public health (Laudadio *et al*, 2012). These antibiotics may increase the incidence of antibiotic resistance in both poultry and consumers (Apata, 2009). Consequently, there is an increased interest in the use of eco-friendly materials such as phytoproducts from plants tissues and organs based extracts in the livestock production. These compounds contain many bioactive phytochemicals (Edeoga, 2005; Krishnaiah *et al*., 2009) which have been found to have valuable effects on immune, appetite, and the growth status of the animal (Barouch *et al*., 2013). Pumpkin is one of the plants that have bioactive compound, for example, cucurbitacins which have antimicrobial and anti-inflammatory activity. Moreover, studies have also proved that pumpkin seeds are a valuable source of fats and proteins (Nawirska-Olszańska *et al.*, 2013).

Mathematical methods have been used to establish the relationships between pumpkin extracts and the weight gain of chicken. However, most of these studies have focused on the effect of the application of pumpkin extract levels on the weight gain of chicken. For instance, Nworgu *et al.,* (2007), using a completely randomized design to assess the weight gain and the economic importance of broiler chickens served with fluted pumpkin leaf extract, found that body weight gain for birds served with different levels of fluted pumpkin leaf extract increased significantly. However, due to the limitation of this design, the study only established the best treatments among the treatment range applied. The studies did not attempt to optimize the level of fluted pumpkin leaf extract that can lead to optimum broiler chickens weight gain. Another study on the effect of two dietary fats, linseed oil and lard, supplemented with pumpkin seeds showed that adding pumpkin seeds to the linseed oil diet improved the oxidative stability of the muscle (Meineri *et al*., 2018). However, this study did not also attempt to optimize levels of pumpkin extract using the treatment range applied.

Extract from other plants have also been used to investigate the performance characteristics and physiological response of broiler chicken to oral supplementation. For example, using a randomized complete block design, Telfairia occidentalis leaf extract were applied at the finisher stage and there was a significant effect on the growth rate of broiler chicken (Alabi *et al*., 2017). Telfairia occidentalis leaf extract was found to affect average body weight gain, average daily feed intake, and feed conversion ratio (Alabi *et al*., 2017). However, no optimization was done in this study and thus the levels of the Telfairia occidentalis leaf extract that would yield the best chicken performance parameters remained unknown.

Thus various experimental designs and statistical analyses clearly show a positive relationship between the pumpkin extracts and chicken growth rate. However, most of these studies only established a positive relationship between extracted bioactive compounds and chicken performance (Nworgu *et al.*, 2007; Alabi *et al*., 2017; Meineri *et al*., 2018). Therefore, to maximize on the chicken performance and productivity using locally available plant materials, there is a need to optimize the application of such plant bioactive compounds. Consequently, there is limited information on optimum levels of application of pumpkin extracts that would significantly affect the weight gain of chicken and lead to efficient use of available resources. This is partly because most of the studies only use models that are not amenable with optimisation process. These models include generalized linear models that can only establish the effect of treatment ranges applied in a given study (Alabi *et al*., 2017; Meineri *et al*., 2018). Hence, there is a need to come up with a modelling technique and data analysis procedures that can optimize the effect of the pumpkin seed extract on the weight gain in chicken. Such a model should indicate the optimal amount of the pumpkin seed extract that would optimize the chicken weight gain over a range of independent variable(s) levels. One of such technique is the response surface methodology (RSM).

Response surface methodology is useful for improving and optimizing the response of interest influenced by several variables, with reduced experimental runs and increased accuracy (Mayers *et al.,* 2004; Tan *et al.,* 2009). Response Surface Methodology is an effective method to optimize process conditions, and it can determine the influence of various factors and their interactions on a response variable (Mayers *et al.,* 2004). It is a widely used mathematical and statistical method for modelling and analysing a process in which the response of interest is affected by various variables (Refinery & Braimah, 2016). Generally, the objective of response surface methodology is to optimize the response (Montgomery, 2005). It can be used to fit a complete quadratic polynomial model through a central composite design, presenting a more excellent experiment design and result expression (Wang, 2016). It also allows for estimation of the effects of multiple factors and their interactions on one or more response variables as well as quadratic effect as opposed to the linear regression model (Muriithi *et al.,* 2017; Aydar, 2018).

By applying response surface methodology in the optimization process only a short period is required to test all of the variables pertaining to the response, making the experimentation more efficient (Whitcomb & Anderson, 2004). Appropriate experimental design should be used to collect the data to develop a more efficient model. Parameter estimation can identify the variable that mostly affects the model, which helps the study to focus on those particular variables. Response surface methodology has been applied in many fields such as in the process industries, clinical studies and biological sciences. However, it has not been widely used in the agricultural sciences (Muriithi *et al.,* 2017). It has been applied in various studies, for example, optimization of calcium determination by flame atomic absorption Spectrometry, optimization of multiple responses of watermelon to organic manure, and optimization of potato tuber yield (Kokya & Farhadi, 2009; Muriithi *et al*., 2017; Aydar, 2018).

Application of response surface methodology models to optimize outputs in poultry production is important since may enable the farmers to reduce production cost. Several studies have used statistical models to investigate the effects of various variables on poultry production. For instance, Plackett-Burman Design, Central

Composite Design, Canonical Analysis, and Steepest Rising Ridge have been used to optimize chicken feathers (Embaby *et al*., 2015). The results showed that Plackett-Burman Design addressed three key determinants out of eight tested factors imposing significant influence ($p \leq 0.006$) on soluble protein production (Embaby *et al*., 2015). Simple linear programming has been used to optimize poultry farm products (Oladejo *et al*., 2019). The results showed total reduction cost by linear programming model for the least cost starter ration in layer starter feed formulations compared with the cost of the existing method in the farm (Oladejo *et al*., 2019). The studies by Embaby *et al*, (2015) and Oladejo *et al*, (2019) show that several aspects of poultry production can be optimized through mathematical models. Therefore this study opted to use response surface methodology to optimize the weight gain of the chicken. This study employed response surface methodology to optimize the weight gain of the Kenbro chicken served with pumpkin seed extract. Central Composite Design was used to fit a complete quadratic polynomial model. In this study, the response (weight gain) was expressed as a function of factors (pumpkin seed extract and time), which gave the idea of the nature of the response surface under consideration.

1.2 Statement of the Problem

Extensive and prolonged use of synthetic growth - promoting antibiotics have become a threat to public health. Therefore, there is a need to use alternative bioactive natural phytochemicals that have positive effects on immune, appetite and growth status of the chicken. This will necessitate evaluation and optimization of locally available natural plant products, such as pumpkin extracts for their nutritional and anthelmintic potential for nutritional and effective gastrointestinal parasites control. Consequently, leading to sustainable and environmentally friendly poultry production. Though many studies have been carried out using pumpkin extracts, these studies focus on getting the effects of amount of pumpkin extracts on the response variable within the treatment ranges used. Thus, studies that try to optimize the effect of pumpkin extracts on growth of poultry are limited. This can be attributed to experimental designs that are generally used in agricultural experiment, which are inappropriate in optimisation of response variable. This requires application of appropriate experimental designs that have potential to optimise a response variable over a range of independent variables. Response surface methodology is one such statistical model that can improve and optimize the response of interest influenced by several independent variables.

1.3 Objectives of the Study

1.3.1 General Objective

The general objective of this study was modelling and application of response surface methodology for optimization of weight gain of eight-week-old Kenbro chicken served with pumpkin (*Cucurbita pepo L*) seeds extract.

1.3.2 Specific Objective

The following specific objectives guided this study.

i. To determine the effect of pumpkin seed extract and the number of weeks on the weight gain of Kenbro chicken.
ii. To fit appropriate second-order polynomial model using the data obtained.
iii. To determine the combination of pumpkin seed extract and the number of weeks that would optimize the weight gain of Kenbro chicken.

1.4 Hypothesis and Research Question

1.4.1 Hypothesis

$H0_1$: There is no statistical significant effect of pumpkin seed extract and the number of weeks on the weight gain of Kenbro chicken.

$H0_2$: There is no significant combination of pumpkin seed extract and the number of weeks that would optimize the weight gain of Kenbro chicken.

1.4.2 Research Question

Was the appropriate second-order polynomial model fit for the data collected?

1.5 Significance of the Study

The findings of this study would guide the farmers on the optimization of Kenbro chicken production without incurring an extra cost in the input. The research also highlights the factors that should be considered when making feed and rearing Kenbro chicken. Researchers interested in optimization modelling also benefit from this study since it can be concluded that response surface methodology is useful when the main interest of research is to optimize the response. The study also provides an insight into the policy makers on the choices of policies as well as providing guidelines on the implementation of such policies that are concerned with the improvement of poultry farming. Finally, the findings of this study will add to the documented evidence on the maximization of chicken production in Kenya. Such evidence will be available for use by donors, government agencies and other institutions to improve the production of the chicken.

1.6 Operational Definition of Terms

Kenbro: Domesticated birds that are used mainly as a source of meat and eggs to human beings.

Optimization: The act of making the best or most efficient use of a resource.

Central Composite Design: Design used for fitting the second order models.

Response Surface Methodology: Is a collection of mathematical and statistical techniques in which several variables influence a response of interest, and the aim is to optimize the response.

CHAPTER TWO
LITERATURE REVIEW

2.1 Overview of Poultry Production in the World

Chickens (*Gallus Gallus domesticus*) are normally considered to have evolved from the jungle fowl (*Gallus gallus*) inhabiting South China, Indonesia, India, Indio-China and Philippines (Blench & MacDonald, 2000). Chickens (*Gallus gallus domesticus*) have been reared in Kenya for several centuries (Upton, 2000). Kenya has an estimated poultry population of 29 million birds, out of which about 28.7 m (98%) are domestics' chickens. For the last 20 years, the chicken population has increased by more than 75% and their meat and egg products by more than 34% and 79%, respectively (MOLD, 2006). Despite a lack of quantifiable indicators for its impact to the gross domestic product, the poultry sub-sector in Kenya has been recognized as a significant economic tool for rural poverty eradication and household nutrition and food security (Upton, 2000; MOLD, 2008). Apart from generating income, chicken eggs and meat are readily available and cheap sources of food for the household. It has been revealed that with only three mature hens a household is nutritionally secure within one year (Juma & Wasy, 2002). Besides, the chickens are useful in several social, cultural and spiritual activities such as entertainments, gifts, funeral rights and spiritual cleansing (Njenga, 2005).

Currently, the human population has been increasing rapidly over the world. Therefore there is a need for expanding poultry farming. Increased demand for poultry meat and eggs has created the ready market for chicken feeds. One of the difficult tasks the manufacturers face is to satisfy the nutritional requirements for a particular class of livestock. This is caused by escalating cost and scarcity of feed ingredients. Meeting the protein needs of boilers represent a very substantial part of the cost of feeding (Oyedeji & Atteh, 2003). The productive and reproductive performance of chickens is affected by the type of feeds and feeding management. Feeds defined in terms of energy density and protein exhibit varying effects on the growth parameters such as weight gain, growth rate and feed conversion rate (Kuietche, 2014). Right proportions and adequate protein, energy, vitamins, and minerals are essential for the efficient production of any animal. Energy and protein levels are of major importance in chicken nutrition.

Pumpkin leaf has revealed their potential for supplying good quality proteins greater than would be obtained from legumes and cereal (Subba *et al*., 1972). The protein from leaves may be recovered and fed to farm animals as solution informs of protein concentrates (Farinu *et al*., 1992). A study by Ladeji *et al*., (1995) revealed that leaves of fluted pumpkin have high nutritive values with 30.5 grams crude proteins, 3 g crude lipid, 8.4 g total ash per 100 g dry matter and 8.3 g crude fibre. A study on the performance of broiler chicken served heated- fluted pumpkin leaves extract supplement showed that leaves of fluted pumpkin had low levels of tannic acid, oxalate and phytic acid. It was further reported that fluted pumpkin leaves were rich in magnesium, potassium and calcium. Leafy vegetables supply vitamins, proteins and minerals, thereby supplementing the inadequacies of most foodstuffs (Ifon & Basir, 1980). Thus pumpkin extracts have been shown to be a good supplement for protein diet and antihelminthics in birds (Brooker & Acamovic, 2005).

Several studies have revealed poultry farming is largely affected by diseases and parasites in Kenya. The most common diseases are Newcastle disease (NCD), fowl pox, chronic respiratory disease (CRDs), coccidiosis and fowl typhoid (Sonaiya & Swan, 2004). Both internal and external parasites are also common in poultry farming which includes lice, ticks mites, etc. A recent study revealed that 93.3% of adult chicken in semi-arid Kenya was infested with at least one type of helminth (Mungube *et al*., 2008). Most rural farmers are not aware of the incidence of worms in their chicken hence virtually no control methods are taken (Ndegwa *et al*., 1998). Herbs are sometimes used to treat sick birds, with the most commonly used herbs being Aloe Vera, croton, milk need and hot pepper (Njenga, 2005). Information on the effectiveness and efficacy of these herbs in the treatment and control of the various diseases is scarce. Therefore, there is a need to provide more information on the effectiveness of these herbs. A study by Levin & Racheal, (2008) showed that pumpkin seed oil contains fatty acids that help maintain healthy vessels, nerves, and tissues. Pumpkin and other species of Cucurbita family possess an unusual amino acid known as Cucurbitin, chemically defined as (-)-3- amino -3- carboxypyrrolidine. It is the main active chemical compound in the pumpkin seeds responsible for the anthelmintic effect capable of eliminating worms. This amino acid, Cucurbita species plants and the concentration varies from the seed of one individual fruit to the other.

This is even within plants belonging to the same species (Foster & Vavo, 1999). The pumpkins seeds are well known as natural and safe deworming agent. The seeds can rid the body off all intestinal helminthes and parasites when used (Herbs, 2002).

These worm infections may cause considerable damage and great economic loss to the poultry industry due to malnutrition, decreased feed conversion ratio, weight loss, lowered egg production and death in young birds. Study conducted in different parts of the world indicated that the proportion of chicken infected with gastrointestinal helminths is high; therefore helminths are considered to be an important cause of ill-health and reduction in poultry productivity (Uhuo *et al*., 2013). Phytotherapy in the treatment of animals is a possible alternative for the control of gastrointestinal parasites (Hördegen, 2005). Pumpkin is one of the plants with compounds that fights against worms. The pumpkin seed oil contains fatty acids such as oleic (C18:1), palmitic (C16:0), stearic (C18:0), and linoleic (C18:2) (Kulaitiene *et al*., 2007), vitamins E and A, essential nutrients: magnesium, potassium, niacin, folic acid, zinc, riboflavin, phosphorus, copper thiamine and high-quality proteins (Eleiwa *et al*., 2014). The complexity and extent of bioactivity of the seeds may offer sustainable natural alternatives for the control of pathogenic/parasitic organisms, stimulation of nutrition or enhanced resistance to disease infections (Brooker & Acamovic, 2005). The bioactivity of the pumpkin seeds also helps to moderate abdominal serum and fat levels of harmful lipids, while increasing the serum levels of valuable lipids (Brooker & Acamovic, 2005).

2.2 Statistical Analysis of the Effect of Pumpkin Seeds Extract on the Weight Gain of Chicken

A study to evaluate the effects of feeding varying levels of pumpkin seed meal on the growth performance and carcass characteristics of broiler chickens, showed significant results (Wafar *et al*., 2017). The statistical method used was a complete randomized design. The growth performance showed that the final body weight, total body weight gain and average daily weight gain significantly increased ($p < 0.05$) as the levels of pumpkin seed meal increased in the diets (Wafar *et al*., 2017). On the other hand total feed intake and feed conversion ratio did not differ significantly. Among dietary treatments, there were significant differences for live weight and dressed weight, though no particular pattern was observed (Wafar *et al*., 2017). The study demonstrated that pumpkin seed meal is a good source of crude protein and can substitute soybean meal in a broiler chicken diet up to 20.00% since the study did not record any adverse effect on the internal organs (Wafar *et al*., 2017). In Utilization of pumpkin (*Cucurbita moschata*) seed in broiler chicken diets, there was no significant differences between treatment viability, final live weight, feed intake, feed conversion, carcass weight, breast yield and weight of thigh (Martínez *et al*., 2010). This suggested that the utilization of up to 10% of pumpkin seed meal in broiler chicken diets, leads to no change in the productive performance and the sensorial quality of the meat (Martínez *et al*., 2010).

A complete randomized design was used to evaluate the effects of dietary inclusion with linseed and pumpkin seed meals on growth performance, carcass traits and breast meat fatty acids profile of helmeted guinea fowls, results showed no significant effects (Chiroque *et al*., 2018). Linseed and pumpkin seed meals had no significant effects on the liability, initial live weight and feed intake, though, final live weight and feed: gain ratio, carcass yield, breast weight and yield of leg improved significantly (Chiroque *et al*., 2018). Also, the experimental diets had no significant effects on the carcass weight, breast yield and sensory quality of meat. This suggested that the inclusion of pumpkin seed meals and linseed significantly improved the yield of the edible portions, weight, live weight and the essential fatty acids in breast meat of guinea fowl, without affecting the sensory quality of the meat (Chiroque *et al*., 2018). To determine the effect of nettle root and pumpkin seed on production traits and intestinal microflora of broiler chickens, significant results was obtained (Tabari *et al*., 2016). Nettle root and pumpkin oil significantly affects the overall feed conversion ratio (Tabari *et al*., 2016). The results suggested that supplementation with nettle root and pumpkin oil, particularly their combination has a positive effects on broiler chickens (Tabari *et al*., 2016).

Investigation on performance of broiler chickens served heat-treated fluted pumpkin (*Telfaria occidentalis*) leaves extract, showed significant effects (Nworgu *et al*., 2007). The results revealed that fluted pumpkin (*Telfaria occidentalis*) leaves extract served to the birds had significant effect on feed intake, weight gain, feed conversion ratio, water intake, protein efficiency ratio and cost of feed per kilogram live weight but birds served fluted pumpkin (*Telfaria occidentalis*) leaves extract in both phases had reduced feed intake (Nworgu *et al*., 2007). A study was conducted to analyse the dietary effect of pumpkin and flaxseed oils on performance parameters of laying hens found that the average egg´s weight increased significantly (Herkeľ *et al.,* 2014). Significantly higher average egg´s weight during the experiment was found after dietary oils supplementation (Herkeľ *et al*., 2014). Egg's production was significant difference only between experimental groups. A study to evaluate the antiatherogenic, renal protective and immunomodulatory effects of purslane, pumpkin and flax seeds on hypercholesterolemic rats, showed significant results (Barakat & Mahmoud, 2011). Cholesterol-enriched diet significantly increased serum urea, creatinine, sodium and potassium levels as compared with a healthy control group (Barakat & Mahmoud, 2011). This suggests that both flax/pumpkin and purslane/pumpkin seed mixtures had anti- atherogenic hypolipidemic and immunmodulator effects which were probably mediated by unsaturated fatty acids present in seed mixture (Barakat & Mahmoud, 2011).

Evaluation of anthelmintic activity and composition of pumpkin (*Cucurbita Pepo* L) seed- in vitro and in vivo studies, showed significant reduction in worm (Grzybek *et al*., 2016). A decrease in faecal egg counts was accompanied by a significant decrease in the worm burden of the treated mice compared to the control group (Grzybek *et al*., 2016). Also, no significant seed extracts on *C. elegans* integrity or motility were found. Therefore pumpkin seed extracts may be used to control gastrointestinal nematode infections. This is a relatively inexpensive alternative to the currently available chemotherapeutic and should be considered as a novel drug candidate in the nearest future (Grzybek *et al*., 2016). Evaluation of pumpkin (*Cucurbita pepo L*) seeds and L- Arginine supplementation on serum lipid concentrations in Atherogenic rats, showed significant results (Abuelgassim & Al-Showayman, 2012). The result showed that treatment of atherogenic rats with pumpkin seeds significantly decreased serum concentrations (Abuelgassim & Al-Showayman, 2012). The findings suggested that pumpkin seeds supplementation has a positive effect against atherogenic rats and this protective effect was not attributed to the high arginine concentrations in pumpkin seeds (Abuelgassim & Al-Showayman, 2012).

2.3 Combination of Weeks and Quantity of Pumpkin Seeds Extract on Weight Gain of Chicken

In the evaluation of the effects of pumpkin seeds oil supplementation to the diet on growth performance, carcass characteristics and some blood biochemical parameters of Japanese quail (*Coturnix japonica*), showed significant results (Abbas *et al*., 2017). The results revealed a significantly increase of pumpkin seeds oil on body weight gain during a 1- 6 week period of the experiment compared to a basal diet without supplementation with pumpkin seed oil. (Abbas *et al*., 2017). The addition of pumpkin seeds oil to the diet showed better feed conversion ratio during the 1-3 week period. Therefore the study suggests that the additional of pumpkin seeds oil to the diet has a significant effect on feed conversion ratio (Abbas *et al*., 2017). Also a study to determine the effects of dietary supplementation of nettle root extract and pumpkin seed oil on production traits and intestinal microflora in broiler chicken, showed the significant result (Tabari *et al*., 2016). There was no significant difference in the total feed consumption of different treatment groups but herbal supplementation affected feed intake from 22 to 42 days (Tabari *et al*., 2016). Birds feed with pumpkin seed oil supplementation had the highest feed intake also body weight significantly increased in birds fed with pumpkin seed oil supplementation. In conclusion, the study revealed that the combination of nettle root and pumpkin seed oil had a positive impact on body weight and was capable of playing an essential role in the health of poultry by maintaining a balanced microflora in the digestive system (Tabari *et al*., 2016).

Application of factorial design and response surface methodology on the growth rate of broiler chickens served with fluted pumpkin leaves extract, showed significant results (Oyegunle *et al*., 2012). The result showed that the average body weight was significant. The use of fluted pumpkin leaves extract in broiler chicken production was most effective from fifth week. The fitted Response Surface Model indicated that the number of weeks and quantity of fluted pumpkin leaves extract with their mutual interaction significantly determined the weight of broiler chickens (Oyegunle *et al*., 2012). Therefore this study suggested that the number of weeks, fluted pumpkin leaves extract and their mutual interaction play a key role in obtaining the maximum weight of broiler chickens (Oyegunle *et al*., 2012). A mathematical model for the estimation of optimum broiler production period under the economic conditions of turkey found that the optimal production period was 5.86 weeks. Hence the study concluded the method is suitable for calculation of optimum production period in broiler farms (Oğuz & Parlat, 2003).

2.4 Response Surface Methodology

Response surface methodology is a collection of statistical and mathematical techniques useful for developing, improving and optimizing processes (Montgomery, 2013). It is mostly applied in the world of industry where some input variables potentially influence some performance measurement usually referred to as the response. The input variables can also be referred to as the independent variables.

Generally, a researcher is concerned with a system involving a response y that depends on the input variables $X_1,\ X_2,\ X_3\ ,\ldots,\ X_k$. The relationship between the response and the input variables is given in equation 1;

$$y = f\ (X_{1,}\ X_2, X_3,, \ldots, X_k) + \varepsilon \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ 1$$

Where the form of the true function f is unknown and ε is the amount of variability not accounted for by f. ε is assumed to be normally distributed with a mean 0 and a variance σ^2. If the mean of the error ε is 0, then E(y) is as shown in equation 2 and 3;

$$E(y) = b = E\ f\ (X_1,\ X_2, X_3,, \ldots,\ X_k) + E\ (\varepsilon) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ 2$$

$$= f\ (X_{1,}\ X_2,\ X_3, \ldots, X_k) \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ 3$$

The variables ($X_1,\ \ X_2,\ X_3, \ldots\ ,\ X_k$) are called the natural variables because they are expressed in the natural units of measurements. The variables ($X_1, X_2, X_3, \ldots, X_k$) are transformed to coded variables ($x_{1,}\ \ x_2,\ x_3, \ldots, x_k$) for convenience in data analysis. The true response can thus be expressed in terms of coded variables as shown in equation 4;

$$b = f\ (x_{1,}\ x_2, x_3, \ldots, x_k) \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ 4$$

The form of the true response is usually unknown and thus it has to be approximated. Therefore success in the application of response surface methodology depends on the ability of the researcher to approximate the form of the true response. In most cases, first order or a second order model is used. If there are two independent variables, then first order model written in terms of coded values will be as in equation 5;

$$b = \alpha_0 + \alpha_1 x_1 + \alpha_2 x_2 + \varepsilon \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \; 5$$

For a particular case of the first order model, the response surface and the contour plots can be shown diagrammatically (Figure 1 and 2)

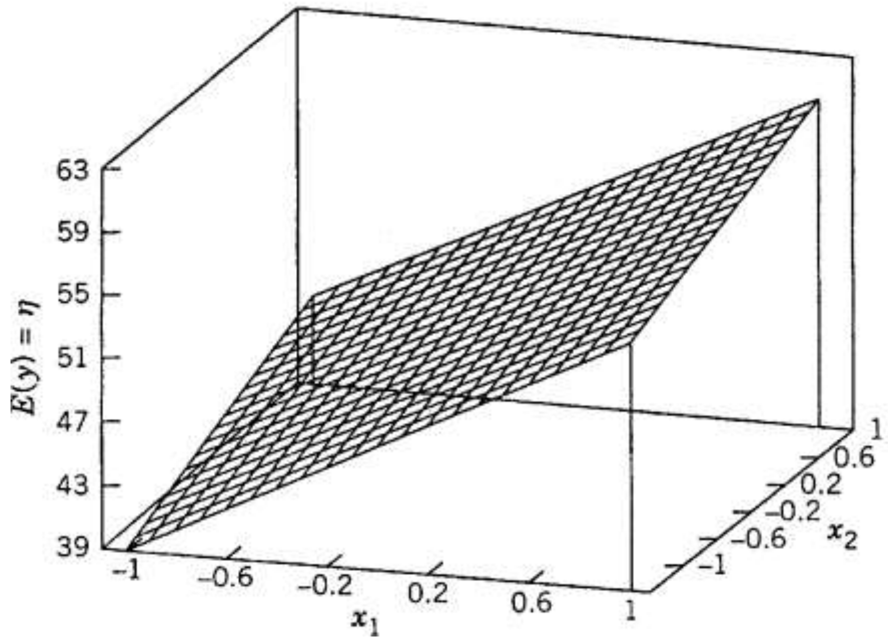

Figure 1: Response surface plot of a particular case of a first order model (Myers *et al*., 2016).

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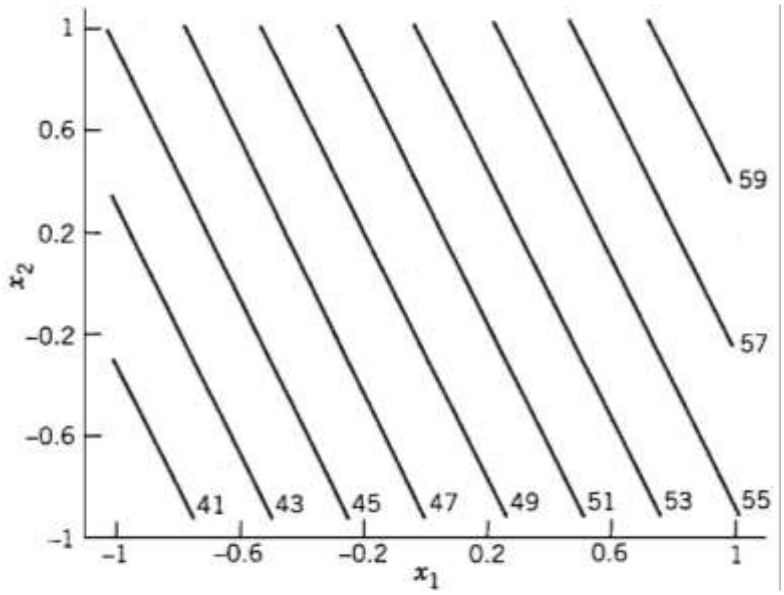

Figure 2: Contour plot for a particular case of the first order model (Myers *et al*., 2016).

First order model is suitable when the interest of the researcher is to approximate the true response surface over a small region of the independent variable space where there is little curvature in (Montgomery 2005). If there is an interaction between the independent variables, the first order model becomes (equation 6);

$$b = \alpha_0 + \alpha_1 x_1 + \alpha_2 x_2 + \alpha_{12} x_1 x_2 + \varepsilon \ldots\ldots\ldots\ldots 6$$

For a particular case of the first order model with an interaction, the response surface and the contour plot can be shown diagrammatically (Figure 3 and 4).

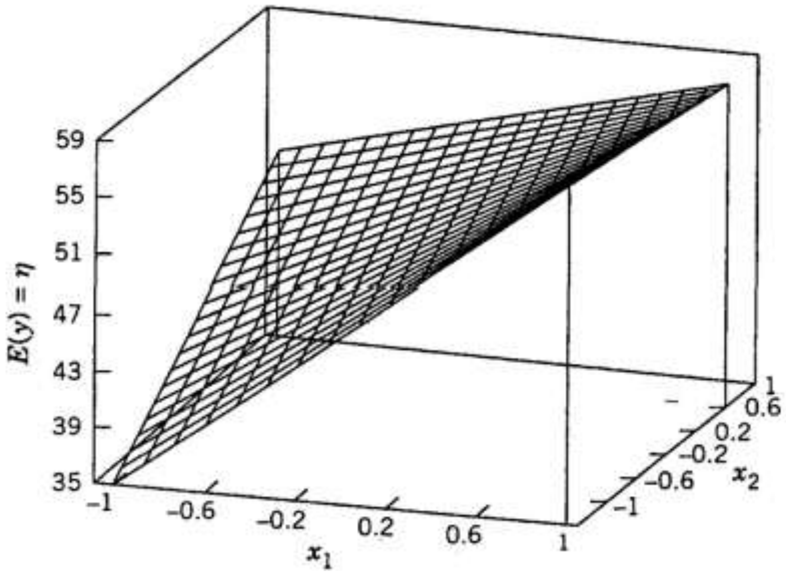

Figure 3: A response surface for a particular case of the first order model with an interaction (Myers *et al*., 2016).

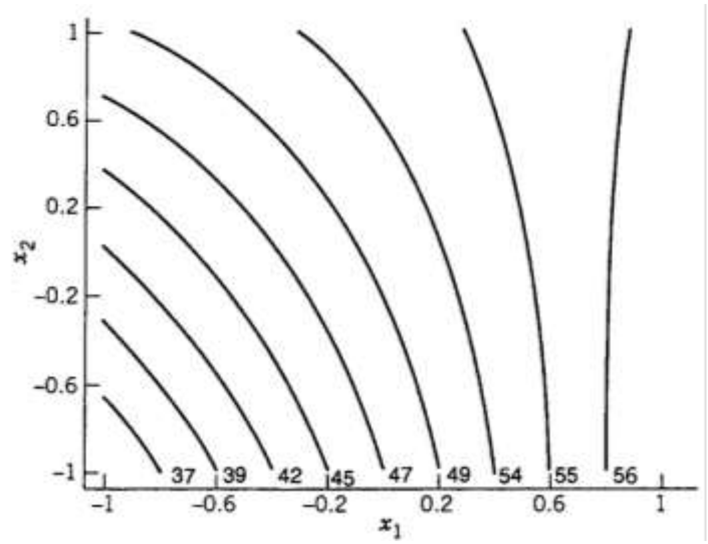

Figure 4: A contour plot for a particular case of a first order model with an interaction (Myers *et al*., 2016).

There are cases where the curvature in the response surface is strong enough such that even the first order model with interaction is inadequate. In such a case, the second order model is preferred (Montgomery, 2013). If there are two independent variables x_1 and x_2, then the second order model will be as shown in equation 7;

$$b = \alpha_0 + \alpha_1 x_1 + \alpha_2 x_2 + \alpha_{11} x_1^2 + \alpha_{22} x_2^2 + \alpha_{12} x_1 x_2 + \varepsilon \quad \ldots\ldots\ldots\ldots\ldots\ldots.. 7$$

A specific case of a second-order model used can be to demonstrate the different forms of the shapes of the response surfaces and the contour plots (Figure 5, 6, 7, 8, 9 and 10).

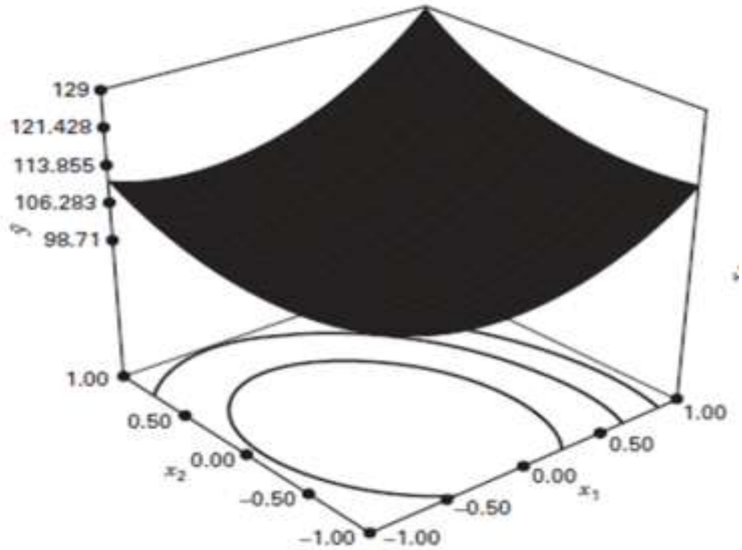

Figure 5: A response surface plot for a particular case of the second order model illustrating a minimum (Montgomery, 2013).

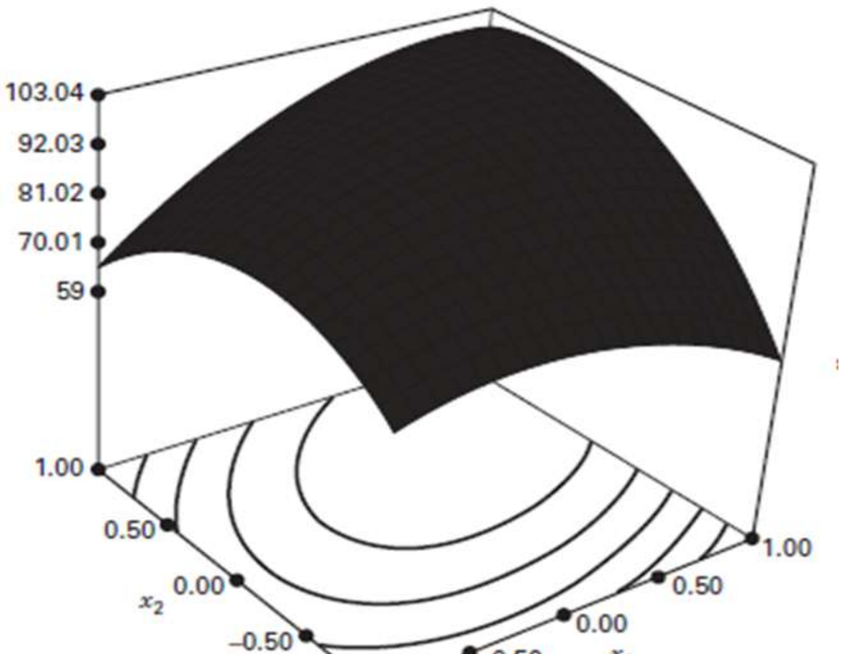

Figure 6: A response surface plot for a particular case of the second order model illustrating a maximum (Montgomery, 2013).

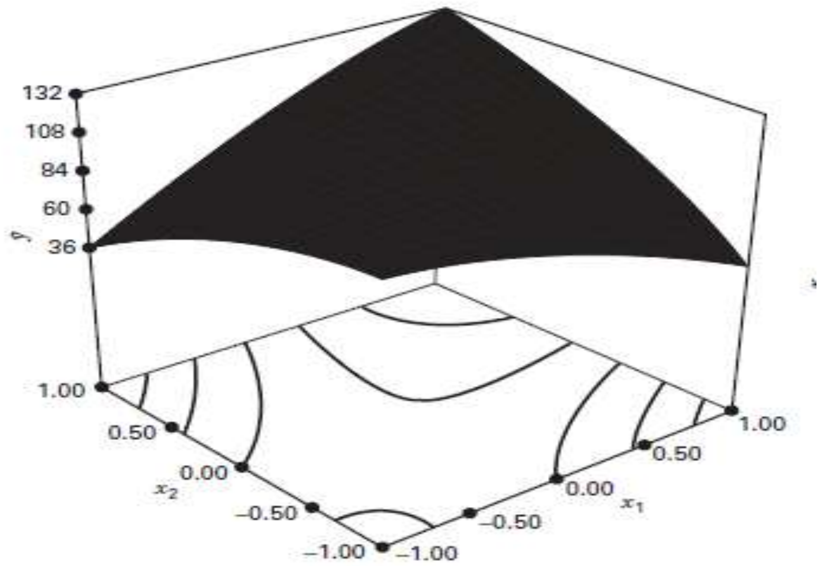

Figure 7: A response surface plot for a particular case of the second order model illustrating a saddle point (minimax) (Montgomery, 2013).

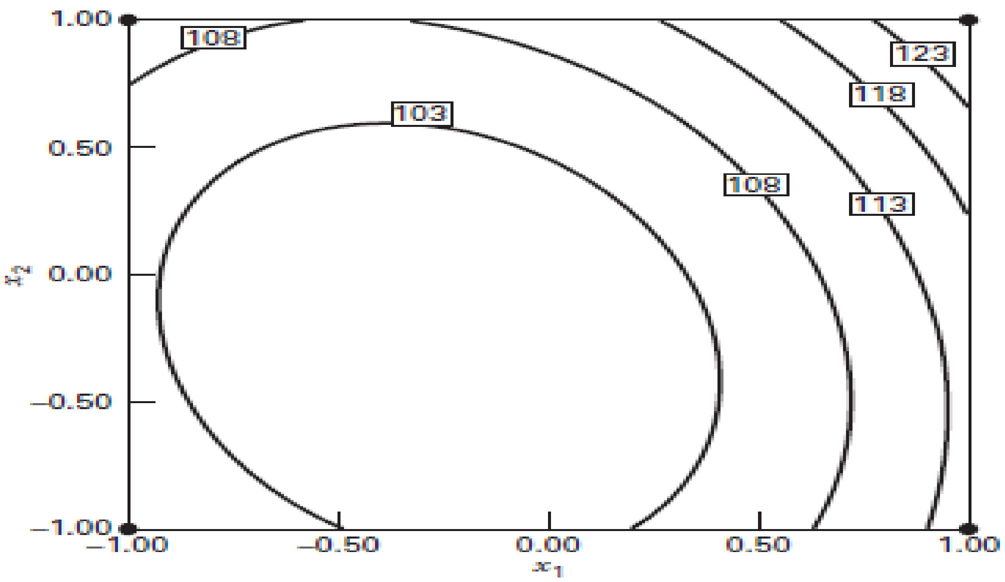

Figure 8: A contour plot for a particular case of the second order model illustrating a minimum (Montgomery, 2013).

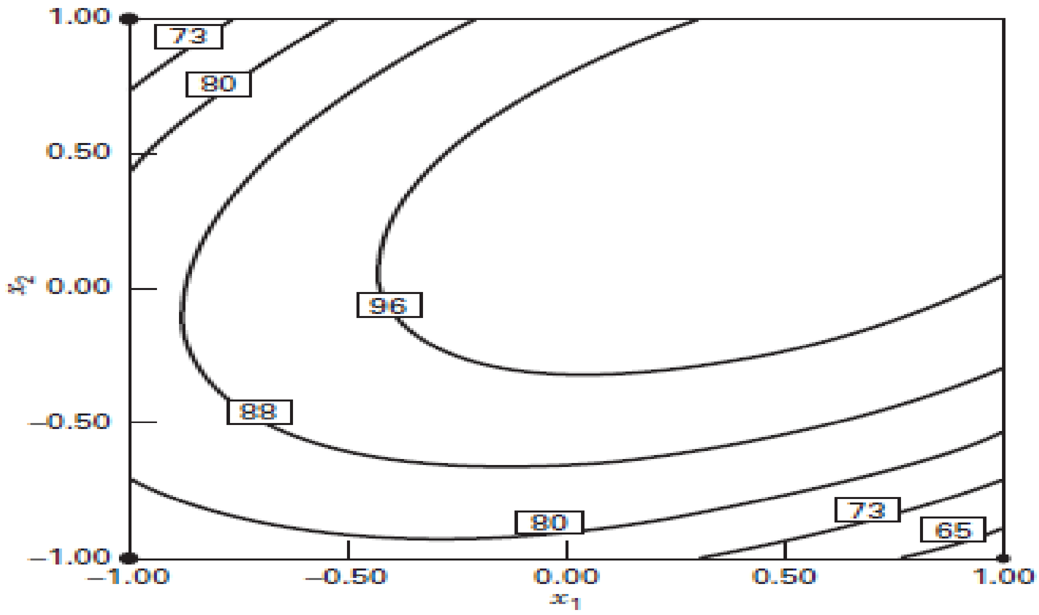

Figure 9: A contour plot for a particular case of the second order model illustrating a maximum (Montgomery, 2013).

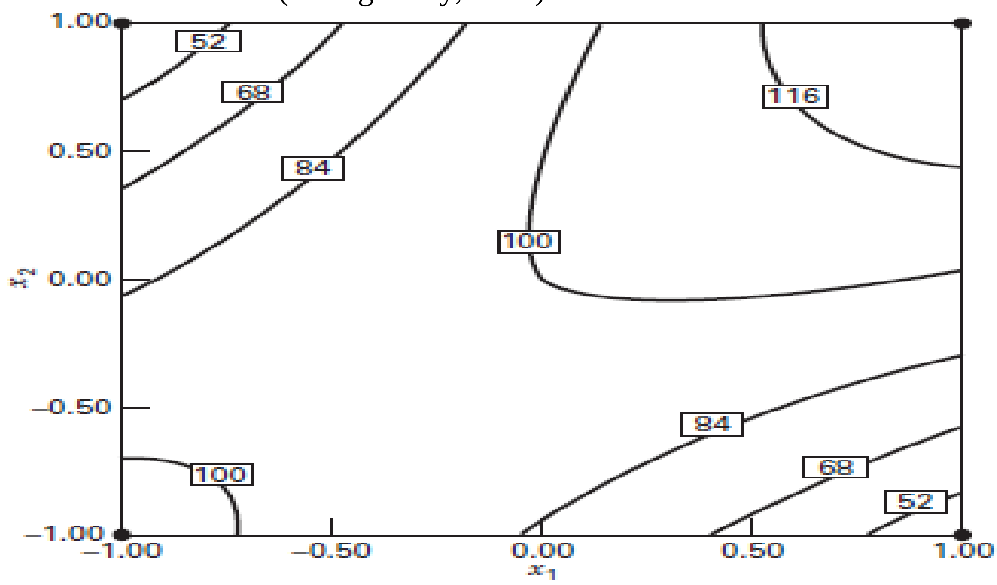

Figure 10: A contour plot for a particular case of the second order model illustrating a saddle point (minimax) (Montgomery, 2013).

The second order is useful when approximating responses such as yield where it is expected that the operating conditions will be near a maximum point on the surface. The second order model is used in response surface methodology because it is flexible and it can fit a variety of functional forms in addition to the ease in parameter estimation in the model (Montgomery, 2013).

After looking at a particular case where there were only two variables, it is important to do a generalization for both the first and the second order models. The first order model can be generalized as shown in equation 8;

$$b = \alpha_0 + \alpha_1 x_1 + \alpha_2 x_2 + \alpha_3 x_3 + \ldots + \alpha_k x_k + \varepsilon \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 8$$

The generalized second order model will be as shown in equation 9;

$$b = \alpha_0 + \sum_{j=1}^{k} \alpha_j\, x_j + \sum_{j=1}^{k} \alpha_{jj}\, x_j^2 + \sum\sum_{i<j=2} \alpha_{ij}\, x_i\, x_j + \varepsilon \ldots\ldots\ldots\ldots\ldots\ldots\ldots 9$$

Where b is the response, α_0 is a constant variable, α_j is the linear coefficient, α_{jj} is the second order coefficient, and α_{ij} is the cross product coefficient between x_i and x_j and is the random error term which is normally distributed with mean zero and variance of one (Montgomery, 2013).

2.4.1 Models for Approximating the True Response Surface

The application of response surface methodology involves developing a model that would approximate the true response surface. This model is mainly based on the collected data and it is known as the empirical model. Empirical models in RSM are built from the multiple regression techniques. For instance, a situation where two independent variables x_1 and x_2 are involved, the first order model is represented as shown in equation 10;

$$y = \alpha_0 + \alpha_1 x_1 + \alpha_2 x_2 + \varepsilon \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 10$$

This model is a multiple regression model with two independent variables. In general, if the response will be related to k independent variables, the first order model would be as in equation 11;

$$y = \alpha_0 + \alpha_1 x_1 + \alpha_2 x_2 + \alpha_3 x_3 + \ldots + \alpha_k x_k + \varepsilon \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 11$$

This model is a multiple regression model with k regressors. The parameters α_0, α_1, α_2, $\alpha_3, \ldots, \alpha_k$ are called the regression coefficients.

If the interaction term is added to the first order model with two independent variables, the first order model will become as shown in equation 12;

$$y = \alpha_0 + \alpha_1 x_1 + \alpha_2 x_2 + \alpha_{12} x_1 x_2 + \varepsilon \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{12}$$

If α_{12} is let to be α_3 and $x_1 x_2$ is let to be x_3 then the 1st order model with interaction will become as shown in equation 13;

$$y = \alpha_0 + \alpha_1 x_1 + \alpha_2 x_2 + \alpha_3 x_3 + \varepsilon \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{13}$$

This model is a standard multiple regression model with three regressors.

If a second order model with 2 independent variables is considered, then, it will be written as shown in equation 14;

$$y = \alpha_0 + \alpha_1 x_1 + \alpha_2 x_2 + \alpha_{11} x_1^2 + \alpha_{22} x_2^2 + \alpha_{12} x_1 x_2 + \varepsilon \quad \ldots\ldots\ldots\ldots \text{14}$$

Letting $x_1^2 = x_3$, $x_2^2 = x_4$, $x_1 x_2 = x_5$ and $\alpha_{11} = \alpha_3$, $\alpha_{22} = \alpha_4$, $\alpha_{12} = \alpha_5$, the second order model will become as shown in equation 15;

$$y = \alpha_0 + \alpha_1 x_1 + \alpha_2 x_2 + \alpha_3 x_3 + \alpha_4 x_4 + \alpha_5 x_5 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{15}$$

This model is a linear regression model

2.4.2 Parameter Estimation in Response Surface Methodology

The statistical techniques useful in building empirical models in response surface methodology are the multiple regressions (Montgomery, 2013). Therefore, the estimation of the regression coefficients in multiple regression is done by the method of least squares. The multiple regression model can be written as shown in equation 16;

$$y = \alpha_0 + \alpha_1 x_1 + \alpha_2 x_2 + \alpha_3 x_3 + \ldots + \alpha_k x_k + \varepsilon \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{16}$$

This model can be summarized in a table in the form of observations as (Table 1);

Table 1: Dependent and Independent Variables of a Multiple Regression Model

y	x_1	x_2	x_3	⋯	x_k
y_1	x_{11}	x_{21}	x_{31}	⋯	x_{1k}
y_2	x_{12}	x_{22}	x_{32}	⋯	x_{2k}
y_3	x_{13}	x_{23}	x_{33}	⋯	x_{3k}
⋮	⋮	⋮	⋮		⋮
y_n	x_{1n}	x_{2n}	x_{3n}	⋯	x_{nk}

The model can then be rewritten using the observations in the table as shown in equations 17 and 18;

$$y_i = \alpha_0 + \alpha_1 x_{i1} + \alpha_2 x_{i2} + \alpha_3 x_{i3} + \ldots + \alpha_k x_{ik} + \varepsilon_i \quad \ldots\ldots\ldots\ldots\ldots\ldots \; 17$$

$$= \alpha_0 + \sum_{j=1}^{k} \alpha_j x_{ij} + \varepsilon_i \quad \ldots\ldots\ldots\ldots\ldots\ldots \; 18$$

To estimate the α's in the above equation the sum of squares due to the errors ε_i is minimized (Montgomery, 2013). The least squares function is represented in equations 19 and 20;

$$S = \sum_{i=1}^{n} \varepsilon_i^2 \quad \ldots\ldots\ldots\ldots\ldots\ldots \; 19$$

$$= \sum_{i=1}^{n} \left(y_i - a_0 - \sum_{j=1}^{k} \alpha_j \, x_{ij}\right)^2 \quad \ldots\ldots\ldots\ldots\ldots\ldots \; 20$$

The sum of squares is minimized with respect to a_0, a_1, a_2, a_3, …, a_k

The least squares estimates must satisfy as shown in equations 21 and 22;

$$\frac{\partial S}{\partial \alpha_0} \Big|_{a_0, a_1, a_2, a_3, \ldots, a_k} = -2 \sum_{i=1}^{n} \left(y_i - a_0 - \sum_{j=1}^{k} a_j x_{ij}\right) = 0 \quad \ldots\ldots\ldots \; 21$$

$$\frac{\partial S}{\partial \alpha_j} \Big|_{a_0, a_1, a_2, a_3, \ldots, a_k} = -2 \sum_{i=1}^{n} \left(y_i - a_0 - \sum_{j=1}^{k} a_j x_{ij}\right) = 0 \quad \ldots\ldots\ldots \; 22$$

The above equations can be written explicitly as shown in equation 23;

$$\text{n}a_0 + a_1 \sum_{i=1}^{n} x_{i1} + a_2 \sum_{i=1}^{n} x_{i2} + a_3 \sum_{i=1}^{n} x_{i3} \;\ldots. + a_k \sum_{i=1}^{n} x_{ik} = \sum_{i=1}^{n} y_i$$

$$a_0 \sum_{i=1}^{n} x_{i1} + a_1 \sum_{i=1}^{n} x_{i1}^2 + a_2 \sum_{i=1}^{n} x_{i1}\, x_{i2} + a_3 \sum_{i=1}^{n} x_{i1}\, x_{i3} \;\ldots + a_k \sum_{i=1}^{n} x_{i1}\, x_{ik} = \sum_{i=1}^{n} x_{i1}\, y_i$$

$$a_0 \sum_{i=1}^{n} x_{i2} + a_1 \sum_{i=1}^{n} x_{i2}\, x_{i1} + a_2 \sum_{i=1}^{n} x_{i2}^2 + a_3 \sum_{i=1}^{n} x_{i2}\, x_{i3} \ldots + a_k \sum_{i=1}^{n} x_{i2}\, x_{ik} = \sum_{i=1}^{n} x_{i2}\, y_i$$

$$\vdots$$

$$a_0 \sum_{i=1}^{n} x_{ik} + a_1 \sum_{i=1}^{n} x_{ik} x_{i1} + a_2 \sum_{i=1}^{n} x_{ik}\, x_{i2} + a_3 \sum_{i=1}^{n} x_{ik}\, x_{i3} \ldots + a_k \sum_{i=1}^{n} x_{ik}^2 = \sum_{i=1}^{n} x_{ik}\, y_i \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \; 23$$

The above equations are called the normal equations and they are k + 1 in number since there k + 1 regression coefficients (Montgomery, 2013). Each normal equation represents one regression coefficient. Once the regression coefficients are estimated by solving the normal equations, the fitted regression model can be represented as shown in equation 24;

$$\hat{y} = \alpha_0 + \sum_{j=1}^{k} a_j x_{ij} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \; 24$$

2.4.3 Model Diagnostics in Response Surface Methodology

Model diagnostic assessment involves a set of procedures available in modelling that seek to assess the validity of a model in any of a number of different ways (Everitt, 2002). In response surface methodology, is done using statistical checks such as ANOVA table, R^2 and R^2_{adj} value, model lack of fit test, and p-value (Trinh & Kang, 2010). It is usually necessary to check the fitted model to ensure it provides an adequate approximation to the real system. Unless the model shows an adequate fit, proceeding with investigation and optimization of the fitted response surface is likely to give poor or misleading results (Trinh & Kang, 2010). R^2 indicates how much of the observed variability in the data was accounted for by the model, while R^2_{adj} modifies R^2 by taking into account the number of covariates or predictors in the model (Trinh & Kang, 2010). The first diagnostic involves checking if the fitted regression is significant ($p < 0.05$). This involves testing if there is a linear relationship between the response variable and the set of independent variables. The hypotheses to be tested are

$H_0 : \alpha_1 = \alpha_2 = \alpha_3 = \ldots = \alpha_k = 0$

$H_1 : \alpha_j \neq 0$ for at least one j

If the null hypothesis is rejected, then it implies that at least one of the independent variables $x_1, x_2, x_3, \ldots, x_k$ has a significant (p < 0.05) contribution in the model. The procedure of testing this hypothesis involves partitioning the total sum of squares into the regression sum of squares and residual sum of squares. That is (equations 25 and 26);

$$SST = SSR + SSE \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \; 25$$

Where

$$SST = \sum_{i=1}^{n}(y_i - \bar{y})^2 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \; 26$$

If the null hypothesis is true, then, $\frac{SSR}{\sigma^2}$ has a chi-square distribution with k degrees of freedom and $\frac{SSE}{\sigma^2}$ has a chi-square distribution with n – k – 1 degree of freedom. It is important to note that the sum of squares due to regression and the sum of squares due to error are independent of each other. The test statistic for testing the hypothesis is computed as in equation 27;

$$F_{computed} = \frac{SSR/k}{SSE/_{n-k-1}} = \frac{MSR}{MSE} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \; 27$$

The null hypothesis is rejected if the $F_{computed}$ exceeds $F_{(\alpha,\ k,\ n-k-1)}$. However, one can also use the p–value approach and reject the null hypothesis if the p–value of $F_{computed}$ is less than the level significance (α). This test can be summarised in the form of a table as (Table 2);

Table 2: Analysis of Variance for Testing the Significance of a fitted regression model

Source of variation	Sum of squares	Degrees of freedom	Mean square	F value
Sum of squares due to regression	SSR	K	MSR	$\frac{MSR}{MSE}$
Sum of squares due to error	SSE	n – k – 1	MSE	
Total sum of squares	SST	n – 1		

Where

$$SST = \sum_{i=1}^{n} y_i^2 - \frac{\left(\sum_{i=1}^{n} y_i\right)^2}{n} = y' \text{ y} - \frac{\left(\sum_{i=1}^{n} y_i\right)^2}{n} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 28$$

$$SSE = y' \text{ y} - \frac{\left(\sum_{i=1}^{n} y_i\right)^2}{n} - \left[\alpha' X' y - \frac{\left(\sum_{i=1}^{n} y_i\right)^2}{n}\right] \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 29$$

Or SSE = SST – SSR

Therefore, the sum of squares due to regression is as shown in equation 30;

$$SSR = \alpha' X' y - \frac{\left(\sum_{i=1}^{n} y_i\right)^2}{n} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 30$$

And the sum of squares due to error is given by (equation 31);

$$SSE = y' \text{ y} - a' X' y \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 31$$

And the total sum of squares is given by (equation 32);

$$SST = y' \text{ y} - \frac{\left(\sum_{i=1}^{n} y_i\right)^2}{n} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 32$$

The goodness of fit of the fitted model is determined using the coefficient of determination (R^2). The coefficient of determination is defined by equation 33;

$$R^2 = \frac{SSR}{SST} = 1 - \frac{SSE}{SST} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 33$$

This value measures the amount of the variability of the response reduced by using the independent variables. The coefficient of determination is always a value between 0 and 1. Large values of the coefficient of determination do not always mean that the fitted model is a good one (Myers *et al*., 2004). This is because whenever an independent variable is added to the model, it always leads to an increase in the value of the coefficient of determination. This is irrespective of whether the independent variable added is significant or not. This weakness leads to the use of adjusted R^2 which is better than R^2. This is because the adjusted R^2 does not always increase whenever an independent variable is added to the model. The value of adjusted R^2 increases only when the independent variable is added to the model. When a variable that is not significant is added to the model, it causes the adjusted R^2 to decrease. The adjusted R^2 is computed as shown in equation 34;

$$R^2_{adj} = 1 - \frac{SSE/_{n-p}}{SST/_{n-1}} = 1 - \frac{n-1}{n-p}(1 - R^2) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \; 34$$

Testing the significance of the individual regression coefficients is important. This is because it helps to know if the model can get better with the addition of more variables or elimination of some variables (Myers *et al*., 2004). The addition of a significant variable in a model can cause the regression sum of squares to increase and the residual sum of squares to decrease. However, whenever a variable that is not significant is added to the model, then mean squared error increases and this decreases the usefulness of the model. To test the significance of the regression coefficients, the following hypothesis is used;

$$H_0 : \alpha_j = 0$$
$$H_1 : \alpha_j \neq 0$$

If the null hypothesis is not rejected, then the variable corresponding to the regression coefficient is deleted from the model. The test statistic for this hypothesis is as shown in equation 35;

$$t_{computed} = \frac{\alpha_j}{\sqrt{\hat{\sigma}^2 \, C_{jj}}} \quad \ldots\ldots 35$$

C_{jj} is the diagonal element in the matrix $(X'X)^{-1}$ corresponding to α. The null hypothesis is rejected if $t_{computed}$ is greater than $t_{(\frac{\alpha}{2}}$, n – k -1). This hypothesis can also be tested using the p–value approach. Precisely, $\sqrt{\hat{\sigma}^2 \, C_{jj}}$ is the standard error of the regression coefficientα_j. That is (equation 36);

$$\text{se}\,(\alpha_j) = \sqrt{\hat{\sigma}^2 \, C_{jj}} \quad \ldots\ldots 36$$

The test statistic can then be written equivalently as in equation 37;

$$t_{computed} = \frac{\alpha_j}{\text{se}\,(\alpha_j)} \quad \ldots\ldots 37$$

The significance of a group of regression coefficients can be tested using the extra sum of squares method. For instance, considering the regression model(equation 38);

$$\text{y} = \alpha \text{ X} + \varepsilon \quad \ldots\ldots 38$$

Where y has dimension n $\times$ 1, X has a dimension n $\times$ p and ε has a dimension n $\times$ 1 and p = k +1. The significance of a group of independent variables sayx_1, $x_2, x_3, \ldots, x_r$ (r < k). This can be done by performing the following partition (equation 39);

$$\alpha = \begin{pmatrix} \alpha_1 \\ \alpha_2 \end{pmatrix} \quad \ldots\ldots 39$$

Where α_1 has a dimension r $\times$ 1 and α_2 has dimension (p – r) $\times$ 1. The hypothesis to be tested is;

$$H_0: \alpha_1 = 0$$

$$H_1: \alpha_1 \neq 0$$

The model can thus be rewritten as shown in equation 40;

$$\text{y} = \alpha \text{ X} + \varepsilon = \alpha_1 X_1 + \alpha_2 X_2 + \varepsilon \quad \ldots\ldots 40$$

X_1 are the columns of X associated with α_1 and X_2 are the columns of X associated with α_2. Considering the full model (equation 41),

$$\alpha = (X'\,X)^{-1}\,X'\,y \quad \text{.......... 41}$$

The regression sum of squares for the full model is also given by (equations 42 and 43);

$$\text{SSR}\,(\alpha) = \alpha'\,X'\,y \quad \text{.......... 42}$$

and

$$\text{MSE} = \frac{y'\,y - \alpha'\,X'\,y}{n-p} \quad \text{.......... 43}$$

The contribution of α_1 to the regression is formed by fitting the model assuming that the null hypothesis is true. That is, fitting the model assuming that α_1 = 0. The fitted model thus becomes (equation 44)

$$\text{y} = \alpha_2\,X_2 + \varepsilon \quad \text{.......... 44}$$

The least squares estimator for the α_2 is as in equation 45 and 46;

$$\alpha_2 = (X_2{}'\,X_2)^{-1}\,X_2{}'\,y \quad \text{.......... .45}$$

and

$$\text{SSR}\,(\alpha_2) = \alpha_2{}'\,X_2{}'\,y \quad \text{.......... 46}$$

The regression sum of squares due to α_1 provided that α_2 is already in the model is given by (equation 47);

$$\text{SSR}\,(\alpha_1 \mid \alpha_2) = \text{SSR}\,(\alpha) - \text{SSR}\,(\alpha_2) \quad \text{.......... 47}$$

To test H_0: α_1 = 0, the test statistic is shown in equation 48;

$$F_{computed} = \frac{\text{SSR}\,(\alpha_1 \mid \alpha_2) / r}{MSE} \quad \text{.......... 48}$$

The hypothesis is rejected if $F_{computed}$ is greater than $F_{(\alpha,\ r, n-p)}$. If the null hypothesis is rejected, the conclusion would be at least one parameter in α is not 0 and it contributes significantly to the model. The P – value approach can also be used in this test. This test is often referred to as the partial F – test. The partial F – test is very useful especially when fitting then second-order response surface (Myers *et al*., 2012). For instance, if the second-order model is considered, the wish can be to test the contribution of the second order terms over and above the first order model. The hypothesis to be tested would be;

$$H_0 : \alpha_{11} = \alpha_{22} = \alpha_{12} = 0$$
$$H_1 : \alpha_{11} \neq 0 \text{ And / or } \alpha_{22} \neq 0 \text{ and / or } \alpha_{12} \neq 0$$

The confidence intervals in multiple regression can also be constructed. In the construction, the errors ε_i are assumed to be normally distributed with mean 0 and variance σ^2. Since α is a linear combination of observation and follows a normal distribution with mean vector α and covariance matrix $\sigma^2\ (X'\,X)^{-1}$. Therefore each statistic $\frac{a_j-\alpha_j}{\sqrt{\hat{\sigma}^2\ C_{jj}}}$ has a student t – distribution with n-p degrees of freedom. C_{jj} is the $(\mathrm{i,j})^{th}$ element of the matrix $(X'\,X)^{-1}$ and $\hat{\sigma}^2$ is the variance estimate. The 100 (1 - α) % confidence interval for α_j is given as equation 49;

$$a_j - t_{\left(\frac{\alpha}{2},n-p\right)}\sqrt{\hat{\sigma}^2\ C_{jj}} \leq \alpha_j \leq a_j + t_{\left(\frac{\alpha}{2},n-p\right)}\sqrt{\hat{\sigma}^2\ C_{jj}} \quad \text{………………………} \ 49$$

Since se (a_j) = $\sqrt{\hat{\sigma}^2\ C_{jj}}$, the 100 (1 - α) % confidence interval for α_j can be rewritten as equation 50;

$$a_j - t_{\left(\frac{\alpha}{2},n-p\right)}\ \mathrm{se}\ (a_j) \leq \alpha_j \leq a_j + t_{\left(\frac{\alpha}{2},n-p\right)}\ \mathrm{se}\ (a_j) \ \text{………..………} \ 50$$

Checking of the adequacy of the overall model is also important. This helps in ensuring that the fitted model is an adequate approximation of the true system and that none of the least squares regression assumptions has been violated. One of the methods of checking the adequacy of the overall model is residual analysis. In this check, the normal probability plot of residuals is constructed. The normality assumption is said to be satisfied if the residuals plot approximately along a straight line (Myers *et al*., 2004). If the normality assumption is not satisfied then, there is a need for transformation of the response variable. Scaling residuals also play an important role in checking for outliers. The scaling residuals can be categorized into standardized and studentized residuals. The standardized residuals are represented as equation 51;

$$b_i = \frac{e_i}{\hat{\sigma}}, \text{ i = 1, 2... n} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 51$$

The standardized residuals lie between -3 and 3 and any observation outside this interval is an outlier.

A lack of fit test is also used in testing the overall adequacy of the fitted model. The lack of fit test involves partitioning the residual sum of squares into the sum of squares due to pure error and sum of squares due to lack of fit. That is;

Residuals sum of squares = pure error sum of squares + lack of fit sum of squares

To perform this partitioning, the start point is (equation 52),

$$(y_{ij} - \hat{y}_i) = (y_{ij} - \bar{y}_i) + (\bar{y}_i - \hat{y}_i) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 52$$

The next step is squaring both sides and summing over i and j. the result is as in equation 53;

$$\sum_{i=1}^{m}\sum_{j=1}^{n}(y_{ij} - \hat{y}_i)^2 = \sum_{i=1}^{m}\sum_{j=1}^{n}(y_{ij} - \bar{y}_i)^2 + \sum_{j=1}^{n} \text{n } (\bar{y}_i - \hat{y}_i)^2 \quad \ldots\ldots 53$$

This clearly shows that the sum of squares due to error is given by (equation 54);

$$\text{SSE} = \sum_{i=1}^{m}\sum_{j=1}^{n_i}(y_{ij} - \hat{y}_i)^2 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 54$$

The sum of squares due to pure error is given by (equation 55);

$$\text{SSPE} = \sum_{i=1}^{m}\sum_{j=1}^{n_i}(y_{ij} - \bar{y}_i)^2 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 55$$

And the sum of squares due to lack of fit is given by (equation 56);

$$\text{SSLOF} = \sum_{j=1}^{n} \text{n } (\bar{y}_i - \hat{y}_i)^2 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 56$$

The number of degrees of freedom for pure error is n–m while the number of degrees of freedom due to pure error is m–p

The mean sum of squares due to lack of fit is obtained by (equations 57 and 58)

Lack of fit mean sum of squares = $\frac{sum\ of\ squares\ due\ to\ lack\ of\ fit}{degrees\ of\ freedom\ due\ to\ lack\ of\ fit}$ …………….. 57

MSLOF = $\frac{SSLOF}{m-p}$ ………………………………………………………………. 58

The mean sum of squares due to pure error is given by (equation 59 and 60);

Mean sum of squares due to pure error = $\frac{sum\ of\ squares\ due\ to\ pure\ error}{no\ of\ degrees\ of\ freedom\ due\ to\ pure\ error}$ 59

MSPE = $\frac{SSPE}{n-m}$ ………………………………………………………………. 60

The test statistic for the lack of fit is obtained as shown in equation 61;

$F_{computed} = \frac{MSLOF}{MSPE}$ ……………………………………………………….. 61

The statistic follows an F – distribution with (m–p, n–m) degrees of freedom. The regression function is said to be non – linear if the $F_{computed}$ is greater than $F_{(\alpha,m-p,n-m)}$. If this conclusion is made, then the model should be abandoned and a more appropriate equation should be found.

2.4.4 Experimental Designs for Fitting Response Surfaces

Suppose first order model with k variables is to be fitted equation 62;

y = $\alpha_0 + \sum_{i=1}^{k} \alpha_i x_i + \varepsilon$ ……………………………………………… 62

Then there exists a unique class of designs that minimises the variance of the parameter estimates $\hat{\alpha}_i$. This class of designs are the orthogonal first order designs. These designs have the off-diagonal elements of the X'X being all zero's. Examples of the orthogonal first order designs are the 2^k factorial and the fractions of the 2^k series and the simplex designs. A simplex design is a regular side figure with k + 1 vertices k dimensions. A case of a simplex design can be shown diagrammatically (Figure 11 & 12).

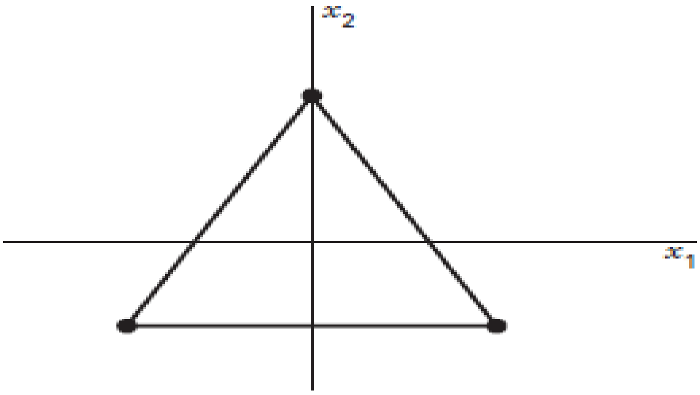

Figure 11: A case of simplex design with k = 2 variables

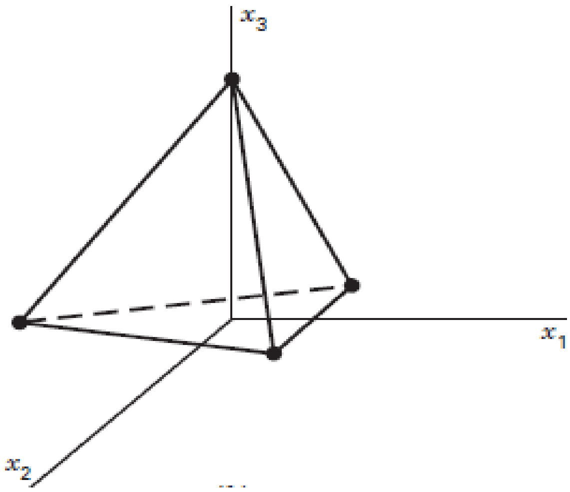

Figure 12: A case of a simplex design with k = 3 variables

One of the most popular designs for fitting a second order model is the central composite design or the CCD design (Montgomery, 2005). A CCD design consists of a 2^k factorial with n_F factorial runs, 2k axial or star runs and n_C center runs. A case of a central composite design can be shown diagrammatically (Figure 13 & 14). The main parameters in the CCD design are: α (which is the distance of the axial runs from the design centre) and n_C (which is the number of centre points).

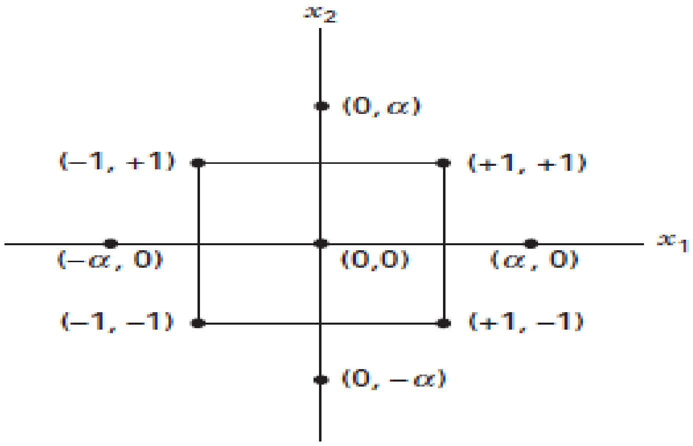

Figure 13: A case of the central composite design for k = 2 variables (Myers *et al.*, 2009).

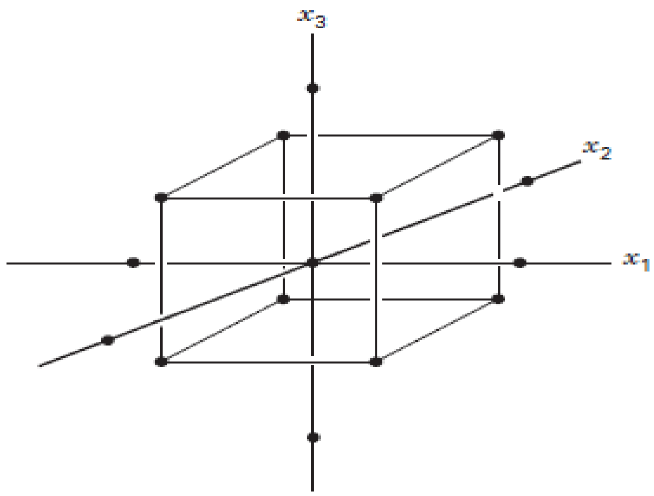

Figure 14: A case of the central composite design for k = 3 variables (Myers *et al.*, 2009).

Another design used in fitting the 2nd order model is the box- Behnknen design. This design is formed by combining the 2^k factorials and the incomplete block designs. The efficiency of the box Behnken design is based on the number of experimental runs and that they are rotatable or nearly rotatable (Montgomery, 2013). The geometrical appearance of the box Behnken design can also be shown diagrammatically (Figure 14 & 15).

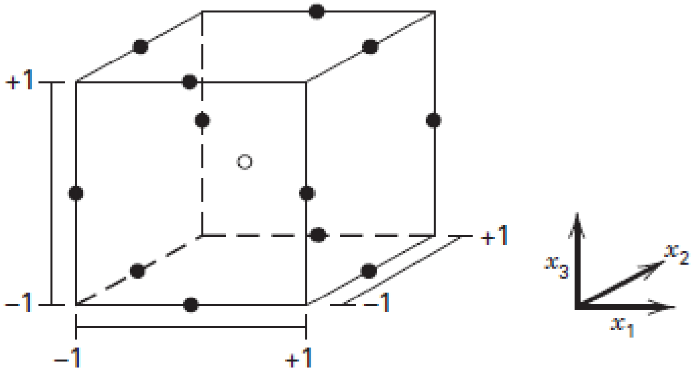

Figure 15: A case of a box – Behnken design with three factors (Montgomery, 2005)

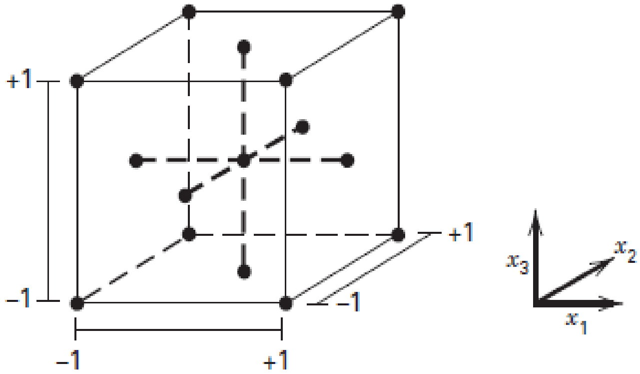

Figure 16: A case of a face centered box – Behnken design for k = 3 (Montgomery, 2005).

2.5 Applications of Response Surface Methodology

Response Surface Methodology is useful for designing, developing, improving and optimizing processes where a response or responses are affected by several variables (Mayers *et al.*, 2004). The Response Surface Methodology was first developed in the 1950s (Box, 1951). Most application of Response Surface Methodology is in industrial, biological and clinical science, social science, food science, physical and engineering sciences. Back in 1951 when response surface methodology was first introduced by Box and Wilson, the experimental runs were reduced enormously compared to the number of runs determined using full factorial design. Besides the reduction of experimental runs, the results obtained from response surface methodology are claimed to be statistically suitable (Tan *et al.*, 2009). By applying the RSM method in the optimization process, only a short period is required to test all of the variables pertaining to the consumer evaluation, making the laboratory test stage more efficient (Moskowaitz, 1994). Besides that, parameter estimation can identify the variables that are largely affecting the model which then helps the researcher to focus on those particular variables that contribute to the product acceptable (Schutz, 1983).

Response Surface Methodology has been applied in various experimental designs involving the extraction process and food preservation (Huang & Zhang, 2008). For example, Liu has chosen to extract anthocyanin from purple sweet potato according to four-factor parameters which were temperature, the ratio of ethanol to ammonium sulphate, time of extraction and pH value (Liu *et al*., 2013). The experimental results were fitted to polynomial equation specifically second order regression. The knowledge of the interaction between the factors is crucial to find the output-input relationship. This is the reason that the interactions are hardly determined using one factor at a time approach (Elksibi *et al*., 2014).

Response Surface Methodology has also been applied in the optimization of leaching parameters for ash reduction from low-grade coal (Behera *et al*., 2018). Cevheroğlu Çıra *et al*. (2016) applied response surface methodology in modelling and optimization of marble surface quality. It has also been applied in biotechnology in the optimization of extracellular glucoamylase production by *Candida guilliermondii* (Mohamed *et al*., 2017). By establishing a model equation, RSM can evaluate the relationship as well as interactions among the multiple parameters using quantitative data. Both Box-Behnken design (BBD) and CCD designs have been used to optimize various processes but many researchers have applied CCD. Some examples of CCD studies are; Separation of β-blockers by ion-pair capillary electrophoresis (Servais *et al*., 2002) and Optimization of Calmium determination by flame atomic absorption Spectrometry (Kokya & Farhadi, 2009).

Response surface methodology model and ANNGA (for experimental data modelling and optimization) may be used to describe the relationship between dietary nutrient concentrations and broiler performance to achieve the optimal target (Ahmadi & Golian, 2011). Mehri (2014) introduced a novel multi-objective algorithm, desirability function, for optimization the bird response models based on response surface methodology (RSM) and artificial neural network (ANN). The study showed that the most desirable function was obtained for an ANN-based model (Mehri, 2014). However, some studies have used both CCD and BBD and gotten the similar results. For instance, the comparative study of the application of CCD and BBD in studying the effect of demographic characteristics on HIV risk in South Africa (Sibanda & Grobler, 2013).

CHAPTER THREE
METHODOLOGY

3.1 Location of the Study

The study was conducted at the poultry demonstration unit of Chuka University. Chuka University is situated in Tharaka Nithi County in Meru South, Ndagani along the Nairobi-Meru highway. It's approximately 186 km North-East from Nairobi city, in a rural setting on the eastern slopes of Mt Kenya. The study site is within approximately 0.33^0 South latitudes, 37.65° East longitude (climate-Data.org) and 1560 m above the sea level. The climate is warm and temperature, with average annual temperatures of about 19.5°C. It receives an annual rainfall of 750 mm. This study was done from December 2018 to April 2019. This region is a potential agricultural area where farming is characterized by both rearing of livestock and growing of crops.

3.2 Research Design and Experiment

3.2.1 Research Design

The study employed the Central Composite Design (CCD) consisting of 13 experimental runs determined by full factorial design, which is an effective design for fitting the second-order model. Central Composite Design was used to optimize the weight gain of Kenbro chicken. A 5-level-2 factor Central Composite Design was employed in the Kenbro weight gain experiment where optimization required 13 experimental runs. Response Surface Methodology was used to determine the "surface" or the association between the response and the factors affecting the response.

Table 3: Full Factorial Central Composite Design Matrix

run	coded values		actual values	
	x1	x2	pumpkin	weeks
1	-1	-1	50	1
2	1	-1	150	1
3	-1	1	50	3
4	1	1	150	3
5	-1.4142	0	0	2
6	1.4142	0	200	2
7	0	-1.4142	100	0
8	0	1.4142	100	4
9	0	0	100	2
10	0	0	100	2
11	0	0	100	2
12	0	0	100	2
13	0	0	100	2

X_1 is the pumpkin seed extract, X_2 is the number of weeks

3.2.2 Method of Preparing Pumpkin Seed Extracts

The seeds were removed from mature fruits (Opica F1 green variety) and dried at room temperature until a constant weight was reached. The seeds were then grounded along with husks using a laboratory blender and stored in the dark at 22°C prior to use (Schinas *et al*., 2009). Extractions will be performed in order to recover the polar and semi-polar molecules. The extracts were prepared by macerating 10 g of powdered pumpkin seeds in 50 ml of an extracting agent, using a thermostated magnetic stirrer for 4 hours (Schinas *et al*., 2009).Then the extract was filtered through soft filter paper and subjected to lyophilization (Labconco, Free Zone 2.5, Kansas, MO, and USA). Then moistening of the sample with the extracting agent for 30 min. The extracts were obtained in the dark. After drying, the extracts were stored in desiccators at a temperature of 22°C in low light conditions.

3.2.3 Experiment

The study used a Random Complete Block Design (RCBD) with two different factors that is pumpkin seed extract (0, 50, 100, 150 and 200 ml) and time in weeks replicated three times. The study used sixty Kenbro chicken in fifteen cages each cage with four Kenbro chicken. In each block, there were five cages (C1, C2, C3, C4, and C5). C1 was the control group (0 ml) while C2, C3, C4, and C5 were feed with 50, 100, 150 and 200 ml of pumpkin seed extracts per litre of water respectively. The pumpkin seed extract was administered for eight weeks.

3.3 Data Collection

The birds were weighed at the beginning of the experiment and thereafter at an interval of three days for eight weeks. The weight was measured in kilograms using the weighing scale (Hook weighing scale). Chicken were provided with feeds and water ad libitum at 8 am. The amount of feed offered to each group was weighed daily at 8 am. The leftovers were collected and weighed in the next morning. The difference between feed offered and leftovers gave the feed intake.

All chicken were vaccinated against Newcastle Disease and Gumboro. Growth performance was computed using live weight and chicken's growth rate. Weight gained and growth rate were evaluated using the formulas (equation 63 and 64);

$$WG = LW_1 - LW_0 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \; 63$$

$$GR = {WG}/{LW_0} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \; 64$$

Where WG is the weight gain (after every three days), GR growth rate, LW_0 live weight for the previous period and LW_1 live weight for a particular period.

Faecal samples will be collected once per week at 8 am for eight weeks during the entire duration of the experiment for each treatment. Modified Mc Master Techniques will be used to count the number of parasites eggs present. Faecal samples will be dried at 55^0C in a forced draft oven for 72 hours, then ground through 1 mm screen using a Wiley mill to determine apparent digestibility.

3.4 Data Analysis

Central Composite Design (CCD) was adopted for this study because it requires a few points and that information on quantitative variables is used effectively. Since the study employed Central Composite Design (CCD) the data on factor was coded according to the formulae (equation 65);

$$X_i = \frac{x_i - \theta}{\Phi} \quad \text{...} 65$$

Where X_i is the coded value of the i^{th} variable, x_i is an encoded value of the i^{th} test variable (equations 66 and 67);

$$\theta = \frac{X_{max} + X_{min}}{2} \quad \text{...} 66$$

$$\Phi = \frac{X_{max} - X_{min}}{2} \quad \text{...} 67$$

The least squared technique was employed to forecast the quadratic polynomial model for the Kenbro weight gain. The response surface plot was generated to illustrate the shape of the surface and trace the optimum with reasonable precision. Analysis of a second-order model was done using computer software (R-program) by implementing the R codes (Appendix 1). R software provides a wide variety of statistical (linear and nonlinear modelling, classical statistical tests, time-series analysis, classification, clustering among others) and graphical techniques, and is highly extensible (Korhoňová *et al.*, 2009). Finally, Analysis of Variance (ANOVA) was used to check the adequacy of the model for the optimization of the weight gain of Kenbro chicken at 95% confidence interval.

3.5 Mathematical Model

3.5.1 Second-Order Model in Response Surface Methodology

The central composite design (CCD) was used to develop the second order model representing the Kenbro weight gain as a function of pumpkin seed extract and time. To describe the response equation, we let X_1 and X_2 define the two factors. The second order model was expressed as follows (equation 68);

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum\sum_{i<j} \beta_{ij} X_i X_j + \varepsilon \quad \text{....................} 68$$

Where Y is the response, β_0 is constant, β_i is the linear coefficient, β_{ii} is the quadratic coefficient, and β_{ij} is the cross product coefficient between x_i and x_j and ε is the random error term which is normally distributed with mean zero and variance of one. In this study Y is the weight gain of the Kenbro chicken, X_1 is the amount of pumpkin seed extract and X_2 is the number of weeks. In matrix form, it is given as in (equation 69);

$$y = X\beta + \varepsilon \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \; 69$$

Where $Y = \begin{bmatrix} y_1 \\ y_2 \\ \vdots \\ y_n \end{bmatrix}$, $\beta = \begin{bmatrix} \beta_1 \\ \beta_2 \\ \vdots \\ \beta_n \end{bmatrix}$ $\varepsilon = \begin{bmatrix} \varepsilon_1 \\ \varepsilon_2 \\ \vdots \\ \varepsilon_n \end{bmatrix}$ and $X = \begin{pmatrix} x_{11} & \cdots & x_{1k} \\ \vdots & \ddots & \vdots \\ x_{n1} & \cdots & x_{nk} \end{pmatrix}$

Where Y is the response vector, X is a matrix of the chosen experimental design, β is the vector constituted by parameters of the model and ε is the residual.

This second order model used for the optimisation of the weight gain was an improvement of the linear models that have been previously used in other studies.
The linear model which have been used in other studies is given by equation 70;

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \ldots + \beta_p x_p \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \; 70$$

where β's are the coefficients, Y is the dependent variable and $X_{1,}$ ……,Xp are independent variables.

The experiment was carried out as a CCD consisting of 13 experimental runs determined by the 2^2 full factorial designs with four axial points and five centre points as shown below. The CCD was adopted for this study because it requires few points and that information on quantitative variables is used effectively (Montgomery, 2005).

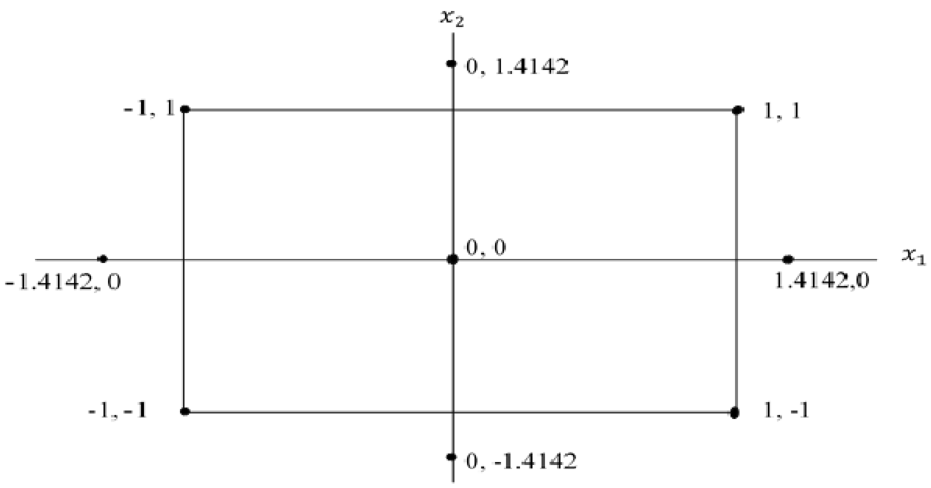

Figure 17: A case of the central composite design for k = 2 variables

3.5.2 Model Validation

Analysis of variance (ANOVA) is a statistical decision-making tool used for detecting any differences in the average performance of tested parameters (Muriithi, 2015). It employs the sum of squares and F-statistic to find out the relative importance of the analysing process, parameter measurement errors and uncontrolled parameters. The analysis of variance for fitting the data to the second- order and contour plots helped characterize the response surface. ANOVA was adopted to check the adequacy of the model for the response in the experiment at 95% confidence level.

3.6 Modelling Process

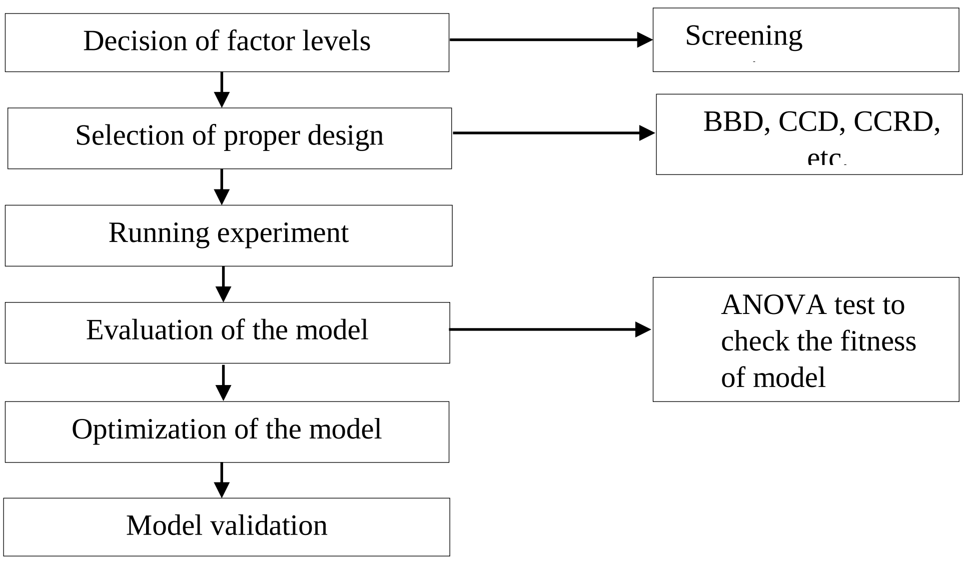

Figure 18: Model Development Process

3.7 Ethical Issues

The study was carried ethically by ensuring that chickens are held with care and also security of the data collected observed. In this regard, all the data collected was used only for the purpose of this study. All secondary data sources involved in this study were acknowledged and cited accordingly. The study ensured that the laid down policies are followed and should there be the need for the use of the study result for policy matters, the information will be released to requesting institution in consultation with Chuka University. Further, the researcher sought permission from the Chuka University ethics committee (Appendix 2) and research permit from National Commission for Science, Technology, and Innovation (NACOSTI; Appendix 3; Appendix 4).

CHAPTER FOUR
RESULTS AND DISCUSSIONS

4.1 Preliminary Analysis

4.1.1 Summary Statistics

The normality test and descriptive statistics of the weight gain of Kenbro chicken were computed and the results presented in the subsequent tables. The normality of the data was tested to assess whether or not the data set is approximately normally distributed. In addition, the skewness goodness of fit test was used to check whether the data set was normally distributed. Skewness is used to determine whether the frequency distribution curve of the data is not a symmetric bell-shaped curve making it stretched more to one side than the other thus implying that the data is not normally distributed. The result of the study showed that the minimum and maximum weight gain of Kenbro chicken was 0.072 kg and 0.254 kg, respectively (Table 4). The average weight gain of Kenbro chicken was 0.180 kg with a median of 0.165 and a standard deviation of 0.48 (Table 4).

Table 4: Summary statistics for weight gain of the Kenbro chicken

Mean	Standard Deviation	Median	Skewness	Max	Min
0.1799	0.4761	0.1650	-0.652	0.2544	0.0724

Since the standard deviation was small it means that most values of the weight gain of Kenbro chicken were very close to the average weight gain. This result is similar to the findings of Leys *et al*. (2013) who indicated that standard deviation is used to tell how other values are spread out from the mean. If the standard deviation is small, it means that most of the values are very close to the average. Regarding the normality test of the data, the data on the weight gain of Kenbro chicken indicated that the data were normally distributed since the skewness test value was falling within the range of ±3. The skewness value for weight gain of Kenbro chicken was 3 > -0.652 > -3 (Table 4). This is in agreement with reports of Aczel and Sounderpadian (2002) who concluded that if the skewness value is between a range of ±3 then the data being analysed is normally distributed.

4.1.2 Error Bars for Block Means

Error bars for block means were generated as graphical representations of the variability of data, which were used on graphs to indicate the error or uncertainty in a reported measurement. The standard deviation error bars give a sense of whether or not a difference is statistically significant. When standard deviation error bars overlap, it's a clue that the means difference is not statistically significant (Payton *et al*., 2000). When standard deviation error bars overlap even less, it's a clue that the difference is probably not statistically significant. When standard deviation error bars do not overlap, it's a clue that the difference may be significant (Cumming *et al*., 2004).

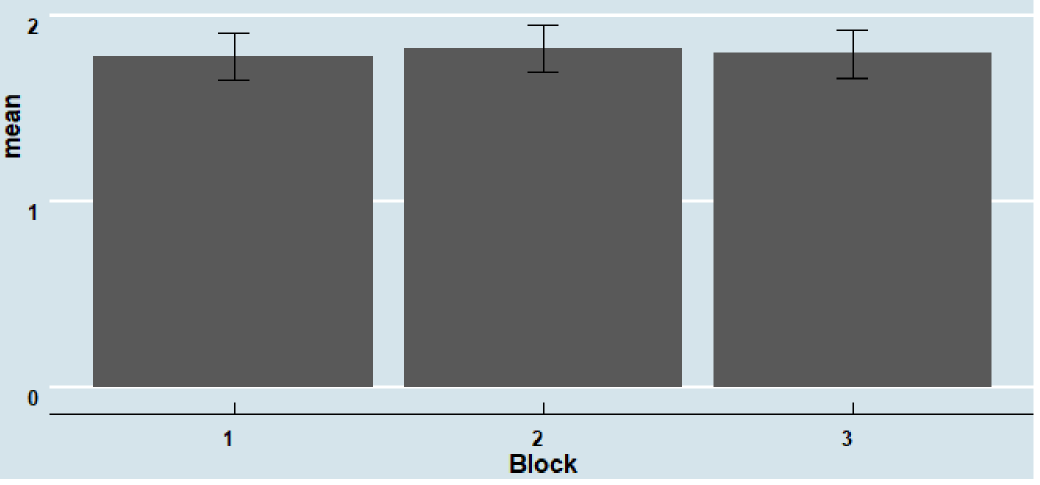

Figure 19: Error bars for block means

The average weight per block shows that the error bars for block overlap at 95% confidence interval. This shows that blocks had no significant effect on average weight gain at 0.05 level of significance (Figure 19). This means that the data from block one is not different from data in block two and block three. The findings implied that the blocking of the experimental units was effective.

4.2 Determining the Effect of Pumpkin Seeds Extract on the Weight Gain of Kenbro Chicken

4.2.1 Summary Statistics and Analysis of Variance for Total Body Weight Gain of Kenbro Chicken

The total body weight gain was computed as the difference between the weights of Kenbro chicken at week seven and the weight at week one of the experiment. The average total body weight gain of Kenbro chicken ranged from 0.77 kg, to 1.55 kg for 0 ml and 200 ml of pumpkin seed extract per litre of water, respectively (Table 5). This indicated that the weight gain increased with increase in amount of pumpkin seed extract.

Table 5: Summary statistics for total body weight (kg) gain of Kenbro chicken

Factor Level	Mean	Standard Deviation	Median	Skewness	Kurtosis
0	0.7672a	1.0815	0.6958	0.4205	-0.451
50	1.1994ab	1.2093	1.0225	-0.1494	-1.1195
100	1.3496ab	1.0875	1.1651	-0.255	-1.1264
150	1.5078ab	0.7082	1.4880	0.5058	-0.0876
200	1.5483b	1.5113	1.4988	-1.8239	0.6922
LSD	0.5112				

[a]means followed by the same letter are significantly different at 5% probability level

Generally, there was a positive relationship between weight gain and amount of pumpkin seed extract used. Kenbro chicken fed with no extract from pumpkin seed had the lowest weight gain while chicken fed with 200 ml of pumpkin seed extract per litre of water had the highest weight gain (Table 5). This pattern of total body weight gain of Kenbro chicken with an increase in the amount of pumpkin seed extract showed that pumpkin seed extract had a positive effect on the total body weight gain of Kenbro chicken. Analysis of variance for the total body weight gain of Kenbro chicken was carried out to determine if the treatment had a significant effect on the total body weight gain of the Kenbro chicken and whether the blocking was effective at $\alpha = 0.05$ level. The data for total body gain was subjected to normality test and the test indicated that the data was normally distributed since their skewness and kurtosis test values were within the range of ±3 and ±1 (Table 5).

The hypotheses were;

$$H_0\text{: } \mu_1 = \mu_2 \ldots = \mu_{35}$$

$$H_1\text{: } \mu_1 \neq \mu_2 \ldots \neq \mu_{35}$$

$$H_0\text{: } \beta_1 = \beta_2 = \beta_3$$

$$H_1\text{: } \beta_1 \neq \beta_2 \neq \beta_3$$

The results indicated that there was significant ($F = 10.11$, $p = 2.01E-10$) treatment effects at 5% probability level (Table 6). Thus rejecting the null hypothesis and concluded that treatments had a significant ($p < 0.05$) effect on total body weight gain of Kenbro chicken. Moreover, the results indicate that the blocking of experimental unit was effective ($F = 0.03$, p-value = 0.62; Table 6). Thus, accepting the null hypothesis that blocking was effective and conclude that the means of blocks are equal. Since the treatments were significant, the least significant difference was used to separate the significant means at $\alpha = 0.05$ (Table 5).

Table 6: Analysis of variance for total body weight gain of Kenbro chickens

Term	df	SS	MS	F value	p-value
Blocks	1	0.028364	0.028364	0.030108	0.623041
Treatments	34	323.9394	9.527629	10.11343	2.01E-10
Residuals	35	32.9727	0.942077		

The results of this study revealed that pumpkin seed extract have significant ($p < 0.05$) effect on total weight gain by the Kenbro chicken (Table 5), with 200 ml of the pumpkin seed extract giving significantly different results from the other pumpkin seed extract treatments (Table 5). The findings of this study are in line with the findings of a study by Wafar *et al*. (2017). Wafar *et al*. (2017) studied the effect of pumpkin seed meal on various growth and performance variables in Broiler chicken and showed that the final body weight gain significantly increased ($p < 0.05$) as the levels of pumpkin seed meal increased in the diets. The increase in total body weight gain with increase in the level of pumpkin seed meal was attributed to balance in nutrients composition and proper metabolism associated with the seed meal (Wafar *et al*. (2017). Therefore, it can be concluded that the increased total weight gain of Kenbro chicken observed in this study may be as results on enhanced crude protein and metabolism associated with the pumpkin seed extract. Pumpkin seeds have been shown to be rich on proteins (24 to 36.5 %) and oil (31.5 to 51%), which can boast chicken growth performance (Al-Khalifa, 1996; Rezig *et al.*, 2012). Moreover, pumpkin seed extract anthelmintic properties (Aziza *et al.*, 2018). The implication is that pumpkin seed extract can be used to control internal chicken parasites. Hence, this can also partly explain why the higher total weight gain on chicken fed with pumpkin seed extract compared to those not fed with it.

4.2.2 Summary Statistics and Analysis of Variance for Final Body Weight of Kenbro Chicken

Final body weight was taken to be the weight of Kenbro chicken at the end of the experiment. The average final body weight of Kenbro chicken was higher for chicken fed with pumpkin seed extract than those not fed with it; 1.91 kg, 2.12 kg, 2.25 kg and 2.42 kg for 0 ml, 50 ml, 100 ml, 150 ml, and 200 ml per litre of water, respectively (Table 7) Regarding the normality test, the results indicated that data for final weight of Kenbro chicken was normally distributed since the skewness and kurtosis test values lied within the range of ±3and ±1 respectively (Table 7)

Table 7: Summary statistics for final body weight of Kenbro chicken at the end of the experiment

Factor Level	Mean	Standard Deviation	Median	Skewness	Kurtosis
0	1.7961a	0.2704	1.5990	0.3623	-0.8443
50	1.9134ab	0.3023	1.7806	-0.1287	-1.3155
100	2.1156ab	0.1770	2.1045	0.4358	-0.5881
150	2.2498ab	0.2719	2.1163	-0.2198	-1.3204
200	2.4157b	0.3778	2.2872	-1.5716	0.0768
LSD	0.4042				

[a]means followed by the same letter are significantly different at 5% probability level

Analysis of variance for the final body weight of Kenbro chicken was carried out to determine if the treatment had a significant effect on the final body weight of the Kenbro chicken and whether the blocking was effective at α = 0.05 level. The hypotheses tested were;

$$H_0: \mu_1 = \mu_2 \ldots = \mu_{35}$$

$$H_1: \mu_1 \neq \mu_2 \ldots \neq \mu_{35}$$

$$H_0: \beta_1 = \beta_2 = \beta_3$$

$$H_1: \beta_1 \neq \beta_2 \neq \beta_3$$

The results indicate that there was significant (F = 10.17, p = 2.71E-10) treatment effects at 5% probability level (Table 8). Thus rejecting the null hypothesis and concluded that treatments had a significant ($p < 0.05$) effect on final body weight of Kenbro chicken. Moreover, the results indicate that the blocking of experimental unit was effective (F = 0.051, p-value = 0.82; Table 8). Thus, accepting the null hypothesis that blocking was effective and conclude that the means of blocks are equal. Since the treatments were significant, the least significant difference was used to separate the significant means at $\alpha = 0.05$ (Table 7).

Table 8: Analysis of variance for final body weight of Kenbro chicken at the end of the experiment

Term	df	Sum Sq	Mean Sq	F value	Pr(>F)
Blocks	1	0.003023	0.003023	0.051331	0.822081
Treatments	34	20.37121	0.599153	10.17445	2.71E-10
Residuals	35	2.061075	0.058888		

There was an increase in the final body weight of Kenbro chicken as the amount of pumpkin seed extract increased. This revealed that there was a significant ($p < 0.05$) effect of pumpkin seed extract on the final body weight of the Kenbro chicken (Table 7). The findings of this study were also in agreement with the findings of a study conducted by Nworgu *et al.,* (2007). Nworgu *et al.,* (2007) investigated the performance of broiler chickens served with heat-treated fluted pumpkin (*Telfaria occidentalis*) leaves extract supplement. The results revealed that fluted pumpkin (*Telfaria occidentalis*) leaves extract served to the broiler chickens had a significant effect on feed intake, weight gain, and, protein efficiency ratio,. Elsewhere, the inclusion of giant pumpkin (squash) seed meal in broilers diets in partial replacement of soybean meal and vegetable oil improved the performance and increased edible carcass parts, while abdominal fat in the carcass was reduced (Aguilar *et al*., 2011). Generally, results from these studies have shown that the extent of bioactivity of the pumpkin seeds may offer stimulation of nutrition and enhancement of resistance to disease (Brooker & Acamovic, 2005). This is similar to what has been seen in this study that pumpkin seed extract has a positive impact on the final body weight of the Kenbro chicken.

4.3 Determination of the Possible Combination of Number of Weeks and Quantity of Pumpkin Seeds Extract on Weight Gain of the Kenbro Chicken.

4.3.1 Summary Statistics for Average Weekly Weight Gain of Kenbro Chicken

The results from this study showed that treatment of 200 ml of pumpkin seed extract in 1 litre of water at 4 weeks had an average weight gain of 0.23 kg with a maximum value of 0.24 kg, a median of 0.23 and a standard deviation of 0.05 (Table 9). The treatment of 0 ml of pumpkin seed extract at 1 week had an average weight gain of 0.075 kg with a maximum value of 0.076 kg, a median of 0.073 and a standard deviation of 0.02 (Table 9). From the results, treatments without pumpkin seed extract gave a consistently lower response to weight gain than other treatments across the time (Table 9). Further, for chicken given treatment of 200 ml of pumpkin seed extract at 4 weeks gave the best performance compared to other treatments of pumpkin seed extract. Therefore 200 ml and 4 weeks were the best combinations of pumpkin seed extract and the number of weeks. This result is similar to the result obtained by Oyegunle *et al*., (2012) who found that combinations of the number of weeks and quantity of fluted pumpkin leave extracts significantly determined the weight of broiler chicken.

In the study on the utilization of giant pumpkin (*Cucurbita moschata*) seed in broiler chicken diets, it was found that the utilization of up to 10% of pumpkin seed meal in broiler chicken diets, had no change in the production performance and the sensorial quality of the meat (Martínez, *et al*., 2010). Elsewhere, results on performances of broiler finisher fed with varying levels of sun-dried *T. occidentalis* leaf meal as dietary supplements revealed that the average daily body weight gain as computed from the differences between average final body weight gain and the average initial body weight of broiler birds were significantly ($p < 0.05$) influenced by the test diet (Imasuen *et al*., 2014). These studies showed that plant materials, just like in this study, has potential to boost weight gain in birds perhaps due to the presence of bioactive phytochemicals which have valuable effects on growth, appetite, immune and nutritional status of the chicken (Alabi *et al*., 2017; Meineri *et al*., 2018).

Table 9: Summary Statistics for average weekly weight gain of Kenbro chicken

Treatment	Mean	Standard Deviation	Median	Skewness	Maximum	Minimum
0 ml - 1 weeks	0.0746l	0.0195	0.0732	-1.1993	0.0762	0.0724
50 ml - 1 weeks	0.1276hij	0.1098	0.12 33	-1.7037	0.1346	0.1150
100 ml - 1 weeks	0.1555fgh	0.4649	0.1261	-1.6655	0.2347	0.1502
150 ml - 1 weeks	0.2037abcde	0.0449	0.1929	-1.1562	0.2096	0.1954
200 ml - 1 weeks	0.2051abcde	0.0444	0.2035	1.4029	0.2101	0.2017
0 ml - 2 weeks	0.0843kl	0.0466	0.0853	-0.8784	0.0884	0.0793
50 ml - 2 weeks	0.1419hi	0.0508	0.1441	-1.5864	0.1455	0.1360
100 ml - 2 weeks	0.1860efg	0.0247	0.1567	-1.6778	0.1571	0.1526
150 ml - 2 weeks	0.2010abcde	0.0175	0.2017	-1.5052	0.2023	0.1990
200 ml - 2 weeks	0.2051abcde	0.0589	0.2112	0.8354	0.2187	0.2071
0 ml - 3 weeks	0.0977jkl	0.1373	0.0929	1.3948	0.1132	0.0871
50 ml - 3 weeks	0.1551fgh	0.0882	0.1576	-1.1758	0.1624	0.1453
100 ml - 3 weeks	0.1911bcdef	0.356	0.1725	1.7076	0.2321	0.1685
150 ml - 3 weeks	0.2131abcde	0.0594	0.2125	0.4892	0.2193	0.1705
200 ml - 3 weeks	0.2184abcde	0.0247	0.2190	-0.9652	0.2205	0.2157
0 ml - 4 weeks	0.1130ijkl	0.1759	0.1099	0.7796	0.1320	0.0972
50 ml - 4 weeks	0.1867defg	0.3028	0.1781	1.3491	0.2224	0.1645
100 ml - 4 weeks	0.1917bcdef	0.1243	0.1902	-1.7319	0.2206	0.1933
150 ml - 4 weeks	0.2155abcde	0.0752	0.2151	0.2286	0.2232	0.1782
200 ml - 4 weeks	0.2322a	0.0536	0.2316	-1.1197	0.2367	0.2263
0 ml - 5 weeks	0.1182hijk	0.1137	0.1191	-0.3812	0.1290	0.1064
50 ml - 5 weeks	0.1883cdefg	0.073	0.1841	1.4263	0.1950	0.1812
100 ml - 5 weeks	0.2005abcde	0.0206	0.2016	-1.7298	0.2017	0.1981
150 ml - 5 weeks	0.2161abcde	0.059	0.2199	1.6807	0.2296	0.2189
200 ml - 5 weeks	0.2263abcd	0.3118	0.2199	-1.5842	0.2485	0.1907
0 ml - 6 weeks	0.1305hij	0.123	0.1281	0.8293	0.1438	0.1195
50 ml - 6 weeks	0.1975abcde	0.0936	0.1955	0.9089	0.2077	0.1893
100 ml - 6 weeks	0.2073abcde	0.0433	0.2069	0.3781	0.2118	0.2032
150 ml - 6 weeks	0.2228abcde	0.2224	0.2206	-1.5705	0.2320	0.1907
200 ml - 6 weeks	0.2306ab	0.3476	0.2275	-1.6677	0.2538	0.1907
0 ml - 7 weeks	0.1489ghi	0.1991	0.1421	1.3585	0.1714	0.1333
50 ml - 7 weeks	0.2023abcde	0.0564	0.2042	-1.3318	0.2068	0.1960
100 ml - 7 weeks	0.2175abcde	0.1242	0.2028	-1.5795	0.2263	0.2033
150 ml - 7 weeks	0.2278abc	0.3313	0.2183	-1.2847	0.2544	0.1907
200 ml - 7 weeks	0.2306ab	0.7567	0.1254	1.5221	0.2022	0.1004
Lsd	0.0402					

[a] means followed by the same letter are significantly different at 5% probability level

4.3.2 Plot for Average Weekly Weight Gain of Kenbro Chicken

The average weekly weight gain had an increasing trend throughout the period. It is also clear that level 200 ml of pumpkin seed extract was superior throughout the period and treatment 200 ml of pumpkin seed extract at 4 weeks had the highest weekly weight gain.

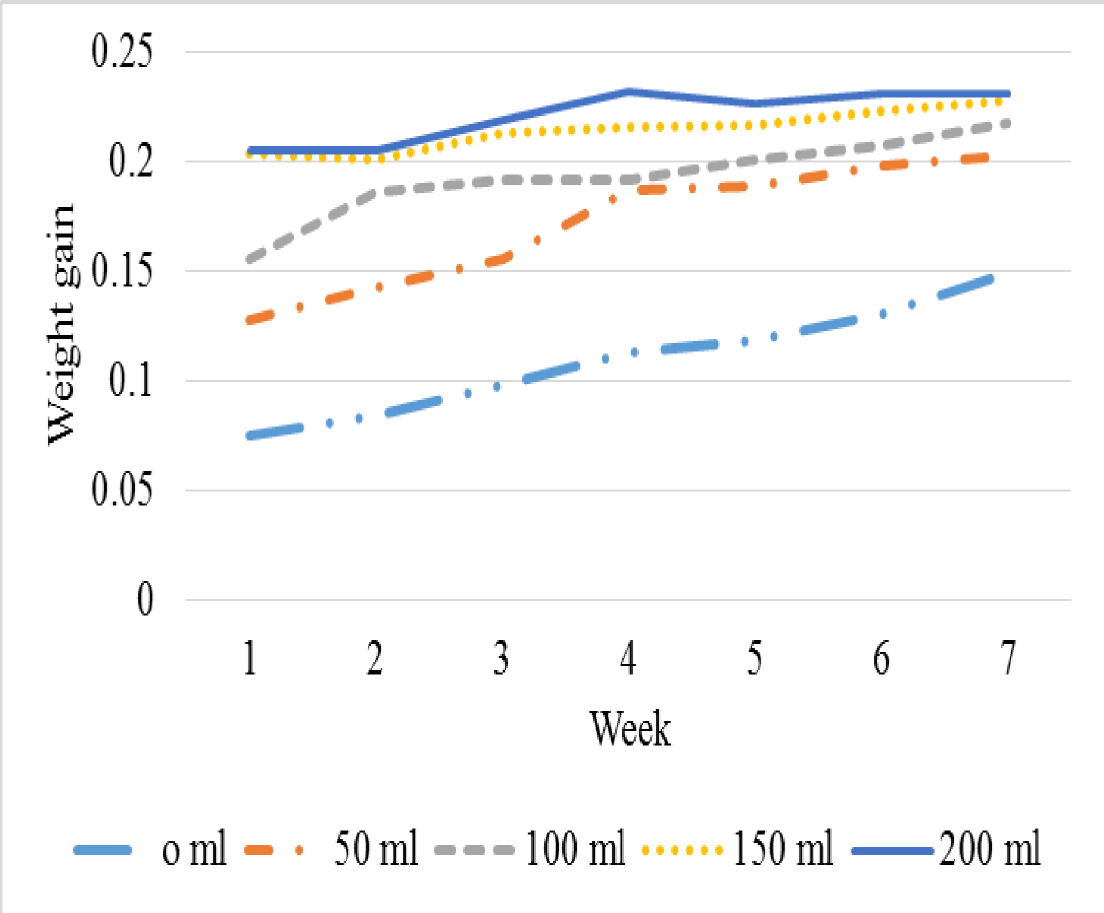

Figure 20: Plot of weight gain against weeks

4.3.3 Factorial Analysis of Variance Model

The F-values for the factors number of weeks, pumpkin seed extract and their interaction were 33.22, 72.89 and 7.18, with corresponding p-values of 1.02E-07, 4.13E-28, and 4.25E-05, respectively (Table 10).

Table 10: Analysis of variance for factors and their interaction

Variable	df	Sum Sq	Mean Sq	F-value	P-value
Pumpkin _ extract	4	15.32482	3.831204	72.88783	4.13E-28
Weeks	1	1.746246	1.746246	33.22196	1.02E-07
Pumpkin _ extract: weeks	4	1.510509	0.377627	7.184274	4.25E-05
Residuals	95	4.993512	0.052563		

The results indicate that there was significant (F-values = 72.89, p =4.13E-28) pumpkin seed extract effects at 5% probability level (Table 10). Thus concluding that pumpkin seed extract had a significant ($p < 0.05$) effect on average body weight of Kenbro chicken. Also the combination of pumpkin seed extract and the number of weeks were significant (F-values = 7.18, p =4.25E-05) at 5% probability level. Therefore, the results obtained in this study were similar to the results obtained in study by Oyegunle *et al*., (2012) who attributed that the main effects of treatment and weeks were highly significant ($p < 0.05$) and also that their interaction was also highly significant and thus there was a strong interaction between treatment and weeks.

4.4 Mathematical Modelling Using the Collected Experimental Data

4.4.1 Fitting a Second Order Model for the Weight Gain of Kenbro Chicken

The coefficient for the second order model for the intercept, pumpkin seed extract, number of weeks, their interactions and quadratic term of pumpkin seed extract and number of weeks were 0.048, 0.102, 0.027, 0.004, -0.002 and 0.001 respectively (Table 11). Their corresponding T values were -2.59, 10.60, 4.21, -4.48, -7.23 and -1.53 respectively (Table 11).

Table 11: Second order response surface model for weight gain of Kenbro chicken

	Estimate	Std Error	T value	Pr(>|t|)
Intercept	0.047992	0.185377	-2.588901	0.011078
Pumpkin _ extract	0.101967	0.096222	10.597047	5.47E-18
Weeks	0.026777	0.063594	4.2104720	5.62E-05
Pumpkin _ extract: weeks	0.003893	0.008681	-4.484032	1.97E-05
Pumpkin _extract^2	-0.002313	0.014674	-7.233783	1.01E-10
Weeks^2	0.001081	0.007088	-1.525584	0.130302

The fitted RSM second order model for weight gain of Kenbro chicken can be presented in a mathematical equation as in equation 71;

$$Y = 0.047992 + 0.101967X_1 + 0.026777X_2 + 0.003893\ X_1X_2 - 0.002313X_1^2 \ \ldots\ldots\ 71$$

Where Y represents the weight gain of Kenbro chicken

X_1 is the pumpkin seed extract

X_2 is the number of weeks

This model is useful for finding the relative influence of the factors by comparing their coefficients.

The study found that both pumpkin seed extract and the number of weeks had positive significant ($p < 0.05$) effects on the weight gain of Kenbro chicken at p-value=5.47E-

18 < 0.05 and 5.62E-05 < 0.05 respectively (Table 11). This model showed that one unit change of pumpkin seed extract or number of weeks influenced Kenbro chicken weight gain by a factor of 0.102 and 0.027 respectively (Table 11). Besides, the study found that combined pumpkin seed extract and number of weeks had a significant effect on the Kenbro weight gain at p-value less than 0.05. The results indicate that a one unit change in combined pumpkin seed extract and number of weeks led to a change in Kenbro weight gain by a factor of 0.004. Finally, it was found that the quadratic terms had a regression coefficient value of -0.002 and 0.001 and a p-value of 1.01E-10 and 0.13 (Table 11). The second-order polynomial model was proposed to explain the degree of weight gain of eight weeks old Kenbro chicken served with pumpkin (*Cucurbita pepo l*) seed extract (Table 11). Since the variables were significant, then the model is also significant and it is adequate in representing the experimental results. This model was an improvement of the models that have been previously used in other studies.

4.4.1 Analysis of Variance for Response Surface Model

Analysis of variance (ANOVA) was used to check the adequacy of the model for the response variable (weight gain) in the experiment at 95% confidence level. The F values for first order, two-way interaction and purely quadratic terms were 99.33, 20.11 and 27.33, respectively (Table 12).

Table 12: Analysis of variance for RSM model

	df	Sum Sq	Mean Sq	F value	Pr(>F)
FO(pumpkin _ extract, weeks)	2	12.57541	6.28770	99.32665945	2.16E-24
TWI(pumpkin _ extract, weeks)	1	1.27281	1.27281	20.10755027	1.97E-05
PQ(pumpkin _ extract, weeks)	2	3.45984	1.72992	27.32751353	3.55E-10
Residuals	99	6.26702	0.06330		
Lack of fit	4	1.27351	0.31838	6.05745814	0.147023
Pure Error	95	4.99351	0.05256		

First order (FO), two-way interaction (TWI) and purely quadratic (PQ)

From the analysis of variance of the second-order response surface model, the first order term; two-way interaction term and the purely quadratic term were significant at 95% confidence level, as p-values are lower than 0.05 (Table 12). This is evidenced by a low P-value of 2.16E-24, 1.97E-05, and 3.55E-10, respectively. Further, the study found that lack of fit was insignificant in the model and so the study concludes that the model is adequate satisfying the adequacy conditions hence the model is suitable for prediction purposes in this study. Enujeke (2013) and Muriithi (2015) also reported similar findings. That the second order model was significant since the lack of fit was insignificant. Also the findings are similar to a study on central composite design (CCD; 5 levels and 4 factors), response surface methodology and artificial neural network-genetic algorithm used to evaluate the response of broiler chicks [feed conversion ratio] to dietary standardized ileal digestible protein, lysine, total sulphur amino acids, and threonine (Ahmadi & Golian, 2011). The results of this study revealed that the platform of CCD (for conducting growth trials with minimum treatments), response surface methodology model, and artificial neural network-genetic algorithm (for experimental data modelling and optimization) may be used to describe the relationship between dietary nutrient concentrations and broiler performance to achieve the optimal target (Ahmadi & Golian, 2011).

4.4.2 Model Summary for the Fitted Second-Order Surface Response Model

Model summary statistics focus on the model maximizing the Adjusted R- Squared and the predicted R-Squared. Adjusted R-Squared is used to adjust the statistic based on the number of independent variables in the model. It compares the explanatory power of the regression model that contains different independent predictors. The values of Adjusted R^2 and R^2 were 0.78 and 0.83 respectively with the corresponding p-value of 2.20E-16 (Table 13).

Table 13: Model Summary Statistics

Source	R^2	Adjusted R^2	F-value	P-value
Quadratic	0.8342	0.7807	54.68	2.20E-16

R-Squared refers to a measure of the proportion of the variation in the dependent variable that is explained by the independent variable for a regression model. In this case, since the multiple regression model has more than one variable, the adjusted R-Squared is the most preferred. The study showed that the quadratic model fitted using the data had an adjusted R-Squared value of 0.78. This implies that the model explains about 78% of the variability in the response variable (weight gain). Therefore only 22% of the variability in the response variable is explained by the other variables outside the model. The adjusted R-Squared is often used to summarize the fit as it takes into account the number of variables in the model. These findings coincide with the result of a study on response surface models to predict broiler performance and applications for economic analysis which concluded that the model was accurate, precise and not biased (Faria *et al*., 2008).

4.5 Response Surface and Contour Plot

Since the response surface is explained by the second order model, it is necessary to analyse the optimum setting. Response surface methodology can be illustrated with three - dimensional plots by presenting the response in the function of two factors. The findings were presented in Figure 21.

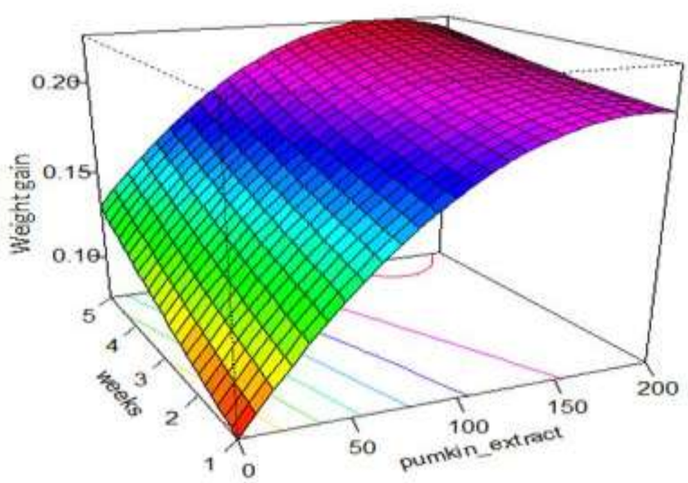

Figure 21: Average Kenbro weight gain as a function of pumpkin seed extract and the number of weeks

Contour plots play a very important role in the study of the response surface. By generating contour plots using R-software for response analysis, the experimenter can usually characterize the shape of the surface and locate the optimum with reasonable precision. This graphical visualization is very helpful in understanding the second order response surface. The findings in figure 21 shows growth of Kenbro chicken in terms of weight gain expressed as a function of pumpkin seed extract and number of weeks (). It was observed that, increasing pumpkin seed extract from low to high resulted in an increased weight gain of Kenbro chicken. The optimal rate of application of the pumpkin seed extract was 192.40 ml while the optimal number of weeks was 3.18 weeks. These are the level of the factors that can guarantee the farmer maximum weight gain of Kenbro chicken without incurring the extra cost of input.

Other studies have found similar results. For instance, a study on the application of factorial design and response surface methodology on the growth rate of broiler chickens served with fluted pumpkin leaves extract (Oyegunle *et al*., 2012). The fitted response surface model indicated that the number of weeks and quantity of fluted pumpkin leaves extracts together with their interaction significantly determined the weight of broiler chickens. Therefore, the study concluded that the number of weeks, fluted pumpkin leaves extract and their interaction plays a key role in obtaining the maximum weight of broiler chickens (Oyegunle *et al*., 2012). Elsewhere, the results from a study carried out to elaborate response surface models using broiler performance data, showed that response surface models are effective to predict the performance of broiler chicken and allow the elaboration of economic analyses to optimize profit (Faria *et al*., 2008). These findings concur with the results obtained in this study.

CHAPTER FIVE

SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1 Summary of the Study Findings

The first objective of the study was to determine the effects of pumpkin seed extract and the number of weeks on the weight gain of Kenbro chicken. The study found that there was an increasing trend of the weight gain of Kenbro chicken with an increase in pumpkin seed extract. This shows that pumpkin seed extract had a positive significant effect on the weight gain of Kenbro chicken. Generally, this implies that chicken that received adequate amount of pumpkin seed extract had higher weight gain not only because of an adequate nutrient but also because of the deworming effect of pumpkin seed extract.

The second objective of the study was to fit an appropriate second-order polynomial model that best fits the data. A second order polynomial model was obtained by employing a least squares technique for the prediction of Kenbro weight gain. This regression model was tested using analysis of variance for residuals minimization that the predicted response model was statistically significant with an adjusted R–squared value of 0.78. The study also found that there was no significant lack of fit in the model and so the study concluded that the response model was adequate.

The third objective of the study was to determine the possible combination of the number of weeks and quantity of pumpkin seed extract on weight gain of the Kenbro chicken. The study found that the interaction between the number of weeks and quantity of pumpkin seed extract had a positive significant effect on the weight gain of the Kenbro chicken. In this study, the optimal levels of pumpkin seed extract and weeks as obtained from the stationary point of the response surface were 192.40 ml and 3.18 weeks respectively. Therefore, this study concluded that if the farmers implement these levels in the production of Kenbro chicken, they would optimize their production without incurring an extra cost in the inputs.

5.2 Conclusion

This research was carried out at the poultry demonstration unit of Chuka University to assess the weight gained by Kenbro chicken served with pumpkin seed extract and also to examine the possible combination of the number of weeks and quantity of pumpkin seeds extract that can result in the maximum weight gain of the Kenbro chicken. The parameters assessed to achieve the objective of the study were total body weight gain, weekly weight gain and final body weight gain. The study found that the main effects (number of weeks and quantity of pumpkin seeds extract) were more influential on the weight gain of the Kenbro chicken. The interactive component was more significant compared to the pure quadratic terms.

Optimal conditions for the weight gain of Kenbro chicken by the application of design of experiments using Response Surface Methodology were investigated. A second order polynomial model was suggested for the prediction of weight gain of Kenbro chicken. The multiple adjusted R-squared value was 78% thus indicating an acceptable fitting to the experimental data. The variance analysis of the model proved that the number of weeks and quantity of pumpkin seeds extract were significant factors.

The optimal settings of number of weeks and quantity of pumpkin seeds extract were obtained by solving the fitted second order models and using the response surface contour plots. The optimal operating conditions were 192.40 ml of pumpkin seeds extract and 3.18 weeks. It can thus be concluded that the number of weeks, pumpkin seed extract and their interactions play a key role in optimizing the maximum weight gain of Kenbro chicken. These factors should be put into consideration in making feed and rearing Kenbro chickens. It can thus be concluded that use of mathematical models for poultry production based on statistics can be useful for predicting and understanding the effects of experimental factors.

5.3 Recommendation

Based on the findings of this study, the following recommendations were made

i. Pumpkin seed extract should be put into consideration when making poultry feeds which would translate to improved production and financial performance of small scale poultry farmers.

ii. In order to create much awareness of RSM on the Agricultural sector the study recommends joint development by agriculturalists and statisticians to model agricultural problems using RSM.

5.4 Suggestion for further Research

i. There is need to find other factors which are determinants of the weight of Kenbro chicken and use them in optimization modelling together with the pumpkin seed extract and number of weeks. This will help in finding out if optimal combinations of more factors would yield more weight gain in Kenbro chicken when compared to just optimizing the pumpkin seed extracts and time.

ii. There is a need to find out also if the optimal combinations of pumpkin seed extract and the number of weeks would yield the same amount of weight in another chicken breed other than Kenbro chicken.

iii. Response surface methodology is a parametric model and its performance would be affected if the data collected or available does not take the functional form of the response. There is, therefore, a need to evaluate the performance of the non-parametric model in optimization as compared to the response surface methodology which is by far the best optimization parametric model.

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APPENDIX 1
R CODES

```
librarweight(pacman)
p_load(data.table, stringr, magrittr, rsm, tidweightverse, readxl)
setwd("E:/mugendi/charles")
load("dat.rda")
hist(dat$weight, col = "black", xlab = "weight", main = "Histogram of weight", breaks
= 5)
sum_stats <- dat %>% summarise(mean = mean(weight), sdparitweight =sd(weight),
md = median(weight),
        skew = skewness(weight), kurt = kurtosis(weight),
        max = max(weight), min = min(weight))
mynames <- c("Mean", "Standard Deviation", "Median", "Skweness", "Kurtosis",
          "Maximum", "Minimum")
names(sum_stats) <- c("Mean", "Standard Deviation", "Median", "Skweness",
"Kurtosis",
                     "Maximum", "Minimum")
sum_stats[, 1:7] <- apply(sum_stats[, 1:7], 2, round, 4)
write.csv(sum_stats, file = "sum_stats general.csv", row.names = F)
dat1 <- dat
dat1[, pumkin_extract := factor(pumkin_extract, levels = 1:5, labels = c(0, 50, 100,
150, 200))]
setorder(dat1, plots)
dat1[, treatments := paste(paste(pumkin_extract, "ml"), paste(weeks,"weeks"), sep =
"-")]
write.csv(dat1, file = "final data set.csv", row.names = F)
summaryTrt <- dat1 %>% group_by(treatments) %>%
   summarise(mean = mean(weight), sdparity =sd(weight), md = median(weight),
           skew = skewness(weight), kurt = kurtosis(weight),
           max = max(weight), min = min(weight))
avg <- sd(summaryTrt$mean)
library(ggthemes)
ggplot(summaryTrt, aes(x=treatments, y=mean))
   geom_bar(stat = "identity")
```

```
  theme_economist()+
  geom_errorbar(aes(ymin=mean - 1.96 *sdparity/sqrt(35),
                    ymax=mean + 1.96*sdparity/sqrt(35)), width=.1)+
  theme(axis.text.x = element_text(hjust = 1, vjust = 1, angle = 45, face = "bold"),
        axis.title = element_text(face = "bold", size = 12),
        axis.text.y = element_text(face = "bold")) +
  ylim(c(0, 2.8))+
  labs(x = "Treatments", y = "mean", title = "Error bars for treatment means Weight")
names(summaryTrt) <- c("Treatment","Mean", "Standard Deviation", "Median",
"Skweness", "Kurtosis",
                       "Maximum", "Minimum")
summaryTrt[, 2:8] <- apply(summaryTrt[, 2:8], 2, round, 4)
write.csv(summaryTrt, file = "summary stats treatments weight.csv", row.names = F)
nms <- c("pumkin_extract", "weeks")
weightlist <- list()
for(i in 1:2){
  this <- dat1 %>% group_by_(nms[i]) %>%
     summarise(mean = mean(weight), sdparitweight =sd(weight), md =
median(weight),
              skew = skewness(weight), kurt = kurtosis(weight),
              max = max(weight), min = min(weight))
  weightlist[[i]] <- this
}
factor_summary <- rbindlist( weightlist)
factor_summary[, 2:8] <- apply(factor_summary[, 2:8], 2, round, 4)
names(factor_summary) <- c("Factor Level","Mean", "Standard Deviation",
"Median", "Skweness", "Kurtosis",
                    "Maximum", "Minimum")
write.csv(factor_summary, file = "factor summary.csv", row.names = F)
summaryBlock <- dat1 %>% group_by(blocks) %>%
  summarise(mean = mean(weight), sdparity =sd(weight), md = median(weight),
          skew = skewness(weight), kurt = kurtosis(weight),
          max = max(weight), min = min(weight), freq = n())
```

```
ggplot(summaryBlock, aes(x=blocks, y=mean)) +
   geom_bar(stat = "identity")+
   theme_economist()+
   geom_errorbar(aes(ymin=mean - 1.96 *sdparity/sqrt(54), ymax=mean +
1.96*sdparity/sqrt(54)), width=.1)+
   theme(axis.text = element_text(hjust = 1, vjust = 1, face = "bold"),
         axis.title = element_text(face = "bold", size = 12),
         axis.text.y = element_text(face = "bold")) +
   ylim(c(0, 2.8))+
   labs(x = "Block", y = "mean", title = "Error bars for block means")
names(summaryBlock) <- c("Blocks","Mean", "Standard Deviation", "Median",
"Skweness", "Kurtosis",
                        "Maximum", "Minimum")
write.csv(summaryBlock, file = "block.csv", row.names = F)
fit_rbd <- aov(weight~blocks*treatments, data = dat1)
summary(fit_rbd)
library(broom)
tidy(fit_rbd) %>%  write.csv(file = "rbd.csv", row.names = F)
fit_factorial <- aov(weight~pumkin_extract*weeks, data = dat1)
tidy(fit_factorial) %>%  write.csv(file = "factorial.csv", row.names = F)
fit_gombertz <- nls(weight ~ SSgompertz(weeks, Asym, b2, b3), data = dat )
summary(fit_gombertz)
tidy(fit_gombertz ) %>%  write.csv(file = "fit_gombertz .csv", row.names = F)
x <- 0:30
df <- data.frame(x,y = predict(fit_gombertz, list(weeks = x)))
wd <- ggplot(dat, aes(weeks, weight)) +
   geom_point()+
   geom_line(data = df, aes(x, y), colour = "blue")+
   labs(x= "Weeks", y = "Weight", title = "Gompertz sigmoid for weight")+
   theme_economist()
wd
rsm_fit <- rsm(weight ~ SO(pumkin_extract, weeks), data = dat)
summary(rsm_fit)
```

```
tidy(rsm_fit) %>% write.csv(file = "rsm model.csv" )
anova(rsm_fit) %>% broom::tidy() %>% write.csv(file = "anova rsm.csv" )
persp(rsm_fit, ~ pumkin_extract + weeks,
    at = canonical(rsm_fit)$xs, main = "Through stationary point", contour="colors")
```

APPENDIX 2
CHUKA UNIVERSITY ETHICS COMMITTEE CLEARANCE LETTER

CHUKA UNIVERSITY

Telephones: 020 2310512
020 2310518

P.O. Box 109
Chuka

OFFICE OF THE CHAIRMAN
INSTITUTIONAL ETHICS REVIEW COMMITTEE

Our Ref: CU/IERC/NCST/18/21 14th March, 2018

THE CHIEF EXECUTIVE OFFICER
NATIONAL COMMISION FOR SCIENCE, TECHNOLOGY AND INNOVATION
P.O. BOX 30623-00100
NAIROBI

Dear Sir/Madam,

RE: RESEARCH CLEARANCE AND AUTHORIZATION FOR CHARLES MUGENDA KINYUA. REG NO SM18/29042/16

The above matter refers:

The Institutional Ethics Review Committee of Chuka University met and reviewed the above MSC, Research Proposal titled Modeling and Application of Response Surface Methodology for Optimization of Weight Gain of Eight weeks old Kenbro served with Pumkin (*Cucurbita pepo L*)" The Supervisor is Dr. Moses Muraya

The committee recommended that after candidate amends the issues highlighted in the Attached Research clearance and authorization check list, the permit be issued.

Attached please find copies of the minutes, research clearance and authorization check list for your perusal. Kindly assist the student get the research permit.

Yours faithfully,

Prof. Adiel Magana
CHAIR
INSTITUTIONAL ETHICS REVIEW COMMITTEE
cc: BPGS

APPENDIX 3
NACOSTI AUTHORIZATION

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY AND INNOVATION

Telephone:+254-20-2213471,
2241349,3310571,2219420
Fax:+254-20-318245,318249
Email: dg@nacosti.go.ke
Website : www.nacosti.go.ke
When replying please quote

NACOSTI, Upper Kabete
Off Waiyaki Way
P.O. Box 30623-00100
NAIROBI-KENYA

Ref. No. **NACOSTI/P/19/19334/30045**

Date: **28th May, 2019**

Charles Mugendi Kinyua
Chuka University,
P.O. Box 109-60400,
CHUKA.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on ***"Modelling and application of response surface methodology for optimization of weight gain of eight weeks old kenbro served with pumkin (Cucurbita Pepo L) seeds extract"*** I am pleased to inform you that you have been authorized to undertake research in **Tharaka Nithi County** for the period ending **27th May, 2020.**

You are advised to report to **the County Commissioner and the County Director of Education, Tharaka Nithi County** before embarking on the research project.

Kindly note that, as an applicant who has been licensed under the Science, Technology and Innovation Act, 2013 to conduct research in Kenya, you shall deposit **a copy** of the final research report to the Commission within **one year** of completion. The soft copy of the same should be submitted through the Online Research Information System.

DR. STEPHEN K. KIBIRU, PhD.
FOR: DIRECTOR-GENERAL/CEO

Copy to:

The County Commissioner
Tharaka Nithi County.

The County Director of Education
Tharaka Nithi County.

APPENDIX 4

NACOSTI PERMIT

THIS IS TO CERTIFY THAT:
MR. CHARLES MUGENDI KINYUA
of CHUKA UNIVERSITY, 109-60400
CHUKA,has been permitted to conduct
research in *Tharaka-Nithi County*

on the topic: *MODELLING AND APPLICATION OF RESPONSE SURFACE METHODOLOGY FOR OPTIMIZATION OF WEIGHT GAIN OF EIGHT WEEKS OLD KENBRO SERVED WITH PUMKIN (CUCURBITA PEPO L) SEEDS EXTRACT.*

for the period ending:
27th May,2020

Permit No : NACOSTI/P/19/19334/30045
Date Of Issue : 28th May,2019
Fee Recieved :Ksh 1000

....
Applicant's Signature

....
Director General
National Commission for Science, Technology & Innovation