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Response Surface Methodology Analysis Of Animal Manure Mixtures On Macronutrient Content

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Abstract
The macro and micronutrient composition, pH, and absorptivity of manure from livestock and poultry differ. To increase the nutrient content of soils, several types of manure must be combined. One strategy to increase manure efficiency is mixing ratio optimization. This study aimed to ascertain the nutrient levels of manure from various farm animals and assess the interactions between the manure and the resulting nutrients. Polynomial equations were created as a mathematical model to tie the factors to the responses. The central composite design (CCD) of response surface methodology (RSM) was utilized to investigate the impact of different animal manures on macro nutrients that were chosen as responses. RSM was also used to determine each manure type's level of significance as well as the interactive effects. According to the findings of the study, goat manure was richest in phosphorus (1.27%), potassium (1.24%), and calcium (4.68%), whereas poultry manure was richest in nitrogen (1.45%). The study showed that while a combination of cattle and swine produced a mixture high in nitrogen, blending goats, poultry, and swine manure gave the highest phosphorus levels. A mixture of cattle, goat, and pig manure was provided an organic fertilizer that was rich in calcium and potassium. To assist in blending different types of well-chosen animal manure for the creation of the intended nutrient-rich organic fertilizer, second and third-order polynomials were devised. Calcium, potassium, and phosphorous fitted best in a third–order polynomial while nitrogen was fitted in a second-order polynomial.
***Key words**: Animal, Central composite design, Macronutrient, Manure, Response surface methodology.*

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I. Introduction

Soil degradation for the agricultural environment and crop production are becoming evident over time. Manure is important in farming practices due to declining soil fertility [1]. However, studies have reported variations in nutrients in different animal manures [2, 3]. Variations in nutrient content have been attributed to the type of animal feed, nature of storage, weather changes, additional water, and microbial decomposers [4]. The variation in nutrients indicates that the quality of animal manure also varies from one animal manure to another. This is the reason why some compost manure has shown an inability to sustain plant growth. Modification of animal manure has shown improvement in manure quality, according to Billah *et al*. [5], the addition of rock phosphate in poultry litter during the process of composting showed an improvement in nutrient availability and biological properties of compost. Application of such manure showed increased yield and yield components of wheat crops compared to inorganic fertilizers. There is a need to explore the best mixing ratios of different manures for maximum nutrient provision and maximum generation of crop yields. Response surface methodology (RSM) is a statistical tool that has been used in optimization to model and determine the interactions of variables and their effects on the responses. As reported by Ebegil *et al*. [6], response surface methodology (RSM) is the best method for optimizing several variables simultaneously. Simultaneous optimization of responses saves on the time and cost of optimization as compared to one factor at a time. RSM is also a statistical and mathematical tool that can best model the interactive effects of several factors under study. it has been used in probing the simultaneous interaction of animal manure in a mixture Central composite design CCD is the most used RSM design, experimental results point out that CCD can best model the interaction of the factors being investigated [7, 8]. This study thus modeled the interaction for the mixture of cattle, goat, swine, and poultry manure that can yield organic fertilizers rich in calcium, nitrogen, phosphorus, and potassium. Central composite designs (CCD) based on polynomial designs were used to understand the interactive effects of variables. The study determined the nutrient content of various animal waste streams and modeled their interactive effects on macronutrient composition. Finally, a mathematical polynomial equation for an optimal mixture of the waste streams for each response was developed.

II. Materials And Methods

Sample collection and pretreatment

Samples of Cattle manure, poultry manure, goat manure, and swine manure were collected from nearby farms. For macronutrient content determinations, 50g of each selected manure was collected. Sample pretreatment was done by oven drying at 60 °C for 48 hours followed by grinding into powder and stored in capped 100 mL plastic bottles at room temperature

Experimental procedures

The experimental procedures included the determination of nutrients in various farm manure streams using analytical instruments and the Dumas method. Extractable cations, phosphorus and total nitrogen in 2.5g of cattle, goat, poultry, and swine manure samples were measured.

XRF determination of P, Ca, and K levels in the samples

Determination of levels of extractible cations Calcium (Ca), Potassium (K), and Phosphorus (P) content in the oven-dried samples were determined using a hand-held calibrated X-ray fluorescence spectrometer (XRF S1 titan 800).

Determination of Extractable nitrogen

Extractable nitrogen was determined using the Dumas method. First, the samples were digested using the potassium persulphate closed digestion method [9] and then analyzed using a Dumas discrete analyzer. The alkaline persulphate digest reagent (0.15 M $K_2S_2O_8$ in 0.15 M NaOH) was prepared by adding 10 mL of 0.15 M NaOH to approximately 80 mL of deionized water, followed by dissolving 4 g of potassium persulphate, in a 100 mL volumetric flask. The solution was sonicated for 10 minutes to dissolve the solid material completely then diluted to a 100 mL mark with deionized water. Samples were prepared by adding 10 mL of deionized water to 2.5 g of oven-dried and ground powdered manure samples each in a plastic sample bottle. Samples and digest reagent were mixed at a 2:1 ratio by adding 2 mL of alkaline persulphate digestion to 4 mL of sample in a glass digestion tube. The digestion was capped and then placed in a heat block set at 120 °C for 60 minutes. After the digestion was complete the tube was allowed to cool to room temperature. The cooled solution was diluted and then injected into a Dumas discrete analyzer (Thermo 200 series).

Response surface methodology

The central composite design (CCD) of response surface methodology (RSM) was employed using Design Expert 13.0.5.0 (Stat-Ease Inc, USA) to design the experiments to study the interactive effects of each manure stream and their effects on each response [10, 8, 11]. The modeled factors were cattle manure, goat manure, poultry manure and swine manure and their effects on Ca, N, P and K content as the responses. Each factor was varied at three levels; low, medium and high coded as -1, 0 and 1 respectively, giving rise to 30 runs. The experiments consisted of sixteen factorial, eight axial and six center points (Table 2.1).

Table 2.1: Experimental design

Runs	Factors/ Manure Stream (kg)			
	A:Cattle	B:Goat	C:poultry	D:Swine
1	1	3	1	1
2	1	1	1	3
3	1	3	3	3
4	1	1	3	1
5	3	3	1	3
6	3	3	3	1
7	2	2	2	2
8	3	1	3	3
9	2	2	2	2
10	3	1	1	1
11	3	1	1	3
12	3	3	3	3
13	2	2	2	2
14	3	1	3	1
15	2	2	2	2
16	1	3	1	3
17	1	3	3	1
18	3	3	1	1
19	1	1	1	1
20	1	1	3	3

21	2	0	2	2
22	0	2	2	2
23	4	2	2	2
24	2	2	2	2
25	2	2	4	2
26	2	4	2	2
27	2	2	2	4
28	2	2	0	2
29	2	2	2	0
30	2	2	2	2

III. Results And Discussions

Analysis of nutrient content for various manure studied

An initial analysis on the nutrient content of each animal waste was carried out to ascertain their nutrient content before preparation of mixtures. The nutrient content in selected animal manure obtained is presented in Table 3.1.

Table 3.1: Analysis of nutrient levels in various manure samples studied

Manure type	**Nutrient concentration %**			
	N	**P**	**K**	**Ca**
Cattle	1.27	0.936	4.453	4.292
Goat	1.27	1.239	5.653	4.677
Poultry	1.45	0.502	2.419	3.125
Swine	0.68	0.593	2.873	4.031

Among the four selected nutrients, nitrogen (N) and phosphorus (P) contents were lower than potassium (K) and calcium (Ca). This was because nitrogen gets easily lost inform of ammonia volatilization [12]. Ease of phosphorus leaching when in kraals and at the time of storage in heaps was also associated with the low phosphorus content [13]. The highest nitrogen content of 1.45% was observed in poultry manure similar to what was observed by Onduru *et al*. [3]. The highest nitrogen concentration was attributed to the fact that the total crude protein content in the feeds of animals is directly proportional to the nitrogen content in the animal manure [14]. The value obtained was slightly lower than the reported value of 3.11% because of the likelihood of volatilization [13]. The levels of phosphorus (1.239%), potassium (5.653 %), and calcium (4.667%) were observed in goat manure in agreement with the findings of Onduru *et al*. [3]. The values of P, K, and Ca concentrations in the goat were linked to animal feed type, manure storage, weather changes, and low amount of water consumed by goats [4].

Interactive effects of cattle, goat, poultry and swine manure on selected responses.

RSM analysis was done on nutrients in the mixture of cattle, goat, poultry and swine to investigate the interactive effect of combining different animal manure on the selected responses. The observed changes were: calcium, potassium, phosphorus and nitrogen content in the mixture.

Interactive effects of selected animal manure on calcium.

The suitability of the model used to analyze the data depends on the p-value and coefficient of determination R^2. Adjusted R^2value (Radj^2) was used because R^2 value was too low [11]. It was observed that, for calcium, the data fitted a cubic model as shown in Table 3.2 with a Radj^2 value of 0.9124. The P-value of 0.0042 implied the significance of the model at a 95% confidence level.

Table 3.2: Fit statistic summary for calcium content

Model	**Sequential p-value**	**Adjusted R^2**
Linear	0.0367	0.2343
2FI	0.0255	0.5183
Quadratic	0.8776	0.4221
Cubic	0.0104	0.9124

Regression coefficients from the ANOVA for the cubic model (Table 3.3) show the interactive effects of combining the selected manures on change in the amount of calcium in the manure mixture.

Table 3.3: Estimated changes on calcium content in the mixture with the change in the selected animal manure.

Factor	Coefficient Estimate	F-value	p-value
model		13.78	0.0042
Intercept	5.54		
A-Cattle	-0.2307	2.79	0.1560
B-Goat	0.3158	5.22	0.0712
C-Chicken	-0.2158	2.44	0.1794
D-Swine	-0.5860	17.97	0.0082
AB	-0.4971	25.86	0.0038
AC	-0.0863	0.7801	0.4175
AD	0.6520	44.49	0.0011
BC	0.0066	0.0045	0.9489
BD	-0.5820	35.45	0.0019
CD	0.0970	0.9847	0.3666
A^2	0.1382	3.43	0.1234
B^2	-0.1238	2.75	0.1582
C^2	-0.0178	0.0568	0.8210
D^2	0.0486	0.4234	0.5440
ABC	-0.2894	8.77	0.0315
ABD	0.4282	19.19	0.0017
ACD	-0.5008	26.24	0.0037
BCD	-0.4288	19.24	0.0071
A^2B	0.3922	5.36	0.0684
A^2C	-0.1421	0.7043	0.4396
A^2D	0.1717	1.03	0.3569
AB^2	0.0821	0.2351	0.6483

The P-values less than 0.0500 indicated the model terms were significant. In this case, D, AB, AD, BD, ABC, ABD, ACD, and BCD were the only significant model terms. Values of P greater than 0.1000 indicate the model terms were not significant. A unit change in cattle, goat, poultry and swine manure led to a change in calcium content by a factor of -0.2307, 0.3158, -0.2158 and -0.5860 respectively. This showed that goat manure was rich in calcium content. However, cattle, goat, and poultry manure with $p>0.05$ was not significant and therefore the most suitable animal manure for calcium content was that of swine. AD had the highest model coefficient of 0.6520 with the least p-value. The highest change in calcium content was therefore observed in the combined manure of cattle and swine (AD). A unit change in a combination of cattle and swine manure led to an increase in calcium content in the blended organic manure by a factor of 0.6520. The highest three-factor interaction was observed in a combination of cattle, goat and swine (ABD) with a factor of 0.4282. Confirming that poultry manure was the least effective in the production of manure rich in calcium content.

Interactive effects of selected animal manure on potassium.

The highest adjusted R^2 value of 0.9722 (Table 3.4) and P-value of 0.0003(Table 3.5) implied that the data obtained fitted best in the cubic model. Table 3.4 shows the fit statistic summary.

Table 3.4: Fit statistic summary for potassium content

Source	Sequential p-value	Adjusted R^2
Linear	0.0033	0.3933
2FI	0.0035	0.7091
Quadratic	0.3247	0.7275
Cubic	0.0040	0.9722

Observing regression coefficients from the ANOVA for the cubic model (Table 3.5), the interactive effects for each term was established. The table also shows the p-values of the model.

Table 3.5: Estimated changes on potassium content in the mixture with the change in the selected animal manure.

Factor	Coefficient Estimate Mean ±SE	F-value	p-value
Model		43.97	0.0003
Intercept	5.32±0.1122		
A-Cattle	-0.5088±0.0972	27.39	0.0034
B-Goat	0.5678±0.0972	34.11	0.0021
C-Chicken	0.0305±0.0972	0.0984	0.7664
D-Swine	-0.4768±0.0972	24.05	0.0045
AB	-0.7246±0.0687	111.13	0.0001
AC	-0.1956±0.0687	8.10	0.0360
AD	0.7263±0.0687	111.63	0.0001
BC	-0.0175±0.0687	0.0648	0.8092
BD	-0.6646±0.0687	93.49	0.0002
CD	0.0059±0.0687	0.0073	0.9352
A^2	0.2197±0.0525	17.51	0.0086
B^2	-0.0681±0.0525	1.68	0.2514
C^2	-0.0764±0.0525	2.12	0.2052
D^2	0.2694±0.0525	26.34	0.0037
ABC	-0.2731±0.0687	15.79	0.0106
ABD	0.5050±0.0687	53.98	0.0007
ACD	-0.2635±0.0687	14.70	0.0122
BCD	-0.3034±0.0687	19.48	0.0069
A^2B	0.4535±0.1191	14.51	0.0125
A^2C	-0.0598±0.1191	0.2519	0.6371
A^2D	-0.1924±0.1191	2.61	0.1671
AB^2	-0.1326±0.1191	1.24	0.3160

The significant model terms based on their P-values were A, B, D, AB, AC, AD, BD, A^2, D^2, ABC, ABD, ACD, BCD, and A^2B. One factor impact on potassium content showed that goat manure was the most effective manure with a mean factor of 0.5678±0.0972 higher than the rest. From the regression coefficients a unit change in potassium led to a change in cattle, goat, poultry and swine manure by mean factors of -0.5088±0.0972, 0.5678±0.0972, 0.0305±0.0972 and -0.4768±0.0972. These factors also showed that while blending animal manure for effective potassium content at least cattle and swine is required with plenty of goat manure. No poultry manure is required since the factor was insignificant. AD had the highest mean model coefficient of 0.7263±0.0687 with the least p-value. This showed that the highest change in potassium content for a two-factor interaction was observed in combining cattle and swine (AD). A unit change in a combination of cattle and swine manure led to an increase in potassium content in the blended organic manure by a factor of 0.7263. The highest three-factor interaction was observed in cattle, goats and swine mixture (ABD) with a mean factor of 0.5050±0.0687. This implied that a mixture of manure from cattle, goats and swine was more effective for higher potassium content in the mixture. A unit change in potassium content led to an increase in combined cattle and goat with a factor of 0.5050. Poultry manure had the least effect on the amount of potassium in the mixture and can be discarded while blending animal manure for effective potassium content.

Interactive effects of selected animal manure on phosphorus content

A p-value of 0.0007 (Table 3.7) and adjusted R^2 value of 0.9574 (Table 3.6) affirmed that the data obtained fitted the cubic model. Table 3.6 shows the summary of the fits.

Table 3.6: Fit statistic Summary for phosphorus content showing suitability of the model

Source	Sequential p-value	Adjusted R^2
Linear	0.0077	0.3424
2FI	0.2766	0.4023
Quadratic	0.4032	0.4142
Cubic	0.0018	0.9574

By taking into account the regression coefficients from the ANOVA for the cubic model (Table 3.7), the interactive effects of combining the selected manure on change in the quantity of phosphorus in the manure mixture were detected.

Table 3.7: Estimated changes on phosphorus content in the mixture with the change in the selected animal manure.

Factor	Coefficient Estimate	F-value	p-value
Model		28.61	0.0007
Intercept	0.9590		
A-Cattle	-0.0203	2.51	0.1738
B-Goat	0.0652	26.09	0.0037
C-Chicken	0.0353	7.62	0.0399
D-Swine	-0.0680	28.34	0.0031
AB	-0.0389	18.52	0.0077
AC	-0.0244	7.28	0.0429
AD	0.0794	77.23	0.0003
BC	0.0095	1.11	0.3410
BD	-0.0175	3.75	0.1104
CD	0.0268	8.77	0.0315
A^2	0.0305	19.57	0.0069
B^2	-0.0042	0.3758	0.5666
C^2	0.0033	0.2248	0.6554
D^2	0.0451	42.83	0.0012
ABC	-0.0434	23.06	0.0049
ABD	0.0134	2.19	0.1987
ACD	-0.0334	13.65	0.0141
BCD	-0.0798	77.96	0.0003
A^2B	-0.0033	0.0432	0.8436
A^2C	-0.1078	47.77	0.0010
A^2D	-0.0305	3.80	0.1087
AB^2	-0.0376	5.78	0.0612

From Table 3.7, B, C, D, AB, AC, AD, CD, A^2, D^2, ABC, ACD, BCD, and A^2C were significant model terms. Cattle manure was not effective for the production of manure rich in phosphorus content because its p-value of 0.1738 was less than 0.05. Goat, poultry and swine manures were effective. A unit change in goat, poultry and swine manure brought about a change in phosphorus content by a factor of 0.06525, 0.03525 and -0.068 respectively. Goat manure was the most effective in phosphorus content with the highest factor coefficient of 0.06525. AD had the highest model coefficient of 0.0794 with the least p-value. The highest change in phosphorus content was observed in AD mixture. A unit change in a combination of cattle and swine manure led to an increase in phosphorus content in the blended organic fertilizer by a factor of 0.0794. The highest three-factor interaction was observed in BCD mixture by a factor of -0.07975. A unit increase in calcium content leads to a decrease in combined goat poultry and swine by a factor of 0.07975. Negative value was attributed to antagonistic effect hence no three factor interaction was effective for phosphorus rich organic fertilizer production.

Interactive effects of selected animal manure on nitrogen.

The data fitted well in both the quadratic and cubic model as shown in Table 3.8 due to proximity of their predicted R^2 values. The highest R^2 values of 0.9929 and 1.000 and p-value <0.0001. However, a quadratic model was used because none of the cubic terms was significant as shown in Table 3.9.

Table 3.8: Fit statistic Summary for nitrogen content showing suitability of the model

Source	Sequential p-value	Adjusted R^2	Predicted R^2
Linear	< 0.0001	0.9501	0.9210
2FI	0.0487	0.9656	0.9554
Quadratic	< 0.0001	0.9982	0.9929

Cubic	< 0.0001	1.0000	1.0000

It was investigated how combining the chosen manure would affect the change in the amount of nitrogen in the organic fertilizer, taking into account the regression coefficients from the ANOVA for the quadratic model (Table 3.9).

Table 3.9: Estimated changes on nitrogen content in the mixture with the change in the selected animal manure.

Factor	Coefficient Estimate	F-value	p-value
Model		1070.03	<0.0001
Intercept	1.17		
A-Cattle	0.0134	445.64	<0.0001
B-Goat	0.0134	445.64	<0.0001
C-Chicken	0.0369	3385.04	<0.0001
D-Swine	-0.0636	10080.20	<0.0001
AB	-0.0034	19.37	0.0007
AC	-0.0064	68.31	<0.0001
AD	0.0064	68.31	<0.0001
BC	-0.0064	68.31	<0.0001
BD	0.0064	68.31	<0.0001
CD	0.0034	19.37	0.0007
A^2	-0.0017	8.30	0.0129
B^2	-0.0017	8.30	0.0129
C^2	-0.0047	63.05	<0.0001
D^2	0.0081	187.75	<0.0001

From Table 3.9, it is clear that A, B, C, D, AB, AC, AD, BC, BD, CD, A^2, B^2, C^2, and D^2 were the significant model terms. None of the four selected animal manures was insignificant for nitrogen content. A unit change in nitrogen content brought about a change in cattle, goat, poultry and swine manure by a factor of 0.0134, 0.0134, 0.0369 and -0.0636 respectively. Both cattle and swine manure (A &D) were the most effective with a coefficient of 0.0134. Swine manure was least effective with a negative factor (-0.0636) implying that its effect is too low. The highest change in calcium content for a two-factor interaction was observed in AD. AD had the highest model coefficient of 0.0064 with the least p-value. A unit change in a combination of cattle and swine manure led to an increase in calcium content in the blended organic fertilizer. None of the three-factor interactions was significant.

Model equations

Model equations were developed based on the regression coefficients of the significant terms. Calcium, potassium, and phosphorus fitted the third-order polynomial, Equations 3.1-3.3. For nitrogen, a second-order polynomial equation was developed in Equation 3.4. The model equations show the stoichiometric relationship between each factor and the response. Based on the relationship farmers can blend various selected animal manure for the production of desired nutrient-rich organic fertilizer. The equations were presented as follows.

$$\boldsymbol{Ca}(\%) = +5.54 - 0.586D - 0.4971AB + 0.652AD - 0.582BD - 0.2894ABC + 0.4282ABD - 0.5008ACD - 0.4288BCD \quad (3.1)$$

$$\boldsymbol{K}\,(\%) = +5.32 - 0.5088A + 0.5678B + 0.0305C - 0.4768D - 0.7246AB - 0.1956AC + 0.7263AD - 0.0175BC - 0.6648BD + 0.0059CD + 0.2197A^2 - 0.0681B^2 - 0.0764C^2 + 0.2694D^2 - 0.2731ABC + 0.5050ABD - 0.26358ACD - 0.3034BCD + 0.4535A^2B - 0.0598A^2C + 0.1924A^2D - 0.1326A^2B \quad (3.2)$$

$$\boldsymbol{P}\,(\%) = +0.959 + 0.06525\,B + 0.03525\,C - 0.068\,D - 0.038875\,AB - 0.024375\,AC + 0.079375\,AD\ \ 0.02675\,CD + 0.0305208\,A^2 + 0.0451458D^2 - 0.043375\,ABC - 0.033375\,ACD - 0.07975\,BCD - 0.10775\,A^2C \quad (3.3)$$

$$\boldsymbol{N}\,(\%) = +1.170 + 0.0134\,A + 0.0134\,B + 0.0369\,C - 0.0636\,D - 0.0034AB - 0.0064AC + 0.0064AD - 0.0064BC + 0.064BD + 0.0034\,CD - 0.0017A^2 0.0017B^2 - 0.0047C^2 + 0.0081D^2 \quad (3.4)$$

IV. Conclusions

This study provided data for the nutrient content of selected livestock and poultry manure. It was established that poultry manure had the highest nitrogen content while goat manure was rich in phosphorus, potassium, and calcium. A combination of cattle, goat, and swine manure was determined to be most effective in the production of a potassium and calcium rich organic fertilizer. Combining goat, poultry and swine manures was more effective for high phosphorus content while a mixture of cattle and swine manure was relatively richer in nitrogen content than the individual manures. The mixing ratios for optimal nutrients concentrations was determined and presented in the form of polynomial equations which could potentially be used in the formulation of organic manures to aid in the provision of the requisite macronutrients. The utilization of RSM in the study was found to be efficient since it uses a limited number of experimental runs to provide all the required information and optimization thereby minimizing the time and resources required for the study while providing all the requisite information.

Credit Author Statement

Simon Magero: Writing - original draft, Writing- Reviewing and Editing, **Linda Ouma:** Conceptualization; methodology, review & editing, Supervision, **Francis Maingi:** review & editing, Supervision.

Declaration Of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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