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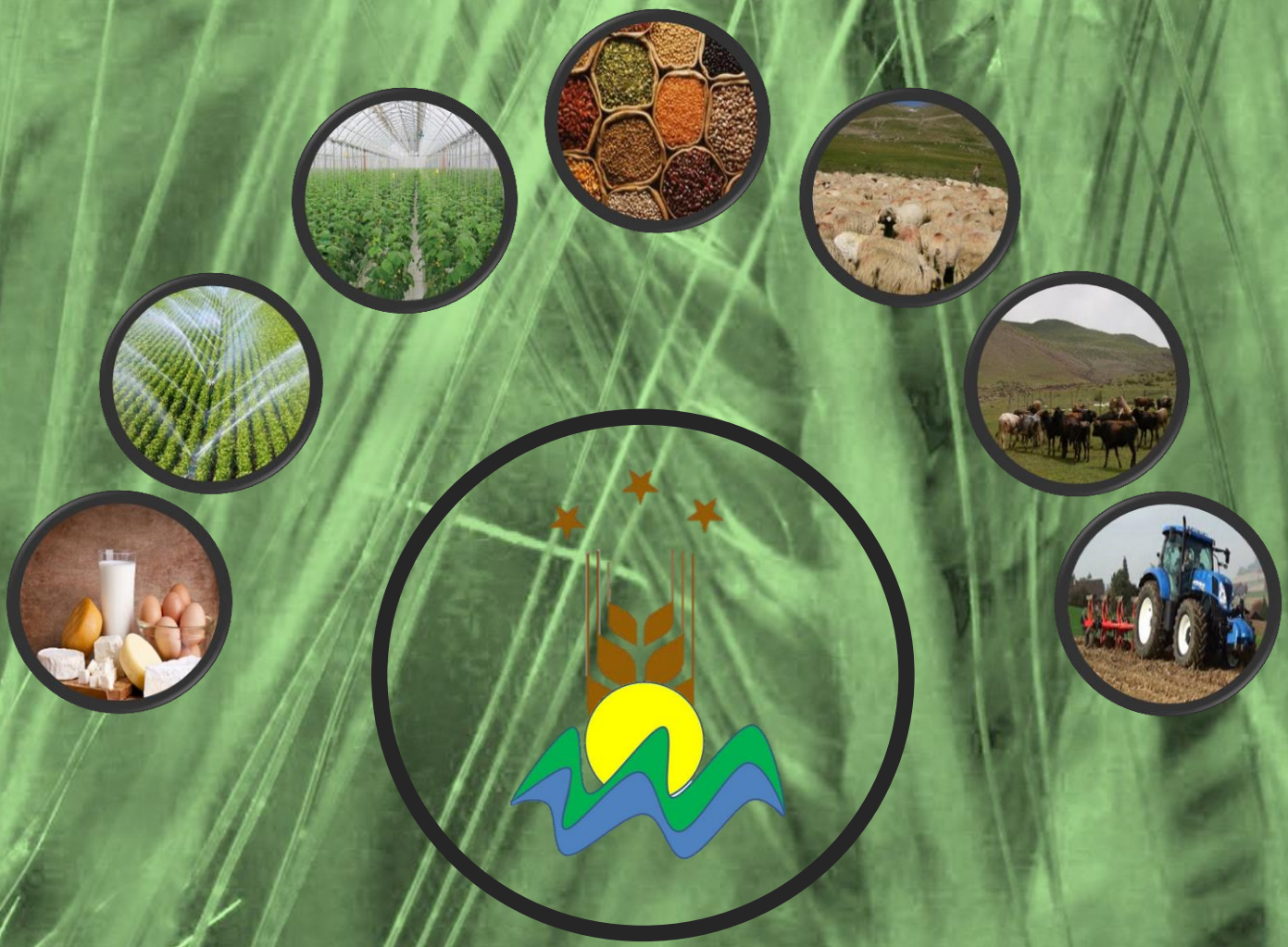

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Research Article
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# BAYESIAN ANALYSIS OF AGRICULTURAL EXPERIMENTS USING PROC MCMC

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**Abstract:** The purpose of this study is to present the general concept of Bayesian analysis and the Markov chain Monte Carlo (MCMC) algorithm and to make some numerical comparisons with frequentist analyses. A factorial randomized complete-block (RCB) experiment is used to analyze the cowpea data set that has four separate single-column replicates, each containing 9 combinations of 3 varieties and 3 spacings. Response is the yield of cowpea hay. Point estimates of variance components obtained in the Bayesian analysis under the priors presented some differences with the Restricted Maximum Likelihood (REML) estimate. The Bayesian method overestimates the variance component compared with the REML estimate. Bayesian method to agricultural experiments is a very rich and useful tool. It provides in depth study of different features of the data which are otherwise hidden and cannot be explored using other techniques. Moreover, SAS software has a power and efficiency to deal with the numerical as well as graphical features of data sets from agricultural experiments.

**Keywords:** Bayesian analysis, Agricultural experiments, Markov Chain Monte Carlo, PROC MCMC

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## 1. Introduction

A main objective of most of agricultural experiments is to determine the effect of different treatments on a particular crop variety. Data collected from agricultural experiments have been analyzed using different statistical methods, such as analysis of variance, maximum likelihood, REML and Bayesian methods. Despite a great number of literatures on the design and analysis of agricultural field experiments, dating back to the early 1900s, and a large uptake of Bayesian methods in many other scientific fields in the last 30 years (Firat, et al., 1997a; Firat, et al., 1997b; Firat, 2001; Karaman, et al., 2014; Cemal, et al., 2016; Firat et al., 2016), Bayesian analysis for agricultural field experiments has not received very much attention.

Statistical methods for the design and analysis of agricultural field experiments were essentially developed by R. A. Fisher, F. Yates and many others. However, most of the modern courses and text-books focus on industrial and medical applications. Field trials are rather different, because a researcher always knows that generally she/he will obtain similar yields on two experimental units that are close together than on units that are further apart (Besag and Higdon, 1999), and also fields trials are conducted real-world settings.

Bayesian methods improve upon frequentist methods by expressing uncertainty regarding the unknown parameters and simplifies the interpretation of the results, especially in ranking and selection of crop varieties. Moreover, an analysis of complex formulations can be carried out with comparative ease, and computation of complicated functionals of high dimensional posterior distributions can be done by using MCMC methods. Although, faster computers and increasing popularity of MCMC methods have allowed Bayesian methods to become widely used in complex data analysis problems, the Bayesian approach has yet to provide a completely satisfactory answer in the analysis of agricultural experiments, since there has been a lack of application in this area.

Besag and Higdon (1993, 1999) and Besag et al. (1995) discussed Bayesian approaches for analyzing agricultural field experiments. They proposed complex formulations for situations when spatial effects were considered, while our approach is for the standard additive mixed model. Our approach has some advantages over other Bayesian approaches; the marginals of complex functions of the unknown parameters can be easily obtained and implementation of high dimensional posterior probability functions can be conveniently done.

In this research, the general concept of Bayesian analysis and the MCMC algorithm are presented. The implementation of the MCMC algorithm using the PROC MCMC procedure of SAS software package (SAS Institute, 2004) is demonstrated through a real data set from an agricultural experiment. Some numerical comparisons with frequentist analyses are made. We shall have rather little to say about MCMC sampling. The analyses in this

paper were all carried out using simple Gibbs samplers.

In organizing the paper, we decided to present the methodology first, followed by an application. We describe some Bayesian formulations for a particular field trial, in which (a) the treatment or variety effects have no special structure, and (b) Gaussian assumptions are appropriate, both in the likelihood and in the prior. We contrast Bayesian and frequentist formulations. Finally, the last section contains some discussion.

## 2. Material and Methods

### 2.1. Data Set and Descriptive Statistics

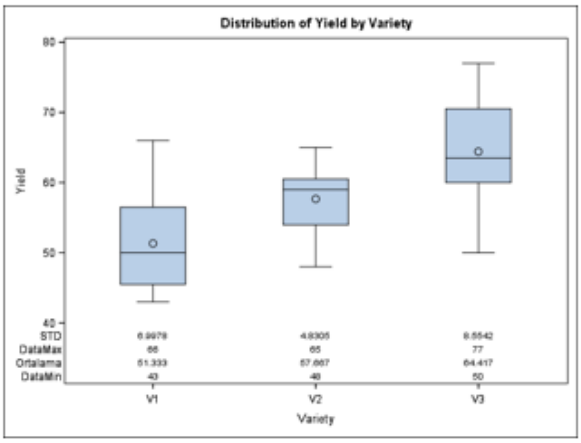
In this section, we analyze a factorial randomized complete-block (RCB) experiment on cowpea. A data set is reported in Snedecor and Cochran (1989), page 308. There are four separate single-column replicates, each containing 9 combinations of 3 varieties and 3 spacings. Response is the yield of cowpea hay (lb/100 morgen plot) and the corresponding yields are given in Table 1. Descriptive statistics of Cowpea data set and boxplots of main factor effects and interactions are displayed in Table 2 and Figure 1, respectively, for the purpose of illustration. We note here that the basic Bayesian formulation, with Gaussian assumptions for variety, spacing and yield but a vague prior applied to varieties and spacings, produces variety and spacing effects that agree closely with those from a superficially similar frequentist extended first-differences analysis. The same holds for the standard deviations and corresponding standard errors for variety differences.

**Table 1.** Data from Cowpea variety trial. The original dataset was published by Snedecor and Cochran (1989, p. 308). This data set is called 'Cowpea data' in this study

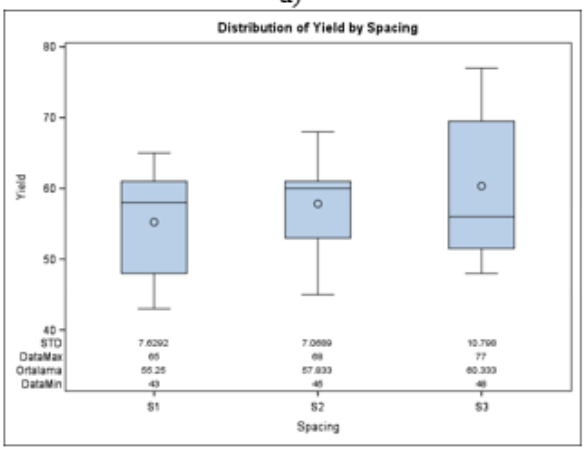
| | | Blocks | | | |
|---|---|---|---|---|---|
| Variety | Spacing | B1 | B2 | B3 | B4 |
| V1 | S1 | 56 | 45 | 43 | 46 |
| | S2 | 60 | 50 | 45 | 48 |
| | S3 | 66 | 57 | 50 | 50 |
| V2 | S1 | 65 | 61 | 60 | 63 |
| | S2 | 60 | 58 | 56 | 60 |
| | S3 | 53 | 53 | 48 | 55 |
| V3 | S1 | 60 | 61 | 50 | 53 |
| | S2 | 62 | 68 | 67 | 60 |
| | S3 | 73 | 77 | 77 | 65 |

**Table 2.** Descriptive statistics of Cowpea data set

| Variety | Spacing | Mean | Stdev. | Min. | Max. |
|---|---|---|---|---|---|
| V1 | S1 | 47.50 | 5.802 | 43 | 56 |
| | S2 | 50.75 | 6.500 | 45 | 60 |
| | S3 | 55.75 | 7.588 | 50 | 66 |
| V2 | S1 | 62.25 | 2.217 | 60 | 65 |
| | S2 | 58.50 | 1.915 | 56 | 60 |
| | S3 | 52.25 | 2.986 | 48 | 55 |
| V3 | S1 | 56.00 | 5.354 | 50 | 61 |
| | S2 | 64.25 | 3.862 | 60 | 68 |
| | S3 | 73.00 | 5.657 | 65 | 77 |




a)




b)

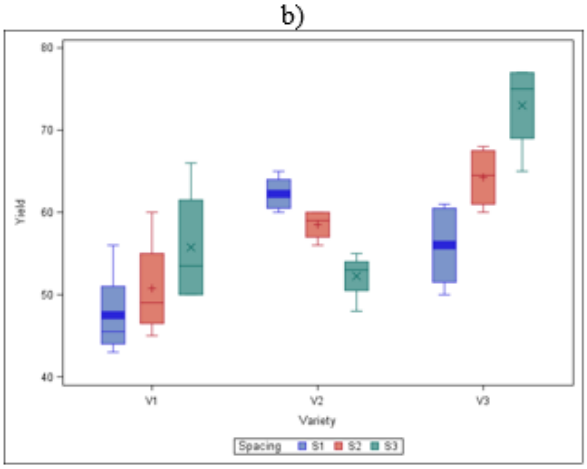



**Figure 1.** Boxplots of main factor effects and interactions: (a) boxplot of variety effect, $\beta_j$'s, (b) boxplots of treatment (spacings) effects, $\gamma_k$ (c) boxplots of interactions $(\beta\gamma)_{jk}$'s.

### 2.2. Bayesian Formulation of Field Experiments

For definiteness, we focus on a full factorial Randomized Block Design (RBD) with two three-level treatment factors (variety and spacing) occurring in a factorial structure. The randomized block design is used to control and reduce experimental error. It is intended to make yield comparisons between the levels of treatment factors of a crop. We assume that measurements are effectively continuous, resulting in an $n$-vector $y$ of yields over $n$ rectangular plots. However, our formulation

extends to more complicated treatment structures and to discrete observations, such as litter size or number of laid eggs per month.

We consider the additive fixed model, with one observation per cell. The model for a two-factor factorial in a randomized complete block (equation 1) is

$$y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + (\beta\gamma)_{jk} + e_{ijk} \qquad \begin{cases} i = 1,2,\ldots,a \\ j = 1,2,\ldots,b \\ k = 1,2,\ldots,c \end{cases} \quad (1)$$

where $y_{ijk}$ is the observed response when factor factor A is at the *j*th level and factor B at the *k*th level in the *i*th block, $\mu$ is the overall mean effect, $\alpha_i$ is the effect of *i*th block, $\beta_j$ is the effect of *j*th level of factor A, $\gamma_k$ is the effect of *k*th level of factor B, $(\beta\gamma)_{jk}$ is the effect of the interaction between factor A and B, and $e_{ijk}$ is a random error component. Both factors are assumed to be fixed. Similarly, the interaction effects are fixed. The experimental errors are assumed independent and normally distributed with zero means and common variance $\sigma_e^2$. To our knowledge, an analysis of the agricultural field data using PROC MCMC has never been done before, and it will be the first time to use the model in equation 1 under new priors to make Bayesian inferences.

The conditional distribution of $\{y_{ijk}\}$ given $\boldsymbol{\mu}$, $\alpha_i$, $\beta_j$, $\gamma_k$, $(\beta\gamma)_{jk}$ and $\sigma_e^2$ is

$$\left(\{y_{ijk}\} \mid \mu, \{\alpha_i\}, \{\beta_j\}, \{\gamma_k\}, \{(\beta\gamma)_{jk}\}, \sigma_e^2\right) \sim N\left(y_{ijkl} - \mu - \alpha_i - \beta_j - \gamma_k - (\beta\gamma)_{jk}, \sigma_e^2\right)$$

Under this assumption of normality, the likelihood function (equation 2) is as follows:

$$f\left(\{y_{ijk}\} \mid \mu, \{\alpha_i\}, \{\beta_j\}, \{\gamma_k\}, \{(\beta\gamma)_{jk}\}, \sigma_e^2\right) = f(\mathbf{y} \mid \boldsymbol{\theta})$$
$$\propto \left(\sigma_e^2\right)^{-\frac{abc}{2}} \exp\left\{-\frac{1}{2}\left[\frac{\sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{c}\left(y_{ijk} - \mu - \alpha_i - \beta_j - \gamma_k - (\beta\gamma)_{jk}\right)^2}{\sigma_e^2}\right]\right\} \quad (2)$$

*Prior distributions for variety or treatment effects*: In order to carry out Bayesian analysis, in addition to the likelihood function we need prior distributions for each parameter of the model, $\mu$, $\alpha_i$, $\beta_j$, $\gamma_k$, $(\beta\gamma)_{jk}$ and $\sigma_e^2$. We consider a conjugate prior for each of these parameters. For the overall mean, $\mu$, a flat prior was used, so that $f(\mu) \propto \text{constant}$, indicating no prior knowledge about this parameter. The simplest choice of prior for the variety effect, $\alpha_i$, in a single trial is either a uniform distribution or a Gaussian distribution, when the data provide little evidence of differences between varieties. In this paper, our prior is the simple Gaussian or uniform distribution, $\{\alpha_i\} \propto \text{constant}$: the latter is useful mainly in making numerical comparisons with standard frequentist analyses. For the priors, a Uniform distribution was also assumed for other fixed effects $\beta_j$, $\gamma_k$ and $(\beta\gamma)_{jk}$, $\{\beta_j\} \propto \text{constant}$, $\{\gamma_k\} \propto \text{constant}$ and $\{(\beta\gamma)_{jk}\} \propto \text{constant}$, respectively. Finally, for the variance component $\sigma_e^2$, a diffuse but proper $\sigma_e^2 \mid \nu_e, S_e^2 \sim IG\left(\nu_e/2, \nu_e S_e^2/2\right)$ prior (i.e. an inverse gamma distribution) was assigned (equation 3).

$$f\left(\sigma_e^2 \mid \nu_e, S_e^2\right) \propto \left(\sigma_e^2\right)^{-\frac{1}{2}(\nu_e+2)} \exp\left\{-\frac{\nu_e S_e^2}{2\sigma_e^2}\right\} \quad (3)$$

where $S_e^2$ and $\nu_e$ are scale and shape (degrees of freedom) parameters for variance component respectively.

*Posterior distribution*: By multiplying likelihood function with the prior distributions of all the parameters, the joint posterior density of parameters (equation 4) is obtained as:

$$f(\boldsymbol{\theta} \mid \mathbf{y}) \propto L(\mathbf{y} \mid \boldsymbol{\theta}) f(\mu) f(\{\alpha_i\}) f(\{\beta_j\}) f(\{\gamma_k\}) f(\{(\beta\gamma)_{jk}\}) f(\sigma_e^2) \propto \left(\sigma_e^2\right)^{-\frac{(abc+\nu_e+2)}{2}}$$
$$\times \exp\left\{-\frac{1}{2}\left[\frac{\sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{c}\left(y_{ijk} - \mu - \alpha_i - \beta_j - \gamma_k - (\beta\gamma)_{jk}\right)^2 + \nu_e S_e^2}{\sigma_e^2}\right]\right\} \quad (4)$$

To implement the Gibbs sampling algorithm, we require the full conditional posterior distributions of $\mu$, $\alpha_i$, $\beta_j$, $\gamma_k$, $(\beta\gamma)_{jk}$ and $\sigma_e^2$. The full conditional posterior distribution of any parameter of interest can be obtained by integrating over the remaining parameters from joint posterior distribution. It is well known that the conjugate priors are very easy to work with because the posterior and prior have the same distributional form and the effect of the data is just to update the parameters from the prior to the posterior. Therefore, the resulting full conditional posterior distributions of $\mu$, $\alpha_i$, $\beta_j$, $\gamma_k$ $(\beta\gamma)_{jk}$ and $\sigma_e^2$ (equations 5-10) are summarized as follows:

$$[\mu|.] \sim N\left(\frac{\bar{y}_{..} - \bar{\alpha}_{.} - \bar{\beta}_{.} - \bar{\gamma}_{.} - \overline{(\beta\gamma)}_{..}}{abc}, \frac{\sigma_e^2}{abc}\right) \quad (5)$$

$$[\{\alpha_i\}|.] \sim N\left(\frac{\bar{y}_{i..} - \mu - \bar{\beta}_{.} - \bar{\gamma}_{.} - \overline{(\beta\gamma)}_{..}}{bc}, \frac{\sigma_e^2}{bc}\right) \quad (6)$$

$$[\{\beta_j\}|.] \sim N\left(\frac{\bar{y}_{.j.} - \mu - \bar{\alpha}_{.} - \bar{\gamma}_{.} - \overline{(\beta\gamma)}_{..}}{ac}, \frac{\sigma_e^2}{ac}\right) \quad (7)$$

$$[\{\gamma_k\}|.] \sim N\left(\frac{\bar{y}_{..k} - \mu - \bar{\alpha}_{.} - \bar{\beta}_{.} - \overline{(\beta\gamma)}_{..}}{ab}, \frac{\sigma_e^2}{ab}\right) \quad (8)$$

$$[\{(\beta\gamma)_{jk}\}|.] \sim N\left(\frac{\bar{y}_{.jk} - \mu - \bar{\alpha}_{.} - \bar{\beta}_{.} - \bar{\gamma}_{..}}{a}, \frac{\sigma_e^2}{a}\right) \quad (9)$$

$$[\sigma_e^2|.] \sim IG\left(\frac{abc + \nu_e}{2}, \frac{\sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{c}(y_{ijk} - \mu - \alpha_i - \beta_j - \gamma_k - (\beta\gamma)_{jk}) + \nu_e S_e^2}{2}\right) \quad (10)$$

As can be seen from equations 5-10, the first five conditional distributions are from the normal and the last one is from the inverse chi-square distributions, therefore only two random number generators are required in this problem, the normal variable generator and the scaled inverse chi-square variable generator. Since every unknown has a closed form distribution, the Gibbs sampler algorithm can be used for the MCMC experiment. The Gibbs sampling algorithm generates random samples from the full conditional distributions of the parameters, without having to calculate the density. Gibbs sampling algorithm requires an initial starting point for the parameters. Then, one at a time, a value for each parameter of interest is sampled given the values for the other parameters and data. Once all of the parameters of interest have been sampled, the nuisance parameters are sampled given the parameters of interest and the observed data. At this point, the process is started over. The power of Gibbs sampling is that the joint distribution of the parameters will converge to the joint probability of the parameters given the observed data.

### 2.3. Bayesian Analysis of Cowpea Data Set Using PROC MCMC

There are two steps involved in data analysis using SAS, (1) First the data step and (2) Second the procedure step. The data step is used to input the data. The statistical analyses are performed in the procedure step via a built-in subroutine within the SAS system. Each subroutine is called a procedure performing some specific tasks. There is one particular SAS procedure called the MCMC which is designed for the MCMC implemented Bayesian analysis and handles problems with a high level of complexity.

SAS Code for the data and procedure steps

```
data cowpea;
input Blok Variety $ Spacing $ Yield;
A=Blok; B=Variety; C=Spacing; y=Yield;
cards;
1 V1 S1 56
2 V1 S1 45
3 V1 S1 43
4 V1 S1 46
1 V1 S2 60
2 V1 S2 50
3 V1 S2 45
4 V1 S2 48
1 V1 S3 66
2 V1 S3 57
3 V1 S3 50
4 V1 S3 50
1 V2 S1 65
2 V2 S1 61
3 V2 S1 60
.....
1 V3 S3 73
2 V3 S3 77
3 V3 S3 77
4 V3 S3 65
run;

*nmc = specifies the number of MCMC iterations;
*nbi =specifies the number of number of burn-in
iterations;
*thin = specifies the thinning rate ;
*plot = produces plots;
*monitor = gives output of a list of symbols;
*array = gives a list of array elements;
*parms = gives a list of parameters in the model;
*prior = specifies the prior distribution of the
        parameters;
*model = specifies the likelihood function;
*call = computes the statistics;

proc mcmc data=recodedb outpost=postb
        propcov=quanew seed=&seed nmc=500000
        nbi=100000 thin=100 plots=all
monitor = (beta1-beta&nvar sigmae diffV1V2 diffV1V3
        diffV2V3 diffS1S2 diffS1S3 diffS2S3 );
array covar[&nvar] intercept &_trgind;
```

```
array beta[&nvar] ;
parms sigmae1  ;
parms (beta1-beta&nvar) 0;
prior beta:~normal(0,var=100000);
prior sigmae~igamma(shape=0.001, scale=0.001);
* Differences between Varieties  ;
 diffV1V3 = beta5;
 diffV2V3 = beta6;
 diffV1V2 = beta5 - beta6;
* Differences between Spacings  ;
 diffS1S3 = beta7;
 diffS2S3 = beta8;
 diffS1S2 = beta7 - beta8;
call mult(covar, beta, mu);
model y ~ normal(mu, var=sigmae);
run;
ods graphics off;
ods rtf close;
```

Statistical analyses for REML estimations were obtained using PROC MIXED procedure, and Bayesian analysis was conducted using the PROC MCMC procedure of SAS software in the analysis of Cowpea data. A single chain of size 500000 iterations was run. The initial 100000 iterations were discarded as a burn-in, and every 100th sample was recorded to reduce the auto-correlation. In total, 5000 samples were stored for each parameter, and means of the sample values were used as an estimate of the parameters.

## 3. Results

The summary statistics for all the variables, including functions of the parameters and the variance component from REML and MCMC approaches for the analysis of Cowpea data set are presented in Table 3. The posterior means are based on 5000 Gibbs sampler.

**Table 3.** Summary statistics for all the variables from REML and MCMC methods

| | | Posterior Summaries | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Posterior Intervals | | | | M. Carlo SEs | |
| Parameter | REML | Mean | SD[1] | HPD[2] Interval | | Equal-Tail Interval | | MCSE[3] | MCSE/SD |
| beta1, $\mu$ | 70.75 | 70.773 | 2.530 | 65.908 | 75.755 | 65.909 | 75.758 | 0.037 | 0.015 |
| beta2, $\alpha_1$ | 6.11 | 6.095 | 2.067 | 1.927 | 9.938 | 2.018 | 10.155 | 0.030 | 0.015 |
| beta3, $\alpha_2$ | 3.33 | 3.296 | 2.070 | -0.655 | 7.413 | -0.723 | 7.368 | 0.032 | 0.016 |
| beta4, $\alpha_3$ | -0.44 | -0.459 | 2.134 | -4.643 | 3.765 | -4.699 | 3.739 | 0.031 | 0.015 |
| beta5, $\beta_1$ | -17.25 | -17.172 | 3.119 | -23.118 | -11.021 | -23.238 | -11.108 | 0.044 | 0.014 |
| beta6, $\beta_2$ | -20.75 | -20.794 | 3.076 | -26.639 | -14.437 | -26.974 | -14.737 | 0.045 | 0.015 |
| beta7, $\gamma_1$ | -17.00 | -17.039 | 3.102 | -23.464 | -11.234 | -23.312 | -11.034 | 0.047 | 0.015 |
| beta8, $\gamma_2$ | -8.75 | -8.744 | 3.183 | -14.837 | -2.349 | -14.967 | -2.419 | 0.047 | 0.015 |
| beta9, $(\beta\gamma)_{11}$ | 8.75 | 8.729 | 4.465 | -0.364 | 17.060 | -0.138 | 17.406 | 0.067 | 0.015 |
| beta10, $(\beta\gamma)_{12}$ | 3.75 | 3.661 | 4.497 | -5.169 | 12.468 | -5.422 | 12.358 | 0.065 | 0.014 |
| beta11, $(\beta\gamma)_{21}$ | 27.00 | 27.111 | 4.381 | 18.173 | 35.323 | 18.606 | 35.789 | 0.068 | 0.016 |
| beta12, $(\beta\gamma)_{22}$ | 15.00 | 15.011 | 4.390 | 6.306 | 23.659 | 6.312 | 23.679 | 0.064 | 0.015 |
| Sigmae, $\sigma_e^2$ | 17.67 | 19.407 | 6.345 | 9.958 | 32.108 | 10.794 | 34.883 | 0.091 | 0.014 |
| diffV1V2 | 3.50 | 3.623 | 3.125 | -2.729 | 9.692 | -2.576 | 9.939 | 0.044 | 0.014 |
| diffV1V3 | -17.25 | -17.172 | 3.119 | -23.118 | -11.021 | -23.238 | -11.108 | 0.044 | 0.014 |
| diffV2V3 | -20.75 | -20.794 | 3.076 | -26.639 | -14.437 | -26.974 | -14.737 | 0.045 | 0.015 |
| diffS1S2 | -8.25 | -8.296 | 3.155 | -14.793 | -2.364 | -14.633 | -2.100 | 0.046 | 0.015 |
| diffS1S3 | -17.00 | -17.039 | 3.102 | -23.464 | -11.234 | -23.312 | -11.034 | 0.047 | 0.015 |
| diffS2S3 | -8.75 | -8.744 | 3.183 | -14.837 | -2.349 | -14.967 | -2.419 | 0.047 | 0.015 |

[1]SD= standard deviation, [2]HPD= (95%), the 95% highest posterior density credible interval, [3]MCSE= monte carlo standard error, REML= Restricted maximum likelihood, MCMC= Markov chain monte carlo.

It can be noted that the Bayesian method overestimates the variance component compared with the REML estimate. The variance component obtained by REML is only marginal with respect to fixed effects but conditionals to other nuisance parameters of the model. The Bayesian analysis allows further marginalization via

Markov Chain Monte Carlo methods. This approach is particularly interesting for models, as the present, with high number of variance components. In consequence, point estimates of variance components obtained in the Bayesian analysis under that priors presented some differences with the REML estimate. These differences are due to the prior information.

The 95% High Probability Density (HPD) interval is the same as the equal tail intervals due to the normality of the posterior distribution. The equal-tail credibility intervals and the HPD intervals all show that all the pairwise differences are significantly different from zero (Table 3). The two varieties and two spacings have significant effect on the yield of Cowpea, i.e., $\beta_1$, $\beta_2$, $\gamma_1$ and $\gamma_1$ are significantly different from zero. Two of the interaction effects are also significant, i.e., $(\beta\gamma)_{21}$ and $(\beta\gamma)_{22}$ are different from zero.

Table 4 shows the Geweke z test for convergence and other diagnostic statistics for all the unknowns. If the result of the Geweke z test is significant, the chain may not have converged. From this table it can be clearly seen that the Markov chains behave very well for all the unknowns, since the p values for the Geweke z-test are larger than 0.05 for all unknowns. Because the autocorrelation is always positive, the effective sample size is always less than the actual posterior sample size. A much smaller effective sample size than the actual size indicates poor mixing of the Markov chain. The concept of effective sample size is much the same as the effective population size in population genetics. Our results show that effective sample sizes are very close to the actual posterior sample sizes.

Figure 2 shows the posterior TAD (trace-autocorrelation-density) panels for $\alpha_1$ (beta2) and $\beta_1$ (beta5), only. The Markov chain converges very well with very low autocorrelation and almost a perfect normal posterior distribution in all TAD panels representing different parameters. Overall, this dataset is sufficient to allow more precise estimates of the parameters.

**Table 4.** Diagnostic test statistics for the Markov chain convergence of the Cowpea data

| | Geweke Diagnostics | | Effective Sample Sizes | | | Posterior Autocorrelations | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Autocorrelation | | | | |
| Parameter | z | Pr>\|z\| | ESS[1] | Time | Efficiency | Lag 1 | Lag 5 | Lag 10 |
| beta1, $\mu$ | 0.851 | 0.3947 | 4593.1 | 1.0886 | 0.9186 | 0.0244 | -0.0292 | -0.0180 |
| beta2, $\alpha_1$ | -0.289 | 0.7725 | 4601.2 | 1.0867 | 0.9202 | 0.0433 | 0.0009 | -0.0092 |
| beta3, $\alpha_2$ | -0.876 | 0.3812 | 4159.7 | 1.2020 | 0.8319 | 0.0164 | -0.0015 | 0.0126 |
| beta4, $\alpha_3$ | 0.178 | 0.8588 | 4684.4 | 1.0674 | 0.9369 | 0.0337 | -0.0001 | 0.0151 |
| beta5, $\beta_1$ | 0.448 | 0.6539 | 5000.0 | 1.0000 | 1.0000 | 0.0035 | 0.0040 | -0.0268 |
| beta6, $\beta_2$ | -0.445 | 0.6562 | 4725.4 | 1.0581 | 0.9451 | 0.0291 | -0.0277 | -0.0213 |
| beta7, $\gamma_1$ | -0.557 | 0.5778 | 4346.6 | 1.1503 | 0.8693 | 0.0521 | -0.0078 | -0.0072 |
| beta8, $\gamma_2$ | -1.542 | 0.1232 | 4536.7 | 1.1021 | 0.9073 | 0.0404 | 0.0072 | -0.0072 |
| beta9, $(\beta\gamma)_{11}$ | 0.322 | 0.7474 | 4463.0 | 1.1203 | 0.8926 | 0.0428 | -0.0058 | -0.0054 |
| beta10, $(\beta\gamma)_{12}$ | 0.541 | 0.5887 | 4818.2 | 1.0377 | 0.9636 | 0.0189 | 0.0231 | 0.0024 |
| beta11, $(\beta\gamma)_{21}$ | 0.476 | 0.6342 | 4160.8 | 1.2017 | 0.8322 | 0.0540 | -0.0348 | -0.0174 |
| beta12, $(\beta\gamma)_{22}$ | 1.119 | 0.2629 | 4741.8 | 1.0545 | 0.9484 | 0.0272 | 0.0036 | -0.0078 |
| Sigmae, $\sigma_e^2$ | 0.468 | 0.6395 | 4825.4 | 1.0362 | 0.9651 | 0.0181 | -0.0051 | -0.0119 |
| diffV1V2 | 0.818 | 0.4136 | 5000.0 | 1.0000 | 1.0000 | -0.0055 | -0.0164 | -0.0016 |
| diffV1V3 | 0.448 | 0.6539 | 5000.0 | 1.0000 | 1.0000 | 0.0035 | 0.0040 | -0.0268 |
| diffV2V3 | -0.445 | 0.6562 | 4725.4 | 1.0581 | 0.9451 | 0.0291 | -0.0277 | -0.0213 |
| diffS1S2 | 0.972 | 0.3309 | 4687.8 | 1.0666 | 0.9376 | 0.0333 | -0.0037 | -0.0088 |
| diffS1S3 | -0.557 | 0.5778 | 4346.6 | 1.1503 | 0.8693 | 0.0521 | -0.0078 | -0.0072 |
| diffS2S3 | -1.542 | 0.1232 | 4536.7 | 1.1021 | 0.9073 | 0.0404 | 0.0072 | -0.0072 |

[1]ESS= effective sample size.

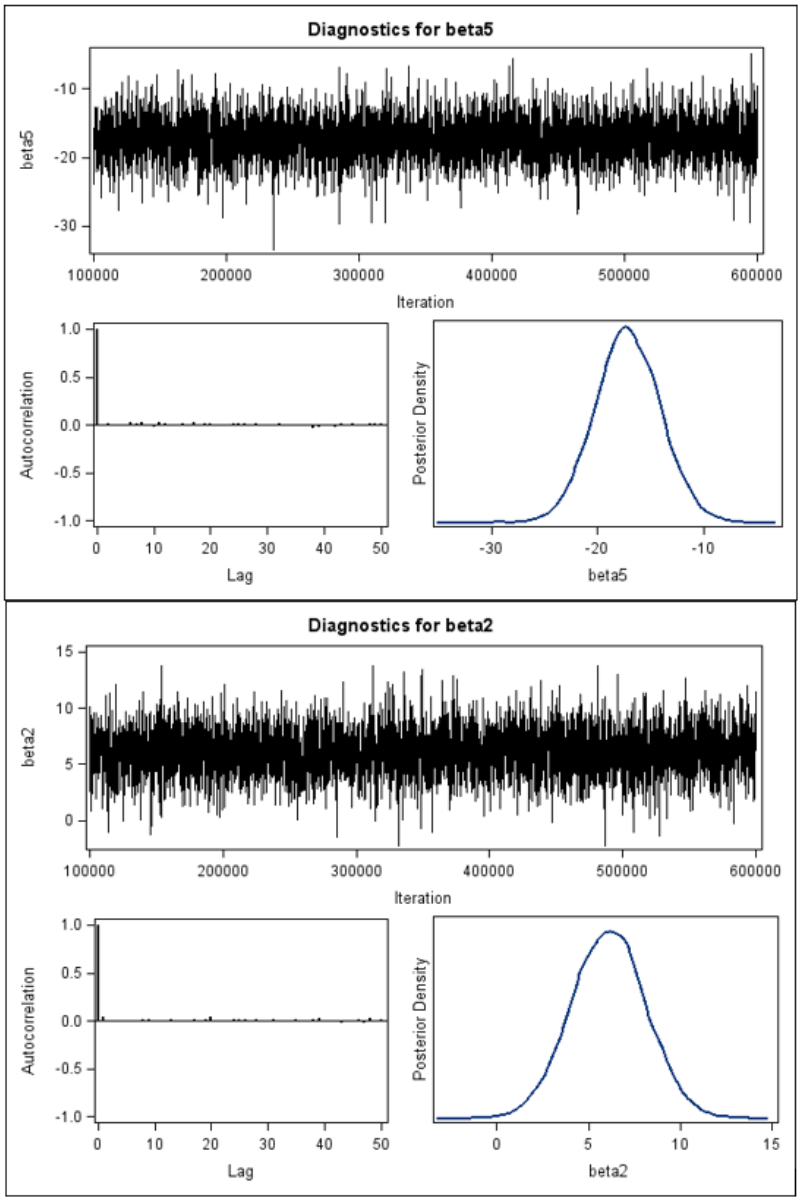



**Figure 2.** Posterior TAD panels for Markov chain convergence diagnosis from the model $\alpha_1$ (beta2) and $\beta_1$ (beta5).

## 4. Discussion

In this paper, we have presented the general concept of Bayesian methodology and the MCMC algorithm for the analysis of agricultural field experiments, a subject that has received not much previous attention despite an enormous number of frequentist literatures, in a way that can be understood by agricultural practitioners. We also demonstrated the implementation of the MCMC algorithm using PROC MCMC procedure of SAS software package to obtain posterior distribution of parameters of interest through a real data set from a two-factor factorial randomized complete-block (RCB) design. Bayesian approach is compatible with factorial experiments when studying interactions. In two factors full factorial experiment, the posterior estimates of the means of mains effects and interactions were obtained and compared with those under the likelihood-based method, REML.

It is always useful to compare and contrast the results of Bayesian analysis with that of the REML analysis. If they are completely different or not comparable in any way, there are at least three approaches to consider; new analyses with different models, the use of different priors and analysis of simulated data to verify the model and the priors. In the agricultural field data analysis, we used a new model under new priors, and produced results using PROC MCMC that have never been reported before. Then, we analyzed the same data using PROC MIXED to obtain the REML estimates under the mixed model. The two results do share some similarity. Based on the results from our data set, REML estimations of the unknown parameters are almost similar with MCMC posterior means. We can conclude that the estimates of REML are accurate but the posterior point estimates from the MCMC algorithm can be overestimated depending on the nature of the data set. The differences in the results of different estimation methods (REML and Bayesian) occurred the most in the estimation of error variance.

Such a comparison increases our confidence in the Bayesian analysis.

The original Bayesian method is more complicated than the classical maximum likelihood-based method REML, because multiple integrals are often involved in obtaining the posterior expectations of the unknown parameters. In most situations, an explicit form of the multiple integrals does not exist, and thus limits the application of Bayesian analysis. Although Bayesian inference was proposed earlier than the likelihood-based inference, it has only recently become popular due to the advent of high computing power and the advanced MCMC algorithms for numerical integrations. With the MCMC implemented Bayesian method, it has become much easier to adopt complicated models. Since it is often very simple to obtain the fully conditional posterior distributions, the MCMC process is much easier to understand than the maximum likelihood method. Thanks to the MCMC algorithm, which has revolutionized the field of Bayesian inference, the non-statisticians can also perform Bayesian analysis. Conducting an MCMC sampling process is no more complicated than doing an agricultural field experiment.

Frequentist approaches to making inferences about the parameters of interest in general linear models have several limitations and may not be able to handle complicated models. These include reliance on asymptotic theory and a failure to account for uncertainty for model parameters. A Bayesian approach to making inferences about the unknown parameters is proposed that circumvents many of the problems associated with alternative frequentist approaches. Markov Chain Monte Carlo (MCMC) and Gibbs sampling are used to obtain posterior point estimates from the posterior distributions. The 95% credible intervals (CI) were also obtained and finally compared with that obtained using classical approach. The Bayesian method for agricultural field experiments is useful to both researchers and students who will appreciate the importance of Bayesian approach when applied to practical statistical problems.

One of the main differences between the Bayesian and likelihood-based approaches is the way in which they deal with nuisance parameters (Smith and Naylor, 1987). This is apparent from our results. The conditional posterior density is obtained by a Monte Carlo numerical integration method, which is known as a Gibbs Sampler, whereas the likelihood function is obtained by maximizing with respect to the nuisance parameters. In certain cases, the two operations may produce sharply contrasting results. The computations required to implement the Bayesian method are of the same order of magnitude as those required for the REML method, and therefore the Bayesian method are likely to be computationally feasible whenever the REML methods are computationally feasible.

Implementation of the Bayesian method not only simplifies the interpretation of the results, especially in ranking and selection of the varieties, but also enables the researcher to analyze complex formulations with comparative ease, by using Markov chain Monte Carlo approaches in any agricultural field trial. Bayesian estimators depend on the information about the parameters contained in the data, and also on prior knowledge. This is one of the potential advantages of the Bayesian methods. Therefore, it is expected that the Bayesian method will do better than the classical procedures when the data contain little information about the parameters of interest. Moreover, the Bayesian method implicitly account for the uncertainty about the values at the parameters of interest.

Finally, we can conclude that the Bayesian method of estimation using the Gibbs sampling approach is suitable for estimating the unknown parameters under a full factorial Randomized Block Design (RBD) with two three-level treatment factors as compared to traditional methods, particularly for small sample data sets. It is also feasible computationally and appears to give much more sensible answer to the inferential problems than likelihood-based estimation methods. Indeed, we have maintained that Bayesian inference has some important practical advantages in analyzing field experiments. For example, the results are easier to interpret, particularly in ranking and selection of animals for next generations and when communicating with non-statisticians; the results from previous experiments can be incorporated in a rather natural manner into the prior for treatments or varieties in a subsequent trial, and there seems more freedom in using MCMC methods to analyze reasonably realistic formulations and to address model uncertainty.

## 5. Conclusion

It is also clear from our study that Bayesian method to agricultural experiments is a very rich and useful tool. It provides in depth study of different features of the data which are otherwise hidden and cannot be explored using other techniques. Moreover, SAS software has a power and efficiency to deal with the numerical as well as graphical features of data sets from agricultural experiments. Our Bayesian method uses Markov chain Monte Carlo (MCMC) approach and conjugate priors and balanced data. Simulating from full conditionals can also be easily done for the analysis of unbalanced data with possibly nonconjugate priors using the SAS PROC MCMC codes presented here, without leading one to consider alternative Markov chain Monte Carlo schemes.

**Author Contributions**

All tasks was done by the single author.

**Conflict of Interest**

The author declare that there is no conflict of interest.

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# AGRONOMIC PERFORMANCE AND PEST RESPONSE OF DIFFERENT MUNGBEAN (*VIGNA RADIATA* L.) GENOTYPES PLANTED DURING DRY SEASON CROPPING IN LEYTE, PHILIPPINES

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**Abstract:** High yielding and pest resistant varieties are among the characteristics of the crops that the farmers are looking. This study aimed to evaluate, determine and assess the profitability of different mungbean genotypes planted during dry season cropping. An experimental area of 416.5 $m^2$ was laid out in Randomized Complete Block Design (RCBD) with ten (10) treatments replicated 3 times. Each treatment plot had an area of 2.0 m x 5.0 m (10 $m^2$) with four rows in each plot. The treatments designated as follows: $T_1$ = EGM 98-419, $T_2$ = LG Mg 28-6-0, $T_3$ = LG Mg 28-6-1, $T_4$ = LG Mg 28-7-1, $T_5$ = Jade Green, $T_6$ = EGM 98-391, $T_7$ = EGM 05-738, $T_8$ = EGM 05-744, $T_9$ = NSIC Mg 17, and $T_{10}$ = PAG- ASA 7. Results revealed that most of the agronomic characteristics of different mungbean genotypes were significantly affected by the different genotypes such as days from sowing to emergence, flowering, maturity and plant height (cm). The genotype EGM 98-419 ($T_1$) emerge earlier compared to others while PAG-ASA 7 ($T_{10}$) flowers early than the other treatments. On the other hand, EGM 98-391 ($T_6$) was the early genotype to mature. However, highest plant height (cm) was obtained from the genotypes LG Mg 28-6-0 ($T_2$), LG Mg 28-6-1 ($T_3$), LG Mg 28-7-1 ($T_4$) and Jade Green ($T_5$). Likewise, number of pods per plant and seed yield were significantly affected by the different mungbean genotypes. Highest number of pods were observed from the genotype LG Mg 28-7-1 ($T_4$) and also obtained the highest seed yield of 1.47t $ha^{-1}$ compared to other genotypes. Highest gross margin of PhP 69622.00 was obtained from LG Mg 28-7-1 (T4) and obtained the highest grain yield. Pest response of all mungbean genotypes were highly resistant to insect pest and moderately resistant to diseases.

**Keywords:** Cultivars, Yield performance, Pest resistance, Insect and diseases, Cropping season, Stability

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## 1. Introduction

Mungbean (*Vigna radiata* L.) is an important legume crop providing vegetable protein for the people throughout Asia (Halimi, et al., 2018). Its dry seeds and fresh green young pods are consumed as vegetables due to its high protein, vitamin and mineral content. Its herbage is used as forage for livestock (Tang et al., 2014). It is used as intercrop in dry and semi-dry regions because of its drought tolerance and nitrogen-fixing abilities (Clua, et al., 2018).

Mungbean is famous as "tawgi" or sprouts and used as raw material in sotanghon manufacturing, hopia making, and ingredients in soups, porridge, bread, noodles and ice cream. It is also beneficial to human health as cholesterol controller, bone strengthener, blood pressure regulator, liver protection, promotes growth to children, anti-viral and anti-cancerous agent (http://www.stylecraze.com/articles/amazing-benefits-of-mung-beans/), thus, high demand for this commodity.

In the Philippines, Bureau of Agricultural Statistics reported that the highest volume of production for the past five years was obtained in 2017, with 45283 metric tons from the total production area of 44324 hectares. In 2018, however production area declined to 32364 hectares with total production of 32364 metric tons, (PSA, 2018). However, national average yield per hectare remains low (0.73 metric tons). Hence, development of more high yielding NSIC varieties is needed.

Planting genotypes adapted to our geographic situation increase crop productivity. Seed Alliance (2018) reported that on-farm trials help the farmers to manage risk and help growers to optimize their operation to avoid a number of common production problems. In this regard, promising mungbean genotypes is needed to be evaluated on their agronomic performance during dry season where mungbean can provide good harvest during this season (Mondal, 2011). Another one important traits of a good variety is its resistance to pest and diseases thus, the response of the different mungbean lines is needed under different agro-climatic conditions before its recommendation to the National Seed Industry Council for release as new variety. Hence,

this study was conducted to determine the performance of the different promising mungbean genotypes in terms of growth, yield and pest response.

## 2. Material and Methods

An area of Umingan clay loam soil, (FAO, 2013) located at the Agronomy Experimental Area College of Agriculture and Food Science, Visayas State University, Babay City, Leyte. The experimental area has a GPS coordinates of 10°44' 59.8668" N, 124°47' 38.1264" E. This was plowed and harrowed twice at weekly interval to provide desirable soil tilth for better growth and development of plant. Furrows were made immediately after the last harrowing at a spacing of 0.5m.

Before land preparation, ten soil samples were collected randomly in the experimental area at 15-20 cm depth. Samples were collected air-dried and sieved through 2 mm wire mesh and brought to the Central Analytical Services Laboratory (CASL), Phil Rootcrops, Visca, Baybay City, Leyte for the initial and final determination of pH (potentiometric method), organic matter (Walkley-Black Method), total N, extractable phosphorus (Olsen's sodium bicarbonate extraction) and exchangeable potassium at the using the ammonium acetate extraction method.

The experimental area was laid out in a Randomized Complete Block Design (RCBD) with three replications following the protocol of conducting NCT trials for legumes. Each replication was divided into ten treatment plots each measuring 2 m x 5 m (10 $m^2$) with four rows per plot. Alleyways of 1 m between replications and 0.5m between treatment plots were provided to facilitate farm operations and data gathering. The following mungbean genotypes evaluated and served as the treatments of the study, were the following: $T_1$ = EGM 98-419, $T_2$ = LG Mg 28-6-0, $T_3$ = LG Mg 28-6-1, $T_4$ = LG Mg 28-7-1, $T_5$ = Jade Green, $T_6$ = EGM 98-391, $T_7$ = EGM 05-738, $T_8$ = EGM 05-744, $T_9$ = NSIC Mg 17, $T_{10}$ = PAG- ASA 7. Seeds of mungbean genotype specified in the treatments were evenly drilled in furrows in each assigned treatment plot. The seeds were covered with thin layer of soil to protect them from ants and birds that may feed on them and disrupt their growth. Thinning was done ten days after seeding in all treatment plots to achieve the desired plant population of 150000-200000 $ha^{-1}$. Complete fertilizer (14-14-14) was applied at the rate of 30-30-30 kg $ha^{-1}$ N, $P_2O_5$, $K_2O$. About 214. 29 grams of complete fertilizer was applied in each treatment plot. The fertilizer was placed in furrows and covered with fine layer of soil about 2-3 cm thick to prevent the seedlings from getting in contact with the fertilizer. The application of fertilizer was done 5-7 days after seedling emergence.

Six weeks after planting, aphids and Cercospora leaf spot disease were observed. No control measure was done to the study since pest resistance parameters were observed and evaluated in the study. The mungbean crop was harvested when about 75% of the pods in each treatment plot reached physiological maturity characterized by black or brown color of pods. All the plants in harvestable area (4.0 $m^2$) of each treatment plots were harvested excluding the two boarder rows. The sample pods in each treatment plots were sundried for three days before necessary data were gathered. For the agronomic characteristics; days from seeding to seedling emergence, days from seeding to flowering, days from seeding to maturity, plant height (cm) at harvest, fresh herbage yield (t $ha^{-1}$). The plot yield was converted to ton $hectare^{-1}$ using the formula (equation 1):

$$HY\ (t\ ha^{-1}) = \frac{PY\ (kg)}{HA\ (4.0\ m2)}\ x\ \frac{10000\ m^2\ ha^{-1}}{1000\ kg\ t^{-1}} \qquad (1)$$

HY= Herbage yield, PY= Plot yield and HA= Harvestable area.

For yield and yield components; number of pods $plant^{-1}$, number of seeds $pod^{-1}$, weight of seeds $plant^{-1}$, weight of 1000 seeds (g) and seed yield (t $ha^{-1}$) was weighed and converted to $hectare^{-1}$ using the formula (equation 2):

$$Y\ (t\ ha^{-1}) = \frac{PY\ (kg)}{HA\ (4.0\ m^2)}\ x\ \frac{10000\ m^2\ ha^{-1}}{1000\ t\ ha^{-1}} \qquad (2)$$

Y= Yield, PY= Plot yield and HA= Harvestable area.

For the pest resistance rating the NCT pest rating manual for legumes (2017) was used. This was determined through the use of a rating scale from 1-5, one is the lowest rating of pests and diseases present in the field while five is the rating that indicated severe number of insect pests and diseases present in the field. Ratings for insect pests was done by selecting (at random) ten sample plants per plot and examine them thoroughly for leaf feeding damage at 25 days after emergence (NCT Manual for Legumes, revised 2017). Other parameters gathered were harvest index (HI), gross margin analysis and meteorological. These were determined using the formula, below (equation 3):

$$HI = \frac{DWG\ (3\ \text{sample plants})}{DWH+DWG\ (3\ \text{sample plants})} \qquad (3)$$

HI = Harvest index, DWG= Dry weight of grains and DWH= Dry weight of herbage.

Gross Margin = Gross Income - Total Variable Cost.

Meteorological Data such as total monthly rainfall (mm), average daily minimum and maximum temperatures (°C) and relative humidity (%) throughout the conduct of the study were taken from the records of Philippine Atmospheric, Geophysical and Astronomical Services Administration (PAGASA) Station, VSU, Visca, Baybay City, Leyte. Likewise, Cost and return was also computed to test the profitable treatment/ mungbean genotypes.

Means were taken and ANOVA was done using Statistical Tool for Agricultural Research (STAR) software. Comparison between treatments was done using the Honestly Significant Difference (HSD).

## 3. Results and Discussion

The climatic data is presented in (Figure 1). The climatic data (total amount of rainfall (mm), minimum and maximum temperatures as well as the percent relative humidity received by the mungbean plants were enough for its normal growth and development, (PCARRD Handbook, 2002).

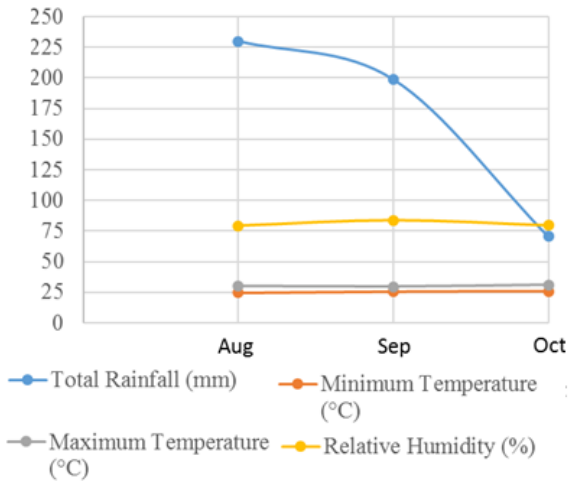


**Figure 1.** Total monthly rainfall (mm), average minimum and maximum temperature (°C) and relative humidity from August 16, 2018 to October 24, 2018 obtained from PAGASA Station, VSU, Visca, Baybay City, Leyte.

### 3.1. Soil Analysis

Results of initial soil analysis showed a pH of 6.17, with 1.897% of organic matter, 0.167% of total Nitrogen, 16.043 mg $kg^{-1}$ available Phosphorus and 0.618 me 100 $g^{-1}$ exchangeable potassium (Table 1). These indicated that the soil was slightly acidic with high amount of phosphorus, very low amount in organic matter and low amount of both nitrogen and potassium (Landon, 1991).

**Table 1.** Soil chemical analyses before and after planting of mungbean genotypes grown in dry season cropping

| | Initial analysis (before planting) | Final analysis (after planting) |
|---|---|---|
| Soil pH | 6.17 | 5.48 |
| % OM | 1.897 | 1.845 |
| Total N (%) | 0.167 | 0.152 |
| Available P (mg $kg^{-1}$) | 16.043 | 25.300 |
| Exchangeable K (me 100 $g^{-1}$) | 0.618 | 0.633 |

In the final soil analysis, the soil pH slightly decreased to 5.48. The organic matter and total nitrogen also decreased from 1.897 to 1.845% and 0.167 to 0.152%, respectively. The decrease in soil pH can be due to due to leaching from high amounts of rainfall. Likewise, there was a decrease in total nitrogen and organic matter and this could be attributed to the consumption of nutrients by the plants (Baldock, 2019). To the contrary, sufficient amount of available phosphorus and exchangeable potassium was observed. This could be due to the added fertilizers into the experimental area and decomposition of leaf litter and other plant herbage that were previously planted in the area (Singh, 2017).

### 3.2. Agronomic Characteristics of Mungbean

Table 2 show the agronomic characteristics of mungbean as affected by the different promising genotypes of mungbean. Analysis of variance showed that the number of days from seeding to emergence, flowering, maturity, plant height (cm) and fresh herbage yield (tha$^{-1}$) were significantly affected by the different mungbean genotypes. Among the genotypes tested, EGM 98-419 ($T_1$) emerged earlier and this was comparable to LG Mg 28-6-0 ($T_2$), LG Mg 28-6-1 ($T_3$), LG Mg 28-7-1 ($T_4$), EGM 98-391 ($T_6$), EGM 05-738 ($T_7$) and PAG-ASA 7 ($T_{10}$) while, EGM 05-744 ($T_8$), NSIC Mg 17 ($T_9$) and Jade Green ($T_5$) were late to emerged. This result can be attributed to the inherent characteristics of mungbean. According to Rehman et al. (2009), different varieties have different genotypic characteristics which resulted to the difference in agronomic and yield performance.

On the other hand, early flowering was obtained by the genotype PAG-ASA 7 ($T_{10}$) and this was comparable to EGM 98-419 ($T_1$), Jade Green ($T_5$) EGM 98-391 ($T_6$), and EGM 05-738 ($T_7$), Mondal et al. (2011) reported that flowering duration was higher in high yielding varieties than the low yielding ones. In addition, flowering duration and flower production had relation with seed yield in mungbean. Furthermore, the genotype EGM 98-391 ($T_6$) and PAG-ASA 7 ($T_{10}$) were considered early maturing among the rest of the treatments. However, EGM 98-419 ($T_1$) was considered late maturing genotype which was comparable to genotypes LG Mg 28-6-1 ($T_3$), LG Mg 28-7-1 ($T_4$) and NSIC Mg 17 ($T_9$). Again this could be accounted to the characteristics of the genotypes (Rehman et al., 2019). Moreover, taller mungbean plants were noted from the genotypes Jade Green ($T_5$), and comparable to LG Mg 28-6-1 ($T_3$), LG Mg 28-6-0 ($T_2$), LG Mg 28-7-1 ($T_4$), NSIC Mg 17($T_9$), EGM 98-391 ($T_6$), EGM 98-419 ($T_1$) while EGM 05-738 ($T_7$), EGM 05-744 ($T_8$) and PAG-ASA 7 ($T_{10}$) were significantly shorter due to different genotypic characteristics of the treatments tested.

### 3.3. Yield, Yield Components and Harvest Index

Table 3 show the yield and yield components and harvest index of mungbean as affected by the different promising genotypes of mungbean. Analysis of variance showed that number of pods per plant, and seed yield (t ha$^{-1}$) were significantly affected by the different treatments but not on the seeds per pod, weight of the seeds per plant, weight of 1000 seeds (g), and harvest index. The genotypes EGM 98-391 ($T_6$) produced higher number of pods comparable to the genotypes EGM 98-419 ($T_1$), EGM 05-744 ($T_8$), LG Mg 28-6-1 ($T_3$), LG Mg 28-7-1 ($T_4$), EGM 98-391 ($T_6$), EGM 05-738 ($T_7$), NSIC Mg 17 ($T_9$), and PAG-ASA 7 ($T_{10}$).

**Table 2.** Agronomic characteristics of different mungbean genotypes grown in dry season cropping

| Treatment | Days from seeding to | | | Plant Height (cm) | Fresh Herbage Yield (t ha$^{-1}$) |
|---|---|---|---|---|---|
| | Emergence | Flowering | Maturity | | |
| $T_1$ - EGM 98-419 | 2.67$^{c}$ | 35.00$^{bcd}$ | 61.33$^{a}$ | 81.10$^{cd}$ | 7.92 |
| $T_2$ - LG Mg 28-6-0 | 3.00$^{bc}$ | 36.00$^{b}$ | 57.67$^{cd}$ | 91.80$^{abc}$ | 9.09 |
| $T_3$ - LG Mg 28-6-1 | 3.00$^{bc}$ | 38.67$^{a}$ | 61.33$^{a}$ | 95.33$^{ab}$ | 7.71 |
| $T_4$ - LG Mg 28-7-1 | 3.00$^{bc}$ | 35.67$^{bc}$ | 60.00$^{ab}$ | 85.47$^{a-d}$ | 6.71 |
| $T_5$ - Jade Green | 3.67$^{ab}$ | 34.00$^{cd}$ | 58.00$^{c}$ | 95.67$^{a}$ | 5.25 |
| $T_6$ - EGM 98-391 | 3.33$^{abc}$ | 34.67$^{bcd}$ | 55.67$^{e}$ | 81.60$^{cd}$ | 9.54 |
| $T_7$ - EGM 05-738 | 3.00$^{bc}$ | 35.00$^{bcd}$ | 59.00$^{bc}$ | 80.97$^{cd}$ | 10.34 |
| $T_8$ - EGM 05-744 | 4.00$^{a}$ | 38.00$^{a}$ | 58.33$^{bc}$ | 76.93$^{de}$ | 10.79 |
| $T_9$ - NSIC Mg 17 | 3.67$^{ab}$ | 39.00$^{a}$ | 60.00$^{ab}$ | 84.17$^{bcd}$ | 10.50 |
| $T_{10}$ - PAG- ASA 7 | 3.00$^{bc}$ | 33.33$^{d}$ | 56.00$^{de}$ | 67.42$^{e}$ | 10.38 |
| Mean | 3.23 | 35.93 | 58.73 | 84.04 | 8.82 |
| C. V. % | 9.78 | 11.90 | 11.05 | 14.62 | 28.06 |

Means within each column followed by the same letter and those without letter designations were not significantly different at 5% level, HSD test.

**Table 3.** Yield and yield components and harvest index of different mungbean genotypes grown in dry season cropping

| Treatment | Number of | | Weight (g) of | | Seed Yield (t ha$^{-1}$) | Harvest Index |
|---|---|---|---|---|---|---|
| | Pods per plant | Seeds per pod | Seeds per 1000 | Plant seeds | | |
| $T_1$ - EGM 98-419 | 25.67$^{a}$ | 11.77 | 9.87 | 69.67 | 1.17$^{ab}$ | 0.31 |
| $T_2$ - LG Mg 28-6-0 | 15.67$^{b}$ | 11.93 | 11.20 | 70.33 | 1.27$^{a}$ | 0.35 |
| $T_3$ - LG Mg 28-6-1 | 24.67$^{a}$ | 12.70 | 12.10 | 73.33 | 1.20$^{ab}$ | 0.36 |
| $T_4$ - LG Mg 28-7-1 | 22.00$^{ab}$ | 12.29 | 13.83 | 70.33 | 1.47$^{a}$ | 0.38 |
| $T_5$ - Jade Green | 15.67$^{b}$ | 11.63 | 9.23 | 68.33 | 0.40$^{c}$ | 0.33 |
| $T_6$ - EGM 98-391 | 26.00$^{a}$ | 12.47 | 11.93 | 70.00 | 1.33$^{a}$ | 0.34 |
| $T_7$ - EGM 05-738 | 21.33$^{ab}$ | 12.83 | 11.93 | 66.67 | 1.43$^{a}$ | 0.32 |
| $T_8$ - EGM 05-744 | 25.33$^{a}$ | 13.03 | 12.00 | 70.33 | 1.43$^{a}$ | 0.33 |
| $T_9$ - NSIC Mg 17 | 24.00$^{a}$ | 12.87 | 10.90 | 69.67 | 1.30$^{a}$ | 0.38 |
| $T_{10}$ - PAG- ASA 7 | 23.67$^{ab}$ | 13.07 | 9.87 | 70.00 | 0.83$^{bc}$ | 0.31 |
| Mean | 22.76 | 12.46 | 11.29 | 69.87 | 1.18 | 0.34 |
| C. V. % | 12.45 | 13.98 | 16.08 | 13.66 | 24.67 | 12.86 |

Means within each column followed by the same letter and those without letter designations were not significantly different at 5% level, HSD test.

On the other hand, lesser number of pods were observed from the genotypes Jade ($T_5$) Green and LG Mg 28-6-0 ($T_2$) but comparable to LG Mg 28-7-1 ($T_4$), EGM 05-738 ($T_7$) and PAG-ASA 7 ($T_{10}$). On the other hand, comparable higher seed yield (t ha$^{-1}$) were observed from all genotypes except Jade Green (0.40 t ha$^{-1}$) and PAG-ASA 7 (0.83 t ha$^{-1}$) which had the lowest seed yield among others. Mondal et al. (2011) added that mungbean varieties that produce more number of pods will also produce higher seed yield in per hectare basis.

**3.4. Response of Insect Pest and Diseases**

Response of different mungbean genotypes to insect pests and diseases is presented in (Table 4). Analysis of variance showed that the insect pest damage and disease did not show significant differences among treatment genotypes. This insignificant result could be due to their genotypic characteristics of the mungbean plants. All treatments were highly resistant to the insect damage. In addition, all genotypes tested were moderately susceptible to *Cercospora* leaf spot disease. In effect, this insect and disease damage did not affect the production of mungbean. Hence, they produce a reasonable yield except Jade Green ($T_5$) and PAG-ASA 7 ($T_{10}$).

Moreover, based on the reaction of insect pest damage of different mungbean genotypes the farmers can minimize the cost of insecticide due to it is highly resistance to insect pests.

**3.5. Gross Margin Analysis**

Gross margin analysis of mungbean in response to different genotypes is presented in Table 5. Highest gross margin of PhP 69622.00 ha$^{-1}$ was obtained from the genotype LG Mg 28-7-1 ($T_4$) followed by EGM 05-738 ($T_7$) and EGM 05-744 ($T_8$) of PhP 66,822.00 ha$^{-1}$. This was due to the high grain yield obtained in the said genotype. However, the genotype Jade Green generated the lowest gross margin of PhP 722.00, due to the very low grain yield (t ha$^{-1}$).

**Table 4.** Incidence of insect pests and diseases of different mungbean genotypes grown in dry season cropping

| Treatment | Insect Pests Damage | Reaction | Disease (CLS) | Reaction |
|---|---|---|---|---|
| $T_1$ - EGM 98-419 | 2.00 | highly resistant | 3.33 | moderately susceptible |
| $T_2$ - LG Mg 28-6-0 | 1.67 | highly resistant | 3.67 | moderately susceptible |
| $T_3$ - LG Mg 28-6-1 | 2.33 | highly resistant | 3.33 | moderately susceptible |
| $T_4$ - LG Mg 28-7-1 | 2.00 | highly resistant | 3.67 | moderately susceptible |
| $T_5$ - Jade Green | 2.33 | highly resistant | 4.00 | moderately susceptible |
| $T_6$ - EGM 98-391 | 2.00 | highly resistant | 3.67 | moderately susceptible |
| $T_7$ - EGM 05-738 | 2.33 | highly resistant | 3.67 | moderately susceptible |
| $T_8$ - EGM 05-744 | 2.33 | highly resistant | 3.67 | moderately susceptible |
| $T_9$ - NSIC Mg 17 | 2.00 | highly resistant | 4.00 | moderately susceptible |
| $T_{10}$ - PAG- ASA 7 | 1.67 | highly resistant | 3.33 | moderately susceptible |

Rating Scale for insect Pest and diseases

| Damage Index | Insects Leaf Damage (%) | Reaction | Damage Index | Scale for Diseases | Description |
|---|---|---|---|---|---|
| 1 | 1-20 | Highly resistant | 1 | 1.00 | Highly resistant |
| 2 | 21-40 | Moderately resistant | 2 | 1.01-2.49 | Moderately resistant |
| 3 | 1-60 | Moderately susceptible | 3 | 2.50-3.49 | Intermediate resistant |
| 4 | 61-80 | Susceptible | 4 | 3.50-4.49 | Moderately susceptible |
| 5 | 80-100 | Highly susceptible | 5 | 4.50-5.00 | Highly susceptible |

**Table 5.** Gross margin analysis of different mungbean genotypes grown in dry season cropping*

| Treatment | Grain yield (t ha$^{-1}$) | Gross Income (PhP ha$^{-1}$) | Production Cost (PhP ha$^{-1}$) | Net Income (PhP ha$^{-1}$) |
|---|---|---|---|---|
| $T_1$ - EGM 98-419 | 1.17 | 81900.00 | 32528.00 | 49372.00 |
| $T_2$ - LG Mg 28-6-0 | 1.27 | 88900.00 | 32528.00 | 56372.00 |
| $T_3$ - LG Mg 28-6-1 | 1.20 | 84000.00 | 32528.00 | 51472.00 |
| $T_4$ - LG Mg 28-7-1 | 1.47 | 102900.00 | 33278.00 | 69622.00 |
| $T_5$ - Jade Green | 0.40 | 28000.00 | 27278.00 | 722.00 |
| $T_6$ - EGM 98-391 | 1.33 | 93100.00 | 33278.00 | 59822.00 |
| $T_7$ - EGM 05-738 | 1.43 | 100100.00 | 33278.00 | 66822.00 |
| $T_8$ - EGM 05-744 | 1.43 | 100100.00 | 33278.00 | 66822.00 |
| $T_9$ - NSIC Mg 17 | 1.30 | 91000.00 | 33278.00 | 57722.00 |
| $T_{10}$ - PAG- ASA 7 | 0.83 | 58100.00 | 27,278.00 | 30822.00 |

*Based on the current price of PhP 70.00 kg$^{-1}$.

## 4. Conclusion

Results of the study found out that different genotypes of mungbean differ significantly in the number of days from sowing to emergence, number of days from sowing to flowering and maturity as well as the plant height. Likewise, and yield components such number of pods per plant and the total seed yield (t ha$^{-1}$). Different mungbean genotypes yields ranges from 1.17-1.47 t ha$^{-1}$ ypes except Jade Green and PAG-ASA 7. Moreover, higher gross margins were obtained from all mungbean genotypes tested except Jade Green which obtain lower gross margin of PhP 722.00 ha$^{-1}$ due to low yield per hectare. Likewise, based on the results of the study, it is recommended that a similar study be conducted in different locations to validate its performance across locations and seasons. While, one genotype Jade Green will be recommended to delete from the entries due to its very low performance in terms of yield.

**Author Contributions**

All authors have equal contributions and reviewed and approved the manuscript.

**Conflict of Interest**

The authors declare that there is no conflict of interest.

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Research Article
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# BIOMASS ENERGY POTENTIAL FROM AGRICULTURAL RESIDUES IN ERITREA

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**Abstract:** This study aimed to estimate the amount of biomass energy that can be acquired from crop residues in Eritrea, a country in the horn of Africa with a population of 6 million and having a national income per capita is around 150 US dollars. It's an agricultural country. The energy potential of crop residues was calculated by considering the calorific values and the amount of available residue. For the year 2015, the total calorific value of agricultural residues was estimated approximately 1332.34TJ. According to the amount of agricultural residues, the most contributing crops were sorghum (50%) and millet (27%). Thus, it can be inferred that knowing the particular and general biomass energy potential of agricultural residues could help in managing energy sources and planning projects.

**Keywords:** Biomass, Agricultural residue, Energy, Eritrea

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## 1. Introduction

Energy supply, specifically the issue of alternative energy sources, has seized the thoughts of people worldwide and has stimulated more research, dispute and action: personal, political and commercial; perhaps as equal as any other environmental issue in the course of time (Onochie et al., 2015). The ever-rising energy demand is directly related to the increase in the standard of living and the advancement of new technologies (Demirel et al., 2014). Considering renewable energy sources such as biomass energy has been one of the principal solutions to the depletion of fossil fuels.

The use of biomass energy dates back to the dawn of history. Followed by food crops, grassy and woody plants, residues from agriculture or forestry, oil-rich algae, and the organic component of municipal and industrial wastes, wood has been the largest source of biomass energy. Every organic matter existing in the biosphere is regarded as biomass. It constitutes of plant and animal origin including the materials obtained as a result of their natural and artificial transformation (Perea-Moreno et al., 2019). Biomass exists in diversified forms such as wood, sawdust, straw, seed waste, manure, paper waste, household waste, and wastewater (Long et al., 2013). By virtue of their nature, some biomass energy sources are used directly as fuel. However, others should be subjected to certain treatments, requiring various technologies before they are used. Converting into a range of valuable biofuels, chemicals, and other products, the application of biomass energy has the potential to significantly minimize the emission of greenhouse gases, reliance on fossil fuels, and eventually support agricultural industries (Mohtasham, 2015). Due to its local abundance and low price biomass appears to be encouraging renewable energy resource. As the main bioenergy resource, it can be produced from natural materials, such as harvest residues, energy crops, algae, and agricultural wastes (Mirkouei et al., 2017). Sustainable bioenergy sources have the capacity to promote economic opportunities, energy security, and environmental benefits (Yang et al., 2017). Bioenergy has been suggested as a sustainable source of energy that has a higher potential to displace the dominant fossil-based energy (Mirkouei et al., 2017).

Employing alternative energy sources is a key factor in improving the livelihood of needy societies and make economic sustainability achievable (Kaygusuz, 2011). Production of charcoal is one common domestic practice of meeting the requirement of energy. When used as fuel this has the potential of reducing indoor air pollution. Furthermore, it has a direct impact on economic growth because people can market the extra charcoal (Zulu and Richardson, 2013).

In Eritrea, like in most developing countries, a major proportion of energy is contributed by biomass sources. A report from the Department of Energy shows that out of the total energy supply 66.3% was derived from local biomass fuels (Semere, 2001). With the growth of the

population in rural areas, it is obvious that the reliance on biomass energy sources will continue. Consequently, the demand for these sources will also keep on increasing (Zemenfes, 2001). Therefore, exploring and evaluating a country's potential energy sources is highly significant so as the standard of living is improved by providing a sufficient source of energy to society. Thus this study aimed to estimate the amount of biomass energy that can be acquired from crop residues in Eritrea.

## 2. Material and Methods

In the assessment of biomass resources two ways namely resources focused and demand-based approaches are used (Van den and Vis, 2014). From the aforesaid methods, the resource-based approach is the most customary approach used worldwide for the calculation of biomass and bio-energy potential. In this method, specific biomass types like agricultural residue, forest residue, and their byproducts are put into consideration (Long et al., 2013). Thus, this study follows the resource-based approach to evaluate the biomass energy potential in Eritrea.

Despite the fact that crop residues have different categories (gross residue sand surplus residues), in this study, only the surplus residues are considered since the farmers use the others for different purposes. Data from the annual report of the Ministry of Agriculture (MOA) of Eritrea for the year 2015 were used to compute the amount of residue from the seasonal year production of main crops cultivated in Eritrea. The formula used for calculating the bio-energy potential from crop residues is adopted from (Hiloidhari and Baruah, 2011; Terrapon-Pfaff et al., 2012). The total residue has to be determined first as the remaining residue depends on it (equation 1).

$$AAR = AAP * RPR * A \quad (1)$$

Where (AAR) is the available amount of agricultural residues of the crop in tons, (AAP) the amount of agricultural product in tons, (RPR) residue-to-product ratio, and (A) the availability of residues relevant for developing countries (Elias and Shabbir, 2018). The RPR values are obtained from different published research works conducted in developing countries in the Sub-Saharan region of Africa as it is represented in Table 1.

**Table 1.** Parameters and their values used in estimation

| FC | R | RPR | A (%) | LHV(MJkg$^{-1}$) | Reference |
|---|---|---|---|---|---|
| Sorghum | Straw | 1.75 | 60 | 12.38 | (Kimutai et al., 2014) |
| Maize | Cob | 0.3 | 100 | 15.5 | (Singh et al., 2008) |
| Millet | Straw | 1.75 | 60 | 18.16 | (Friedl et al., 2005) |
| Barley | Stalks | 2.7 | 60 | 18.6 | (Friedl et al., 2005) |
| Wheat | Straw | 0.8 | 15 | 17.15 | (Singh et al., 2008) |
| Hanfez | Straw | 1.75 | 60 | 17.88 | (Friedl et al., 2005) |
| Sesame | Straw | 0.5 | 56 | 14.35 | (Zabaniotou et al., 2008) |
| Groundnut | Shells | 0.48 | 40 | 15.56 | (Jekayinfa and Scholz, 2009) |

FC= field crops, R= residue type, RPR= residue to product ratio, A= availability, LHV= lower heating value.

The portion of available residues from crop production after other parts are used for different purposes is known as the surplus availability (Hiloidhari and Baruah, 2011). Finally, the bio-energy crop residue potential is estimated from equation (equation 2) as follows;

$$THV = AAR * LHV \quad (2)$$

Where (THV) the total heating value of agricultural residues of the crop in TJ, (AAR) is the available amount of agricultural residues of the crop in tons, and (LHV) lower heating value of air dry residues of the crop in MJ.kg$^{-1}$.

## 3. Results and Discussion

Using the resource focused approach the total amount of agricultural residues of major crops Barley, Groundnut, Wheat, Hanfez (combination of wheat and barley), Sorghum, Millet, Sesame, and Maize was estimated to be 85265.28 tons in Eritrea (Table 2).

As it has been represented in Fig 1, out of the total residue sorghum and millet comprise about 50% and 27% respectively being the major ones.

**Table 2.** Amount of agricultural product and available residues in tones of selected field crops in Eritrea

| FC | R | AAP | AAR |
|---|---|---|---|
| Sorghum | Straw | 32091 | 33695.6 |
| Maize | Cob | 34019 | 10205.7 |
| Millet | Straw | 17412 | 18282.6 |
| Barley | Stalks | 12209 | 19778.6 |
| Wheat | Straw | 8495 | 1019.4 |
| Hanfez | Stalks | 2923 | 1918.2 |
| Sesame | Straw | 1061 | 297.1 |
| Groundnut | Shells | 355 | 68.2 |
| TOTAL | | | 85265.28 |

FC= field crops, R= residue type, AAP= amount of agricultural residues, AAR= amount of agricultural product.

In calculating the total amount of heating value, although the total amount of crop residues is estimated to be 85265.28tons, it should be noted that since residues are used for various purposes the surplus availability factor was considered to estimate the reliable amount of energy. Hence, as shown in Table 3, the total heating value of residues for the year 2015 was estimated to be 1332.34TJ.

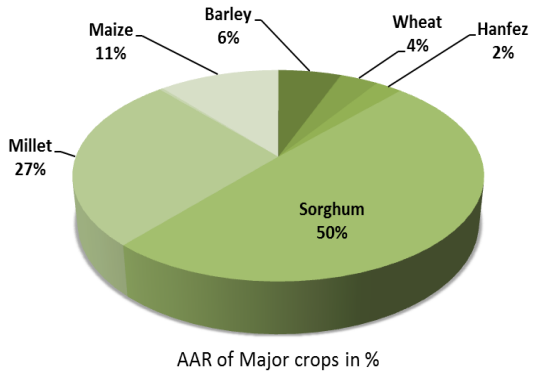


**Figure 1.** Amount of available residue (AAR) of major field crops.

**Table 3.** Total heating values of agricultural residues in Eritrea

| FC | R | THV (TJ) |
|---|---|---|
| Sorghum | Straw | 417.15 |
| Maize | Cob | 158.19 |
| Millet | Straw | 332.01 |
| Barley | Stalks | 367.88 |
| Wheat | Straw | 17.48 |
| Hanfez | Stalks | 34.30 |
| Groundnut | Shells | 4.26 |
| Sesame | Straw | 1.06 |
| TOTAL | | 1332.34 |

FC= field crops, R= residue type, THV= total heating value

## 4. Conclusion

The study was done to estimate the biomass energy potential in Eritrea. Like many other countries, Eritrea's source of energy depends on the import of fossil fuels. The subject of energy demand remains to be a solution-seeking challenge. Energy scarcity and energy-related cases are problems that need to be addressed at the soonest possible. In facilitating the possible solutions knowing the capacity and potential of every alternative source of energy is crucial. Thus, knowing the biomass energy potential could help in managing energy sources, planning projects, and policymaking as a whole. The total heating value of crops in Eritrea was found to be 1332.34TJ. It is easy to infer that this considerable amount of energy is significantly contributing to the energy demand of the country. Additionally, establishing systems to change this potential into other kinds of energy sources such as biogas would definitely pay off.

**Author Contributions**

All authors contributed equally to this work. All authors reviewed and approved the manuscript.

**Conflict of Interest**

The authors declare that there is no conflict of interest.

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# AVAILABILITY OF SOME TROPICAL PLANTS AS ALTERNATIVE ROUGHAGE SOURCE IN RUMINANT FEEDING

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**Abstract:** Roughages have vital importance in the diet of ruminants because they are cheap and absolutely necessary for digestive physiology. Ruminant nutrition requires quality feeds to obtain high amounts of product. As more than half of the business inputs are made up of feed expenses, the necessity of high-quality roughage sources, which are cheaper compared to concentrate, arises. The high quality of roughage means that the amount of mixed feed that can be put into the ruminant ration to meet the nutrient requirement is less. Thus, the cost of the product to be obtained will decrease and the net profit will increase. Since the leaves of some plants grown in the tropical region and the fruits and shells that cannot be used as human food are not utilized, they cause environmental pollution, and the feed cost cannot be reduced because the vegetable waste is not used in animal feeding. Many tropical plant leaves and waste are rich in protein and crude fiber. Crude protein levels in the leaves of some tropical plants can be up to 30%. The usability of tropical plant leaves and fruit peels, which are rich in nutrients, as roughage has not been adequately studied. These plants can be used as an alternative roughage source for ruminants in times of shortage of quality roughage and in times of famine, increasing animal production and preventing problems in the environment. The purpose of this review is to examine the possibilities of using leaves and fruits and wastes of some tropical plants (Guava, papaya, banana, mango, pineapple, cassava, moringa and avocado) as an alternative roughage source in ruminants.

**Keywords:** Ruminant, Tropical plants, Roughage

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## 1. Introduction

One of the main factors limiting animal production in the world and especially in developing countries is the high cost of feed inputs. Roughages are bulky feeds rich in crude fiber (CF), with low digestibility, energy density and nutrient composition. One of the most important problems to be solved in the feeding of ruminants is to meet the quality, cheap and easy to obtain roughage requirement of the animals. Roughage is essential for meeting the life and productivity needs of animals economically and for rumen physiology (Saricicek, 2007). In case the needs of animals cannot be met with fodder crop (high quality roughages), it is tried to be met with low quality roughages such as hay, stalk, husk and cut. In this case, the energy, protein and mineral needs of ruminants that cannot be covered with these feeds are met with expensive concentrate feeds. High use of concentrated feed in animal nutrition increases the costs of animal products and causes some metabolic disorders (Gemalmaz et al., 2016).

Some subtropical and tropical plants grown in tropical climates are grown in the Mediterranean region in Turkey. Recently, there has been increased interest in the use of tropical plants as feed for animal nutrition due to the richness of nutrient contents. The importance of tropical plants for animal production is that leaves, flowers, boughs, seeds, fruits and pods can be used by animals as concentrated feed or roughage. There are a variety of tropical legume plants that can be used as a protein source in the diet of ruminants. Tropical legumes are very important sources in terms of their seeds and the amino acid (AA) contents. However, it also has disadvantages such as being produced in small quantities, being used directly as human food and requiring expensive processing to be used in animal feed. Plants grown in the tropics have the potential to produce large amounts of leaves rich in nitrogen compounds on farms, and this potential can be an alternative roughage source to meet the roughage needs of ruminants. In some tropical regions (Benin, Niger etc.) where pastures are insufficient, the leaves of shrubs and trees that are rich in tannins and agricultural industry by-products (bark, pulp) are used as feed. Fadiyimu et al. (2010) state that the potential of using wastes such as tropical plants, leaves of trees and fruit pods as feed sources in animal feeding should be investigated in places where forage cultivation is insufficient. Tree branches and leaves can be an important part of the diet for ruminants such as goats, sheep and deer (Kamalak et al., 2005). Tree leaves, which are in the class of roughages, are materials that can be obtained from various trees grown in or around livestock enterprises. Especially sheep and goats fondly

consume tree leaves. However, very little is known about the nutritional and fodder value of these trees, shrubs and leaves, some of which have traditionally been used for many years. It will be very useful to reveal the nutritional values of branches and leaves, which have not been studied before.

In this review, the potential of some tropical plant leaves and wastes to be used as roughage sources in ruminant feeding is discussed in order to reduce the cost of feed and to prevent environmental pollution in areas where tropical plants (Guava, papaya, banana, mango, pineapple, cassava, moringa and avocado) are grown, as there is a shortage of forage due to the lack of forage crop cultivation in many tropical countries.

## 2. Guava (*Psidium guajava* L.)

Guava (*Psidium guajava* L.) belongs to the genus Psidium of the Myrtaceae family. Guava (*Psidium guajava* L.), also known as the apple of the tropics, is native to tropical Central America that extends from Mexico to North and South America, but also grows in other tropical and subtropical regions around the World. This fruit tree is an evergreen shrub-type small tree, 3-10 m tall (Gonzalez-Gaona et al., 2010). Guava flowers are fragrant and a good source of nectar for bees. In addition, guava leaves are reported to be effective in medicine against digestive system, respiratory tract, mouth / tooth and skin infections, diabetes, cardiovascular / hypertension, cancer, gynecological diseases, pain, fever, liver and kidney problems (Daswani et al., 2017). Guava fruits are extremely delicious. It is stated that the fruits of guava trees grown on pastures in tropical regions are consumed by farm animals, and up to 11 kg of fresh guava can be given daily to cattle (Somarriba, 1985). Guava waste is made from shells, seeds and stone cells in various proportions. The seeds contain moderate levels of ether extract (14%) and protein (15%), rich in crude fiber (42%). Stone cells are rich in lignin (37%) and cellulose (54%) (El Boushy et al., 2000). Wastes are poor in protein (7-11%) and rich in crude fiber (ADF 48-70%), especially lignin (16-22%) in dry matter (DM). Cattle, sheep and goats traditionally consume guava leaves in Hawaii and South Africa. Guava leaves have weak to moderate protein (10-14% DM) and high fiber content (ADF 27-39% DM). In a study conducted in Thailand, it was determined that the DM and protein degradability in guava leaves are high (71%) and it is a high-value feed for cattle (Paengkoum et al., 2012). Al-Sagheer et al. (2018) stated that when 25% of guava leaves are used in the diet, there is no harmful effect on ruminal degradability of nutrients and may be an alternative contribution in reducing $CH_4$ production. Hassan et al. (2016) state that dried guava wastes can be used effectively in the diet at a rate of 20% without adversely affecting the performance, digestibility, carcass characteristics or health parameters of Ossimi lambs.

## 3. Papaya (*Carica Papaya*)

*Carica papaya* L. (melon tree) belongs to the Caricaceae family. It is widely grown in Mexico and Central and South America, the Caribbean and Southeast Asia and Africa. The papaya tree is a perennial unbranched tree that grows up to 10 m, with large leaves and clusters of fruits. Papaya fruits are delicious, and leaf and fruit by-products can also be used in animal nutrition. In tropical countries, papaya leaves and pods are fed to animals as fresh and dried by growers. Papaya leaf has high protein content (23.9%) and low fiber (10.5%) ratio (Jayanegara et al., 2013). It has been stated by Melesse et al. (2018) that papaya by-products are a good source of energy for ruminants as they are rich in carbohydrates, and leaves are a good source of protein.

## 4. Mango (*Manguifera indica*)

Mango (*Mangifera indica* L.) is a tree grown for its fruit from the Anacardiaceae family. Mangoes are grown in South Asia, East Asia, East Africa, Brazil, the West Indies and Mexico. Mango Tree, has begun to grow in coastal areas like Antalya, Mersin in Turkey (Gübbük et al. 2017). The mango tree can stay green throughout the year. Mango leaves are delicious for ruminants and are loved to be consumed. CP, ash, ether extract, neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) of mango leaves were found to be 13.60, 12.61, 3.92, 35.32, 34.98, and 12.86%, respectively (Jhaumeer et al., 2018). Therefore, it can be used as fresh and dried for animal feeding. Kumar et al., (2011) determined that mango leaves have the potential to inhibit methane formation in the rumen. It has been concluded that when 6% of mango leaves are used in lamb ration, gas and methane emission is significantly reduced, so that it can be used in lambs' rations at the rate of 6% without any harmful effects (Hassan et al., 2020).

## 5. Pineapple (*Ananas comosus*)

Pineapple (*Ananas comosus* (L.)) is from the Bromeliaceae family. Pineapple fruit canned producers are mostly in Asian countries, including Thailand, the Philippines, Indonesia, and Malaysia. In Africa, Kenya, Benin and Nigeria are also important producers (Achigan-Dako et al., 2014). Pineapple fruit is very sweet, it is an excellent source of vitamins and minerals. It is a very rich fruit especially in terms of Mn, B1 and C vitamins. In addition to fruits, high-quality leaves are produced in large quantities that can be used as roughage for ruminants (Wakasa, 1989). Pineapple leaves are generally given to cattle by cutting. Pineapple leaves can also be used fresh, dried or silaged in ruminant diets. Pineapple leaves are low in crude protein content (4 to 7% DM) but very rich in fiber (NDF 58-73% DM) (Heuzé and Tran, 2015). Pineapple leaves are suitable for dairy cows due to their high fiber content, but it must be chopped before use (Buliah et al., 2018). When pineapple

leaves were fed to beef cattle with fresh herbs or total mixed rations, it was determined that feed consumption and daily live weight increased, so the producer made more profit (Prachyalak et al., 2001). Since the silage of pineapple leaves is low in protein and very rich in fiber, it can easily be given to ruminants 15-20 kg per day (Göhl, 1982). It has also been revealed that silage made from pineapple fruit residues will be an alternative to traditional green food, reducing the cost of feed, and also helping to prevent environmental pollution from pineapple fruit waste (Elias et al., 2017).

## 6. Banana (*Musa spp*)

Banana (Musaceae) is a family belonging to the order Zingiberales. It is grown in tropical and subtropical regions in Asia, Africa and Australia, the Philippines, the Pacific Islands, West Africa, the Caribbean and Central America. In Turkey it is mostly cultivated between Bozyazı and Anamur. Banana protein, cellulose and carbohydrates are especially high in sugar.

Banana leaves have high moisture content (85%) and low protein content (10-17%). The body of the tree has a low protein content (2.8-7.6%) and a high-moisture content (92-95%). Banana peel is rich in starch, sugar, cellulose, minerals (K, P, Ca and Na) and some vitamins, except for its high moisture (73.8%) content (Bouafou et al., 2012). Since banana fruit has a high moisture and carbohydrate content it causes digestive disorders. Therefore, fresh banana should not be used less than 70% in the ration and should not exceed 8% of live weight. 5 kg should be given to heifers for 100 kg body weight (Geoffroy, 1985). In the study conducted with rams, the dry matter digestibility of fresh green banana fruit and silage was determined as 66.4% and 68.2%, respectively. It is suggested that banana peel can be used in ruminant rations with a profit of 7.5% (Gourdine et al., 2011). It is claimed that in ruminant rations where the digestibility of banana leaves is around 65%, it can be given to dairy cows at a rate of 15% per day, and that ruminants can meet 60 to 80% of their total needs from banana leaves, even at a very early age (Geoffroy, 1985).

## 7. Cassava (*Manihot esculenta*)

Cassava (*Manihot esculenta*) is from the Euphorbiaceae family. Cassava is native to South America and is widely grown in tropical and subtropical regions, including sub-Saharan Africa and South East Asia. Cassava is grown for tubers that are used as a starch source. Cassava ruminal is a suitable feed for ruminants in terms of in vitro fermentation and organic matter digestibility. DM, CP, CF, EE and ash content of Cassava leaf are 93.0, 21.0, 25.0, 0.55, 8.5% (in DM), respectively (Ravindran, 1993). It is suggested that cassava leaves can be a valuable feed for animals, but the leaves can be fed fresh, but dried or used as silage is more appropriate (Phengvilaysouk et al., 2008). When 50% cassava leaves were used in dairy cattle rations, it had a positive effect on DM consumption, body weight gain, milk production and milk fat content and the by-pass protein effect was also found to be high (Wanapat, 2002). It has been determined that the body weight gain, food digestibility and carcass quality of West African Dwarf rams are increased when cassava leaves are used in feed at a rate of 20% (Odusanya et al., 2017). Harun et al. (2017) suggest that 50% of cassava leaves can be used to increase the nutritional value of feed in the diet of malnourished goats. Kavana et al. (2005) stated that cassava leaves are more suitable to be used as silage, and up to 35% cassava leaves can be used to provide by-pass protein to silage with urea and molasses. It has been determined that cassava silage increases rumen fermentation, feed consumption, milk yield and quality in dairy cows (Wanapat et al., 2018). Noviadi et al. (2017) found that cassava leaves silage increased nutrient digestibility in goats.

## 8. Moringa (*Moringa oleifera*)

Moringa (*Moringa oleifera* Lam.) is a tropical tree species plant from the Moringaceae family. Moringa is a plant grown in a wide area from the southern hills of the Himalayas to Africa, the Caribbean Islands and Central America, India, Ethiopia, the Philippines and Sudan. Moringa, which is used for human food, is also used as a medicinal plant, and has an area of use in animal nutrition (Falowo et al., 2018).

Moringa is delicious and has a high nutritional value. Moringa leaves contain 412.0 g / kg crude fat, 211.2 g / kg carbohydrate, and 44.3 g / kg ash with 21.8% crude protein, 22.8% ADF and 30.8% NDF (in DM) (Sanchez et al. 2006). Moringa leaves are consumed by cattle, sheep and goats in Cuba and Venezuela. When Moringa leaves are given to growing goats alone or as an addition to the diet, it has been observed that daily feed consumption gives better results compared to leucaena (*Leucaena leucocephala*) or gliricidia (Asaolu et al., 2012). Moringa leaves have the highest crude protein digestibility (CPD), followed by branches and roots Moringa leaves have been found to increase daily body weight and digestibility of feed when added up to 50% to low quality feeds (Aregheore, 2002). According to Moyo et al. (2014), meat quality improves when moringa leaves are given to growing goats. According to Li et al. (2017), Moringa leaves have the highest crude protein digestibility (CPD), followed by branches and roots. The root CPD was higher than DM and OM. It is suggested that the leaves of moringa can be given alone, the branches should be mixed with feed with high nutrient content, and the roots should not be fed to cows.

## 9. Avocado (*Perseae Gratissimae*)

Avocado (*Persea americana*) is a tree belonging to the laurel family. Avocado is grown commercially in Central and South America, the West Indies, Polynesia, Philippines, Australia, New Zealand, Madagascar, Mauritius, Madeira, Canary Islands, Algeria, South Africa,

southern Spain and southern France, Sicily, Crete, Israel, Egypt and tropical Africa. The fruit of the avocado plant is also known as "American pear" because it resembles pear (Knight, 2002).

As well as the benefits of the avocado fruit, the leaves of this fruit have many benefits and are used in animal nutrition. In addition, avocado leaves are rich in potassium and vitamin B6. Leaves are high in dry matter (94.67%), protein (25.54%), ether extract (4.01%), ash (19.38%) and crude fiber (38.40% in DM) (Arukwe et al., 2012). Since avocado leaves and peel contain persin, fresh feeding of the leaves can cause poisoning and death, so the leaves should be dried or silaged (Yamassaki et al., 2017). De Evan et al. (2020) reported that Avocado vegetable waste (husk-pulp), which is released in large amounts, can also be used as a source of roughage in ruminant feeding. It was concluded that when avocado wastes (pulp and shell mixture) are added to dairy goats' rations, it can improve the quality of milk fatty acid profile without negatively affecting milk yield (Velarde ve ark., 2020).

## 10. Conclusion

Although many tropical plant leaves and waste have high nutritional values and flavors, in some countries they are partially used as animal feed. Fruit peels, pulp and leaves of many tropical plants grown as human food that are not used as human food are generally not utilized and cause environmental pollution. These tropical plant wastes can be used as an alternative roughage source in ruminant feeding when there is a shortage of feed or when there is excess waste. The use of leaves and wastes with high nutritional value as a source of roughage in ruminant feeding will result in meeting the nutritional needs of animals cheaper. If these wastes are encouraged to be used as roughage in ruminant feeding, both animal production will increase, and feed costs of producers will be reduced. On the other hand, environmental pollution due to wastes will be prevented.

In conclusion, under the light of the literature reports, it can be said that plant leaf and fruit wastes (Guava, papaya, banana, mango, pineapple, cassava, moringa and avocado) grown in tropical regions will be evaluated as a source of roughage in ruminant feeding and will contribute to the solution of quality roughage problem.

**Author Contributions**

All authors have equal contribution and all authors reviewed and approved the manuscript.

**Conflict of Interest**

The authors declare that there is no conflict of interest.

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Review
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# ANTI MULLERIAN HORMONE: A PUTATIVE ENDOCRINE MARKER FOR PREDICTION OF SUPEROVULATION RESPONSE IN CATTLE

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**Abstract:** Anti-Müllerian Hormone (AMH) is a dimeric glycoprotein molecule with a molecular weight of 140-kDa linked to the Transforming Growth Factor-β (TGF-β) superfamily. Research on the use of AMH in livestock has gained momentum in recent years. In particular, it is now widely used in cattle breeding, where embryo transfer technology is used to obtain more offspring from genetically superior females. One of the most important factors that increase the success of embryo transfer is the response of the selected donor to the superovulation protocol. AMH has been successfully used as a biomarker in predicting superovulation response in cattle and in estimating the numbers of oocytes collected by ovum pick up (OPU). AMH plasma concentrations are positively and highly correlated with antral follicle count (AFC) in cattle and can also be used as a marker of ovarian reserve. In addition, AMH was also positively and highly correlated with the number of corpus luteum (CL) and total embryos after superovulation in several studies. It has been also reported via Genome-Wide Association Studies (GWAS) that plasma AMH level is an inherited trait in cattle and can be improved through genomic selection. In this study, we aimed to evaluate the relationship between plasma AMH levels and superovulation response in cattle by compiling the data obtained from various studies in light of current scientific literature.

**Keywords:** Biomarker, Superovulation, Embryo Transfer, Cattle

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## 1. Introduction

In mammals, for normal sexual development to occur, one of the Wolffian or Müllerian ducts found in mammalian embryos must develop while the other regresses. The Wolffian duct differentiates into male reproductive organs, while the Müllerian duct differs into the female reproductive tract (oviduct, uterus, cervix and vagina). While the Wolffian duct differentiates with stimulation of testosterone produced from fetal leydig cells in males, Anti-Müllerian Hormone (AMH) expressed from sertoli cells of fetal testes activates the regression of the Müllerian duct through apoptosis of the epithelial cell. In females, it is secreted by the granulosa cells of the developing follicles and plays an inhibitory role on the primordial follicles in folliculogenesis (Jost, 1953; Josso et al., 1993; Behringer et al., 1994). Also it has been used as a marker of controlled ovarian stimulation response for in-vitro fertilization (IVF) administration especially in the treatment of infertility in women. Recently, plasma concentrations of AMH have been utilized in ovarian pathological conditions such as menopause prediction, ovarian tumours, polycystic ovary syndrome (PCOS) and premature ovarian failure in women (Leader and Baker, 2014).

Multiple ovulation and embryo transfer (MOET) a technology that has the potential to increase the genetic progress and production of beef and dairy breeds, has been applied in cattle for many years. Even though there have been improvements in MOET technology it is still difficult to predict superovulation response to follicle stimulating hormone (FSH) treatment which varies widely between individuals in cattle (Hasler, 2014). One of the most important factors that increase the success of embryo transfer is the response of the selected donor to the superovulation protocol. In addition to determining healthy animals with superior genetic characteristics, the most important and desired criterion in the selection of donor cattle for an economical and efficient assisted reproduction technology is obtaining a large number of transferable embryos per donor (Sağırkaya, 2009). It is important to estimate the superovulation response in cattle breeding, where embryo transfer technology is used to obtain more offspring from genetically superior females. For this purpose, estimating the superovulation response of cattle and thus the selection of suitable donors is an important research area. In recent years, researchers have shown that plasma AMH levels can be used as an endocrine marker for the prediction of superovulation response (Rico et al., 2009).

## 2. Signalling pathways and the role of AMH in granulosa cells

AMH is a dimeric glycoprotein molecule with a molecular weight of 140-kDa composed of 551 amino acids and linked to the Transforming Growth Factor-β (TGF-β) superfamily (Jost, 1953; Cate et al, 1986). It is encoded by the gene on chromosome 7 in cattle (Gao and Womack, 1997) and chromosome 19 in women (Picard et al., 1986). AMH uses a heteromeric receptor system consisting of a single membrane encompassing serine, threonine kinase receptors (termed type I and type II). The type II receptor (AMHRII) conferred ligand binding specificity, while the type I receptor mediates the downstream signal. AMH is secreted by primary and preantral follicles in the ovary and inhibits initial follicle recruitment and FSH-stimulated follicular growth. The study conducted by Durlinger et al. (2002) reported a decrease in the sensitivity of the follicles to FSH following AMH binding, causing an inhibitory effect on the recruitment of primordial follicles into the growing follicle pool in mice. AMH acts as a negative regulator of the early stages of follicular development (Figure 1) (Durlinger et al., 2002; La Marca&Volpe, 2006; Umar et al. 2019).

## 3. Variations in plasma AMH levels

Various studies indicate that plasma AMH levels in cattle show very small changes throughout the oestrous cycle. Souza et al. (2015) found significant positive correlations between AMH concentrations in cows at different stages of the oestrous cycle (random&proestrus: r=0.77, P<0.01; proestrous & diestrous: r=0.79, P<0.01; random & diestrous: r=0.76, P<0.01) and reported the repeatability of plasma AMH as 0.91 (Figure 2). This stability of AMH allows the determination of plasma levels by a single measurement at any stage of the oestrous cycle.

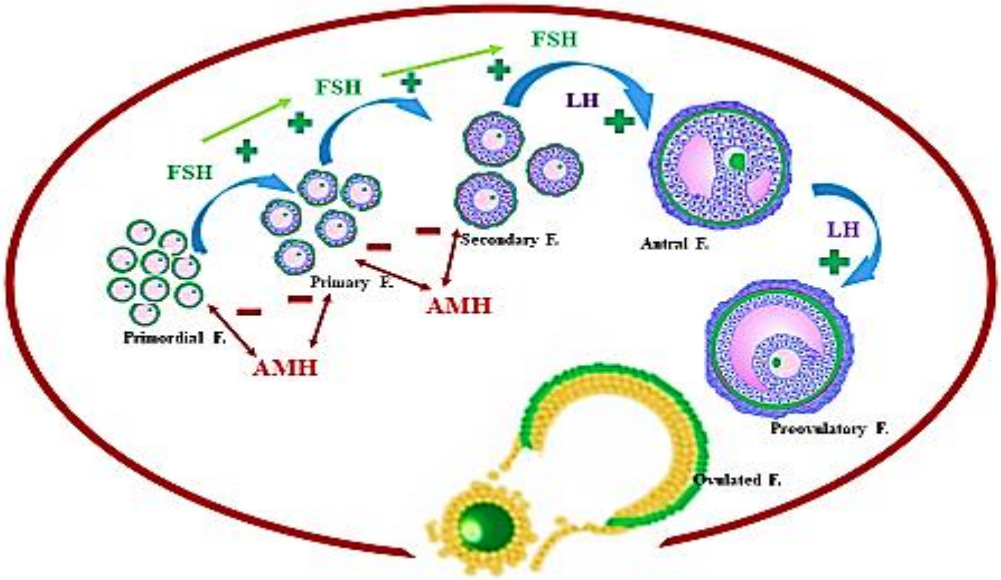


**Figure 1.** Inhibitory effect of AMH on growing follicles.

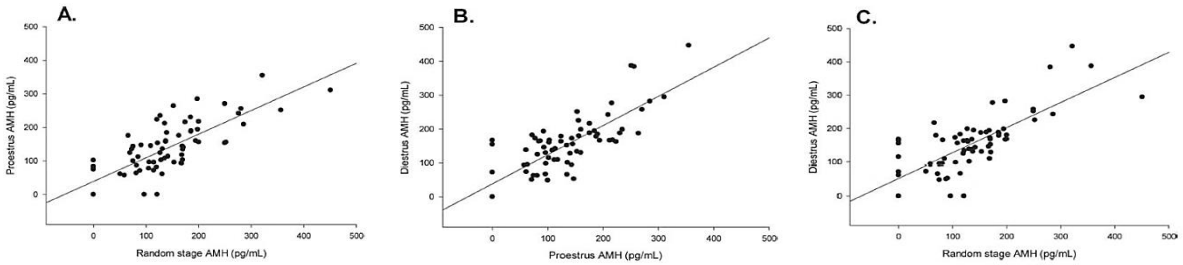


**Figure 2.** Comparison of AMH measurements at different times of the estrous cycle in cows (Souza et al. 2015).

While AMH concentrations in an adult cow show high repeatability, there is a high variation in AMH concentrations among cows (Table 1). In a study conducted by Ribeiro et al. (2014) on 1200 cows, it was found that AMH levels ranged from 10 to 3,198 pg/ml. In the same study, the average AMH concentration was found as 320.3±251.1 pg/ml. In addition, numerous studies show that in-herd AMH concentrations range from 0.01-400 pg/ml and only a few cows reach levels above 400 pg/ml (Rico et al., 2009; Souza et al., 2015). Due to the wide variation between herds and individuals, the reference AMH concentration range for a cow could not be determined. Therefore for the selection of the most suitable donor cows for embryo production, it is

more appropriate to select those with high plasma AMH levels among the animals with high genetic value in the herd.

**Table 1.** Variations of plasma AMH concentrations among cows

| Literature | Plasma AMH concentrations | Material |
|---|---|---|
| Rico et al. (2009) | 25 to 228 pg/ml | Holstein Dairy Cows |
| Ribeiro et al. (2014) | 10 to 3,198 pg/ml | North American Holstein Cows |
| Souza et al. (2015) | 0 to 400 pg/mL | Holstein Cows |
| Jimenez-Krassel et al. (2015) | 6 to 440 pg/mL | Holstein Heifers |
| Hirayama et al. (2017) | 32 to 1,992 pg/mL | Japanese Black Cows |
| Gobikrushanth et al. (2018) | 14 to 1,879 pg/mL | Canadian Holstein Cows |
| Nawaz et al. (2018) | 2 to 2,000 pg/mL | Holstein Heifers |
| Akbarinejad et al. (2019) | 98 to 2110 pg/mL | Holstein Dairy Cows |
| Sevgi et al. (2019) | 233 to 2531 pg/mL | Simental Cows |
| Gobikrushanth et al. (2019) | 15 to 2,863 pg/ml | Irish Holstein Cows |
| Akbarinejad et al. (2020) | 46 to 2089 pg/mL | Holstein Dairy Cows |

Also the effect of the breed of cows and lactation number on AMH levels was described. Many studies indicate that AMH concentrations were lower in dairy cattle than those in beef. Mossa et al. (2017) showed that plasma AMH concentrations, follicle numbers and ovary size were lower (P<0.01) in dairy heifers compared with beef heifers. The analyses of the literature suggested that plasma AMH levels vary not only between dairy or beef breeds but also within individuals in the same breed.

Souza et al. (2015) demonstrated the relationship between circulating AMH levels and the number of corpus luteum in primiparous and multiparous cows (Figure 3) and reported a significant (primiparous: r = 0.67; P<0.01; multiparous: r = 0.63; P < 0.01).

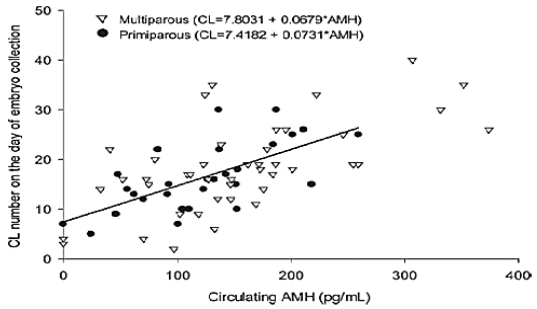


**Figure 3.** Scatterplot showing correlation between CL number and AMH levels in multiparous and primiparous cows (Souza et al., 2015).

The recent studies conducted to show the variations of AMH plasma levels in dairy female calves from birth to puberty shows that AMH concentrations increase in the first 2 months of age, decrease at 5 months of age, and are stable at approximately 8-9 months of age (onset of puberty). Similar results have been reported in beef calves (Mossa et al. 2017). This evidence proves that AMH concentrations start to increase in the first months of life in female calves and decrease before puberty and remain stable during the sexual cycle after the first ovulation (Figure 4).

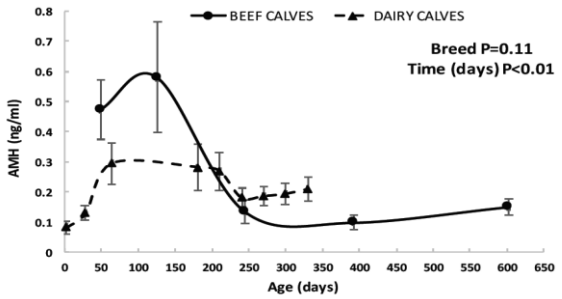


**Figure 4.** Circulating AMH concentrations in dairy and beef calves (Mossa et al., 2017).

## 4. Relationship between AMH and superovulation response

In recent decades, there has been a great deal of literature examining the relationship between plasma Anti-Mullerian Hormone level and fertility in humans and also many studies reported a positive and strong correlation between plasma AMH levels and ovarian reserve and activity in women (de Vet et al. 2002; Van Rooij et al., 2002; Mulders et al. 2004; Tremellen et al., 2005; Broekmans et al., 2006; Visser et al., 2006; Helden and Weiskirchen, 2017). The average number of transferable embryos in the bovine embryo transfer industry is reported to have remained virtually unchanged over the past 40 years, with approximately 6 transferable embryos per superovulation and embryo collection. Consistently some females produce above-average embryos, while others of similar age, breed and management perform worse (Hasler, 2014). Superovulation aims to stimulate the growth and maturation of small antral follicles, resulting in multiple ovulation. Therefore, the small pool of antral follicles available for stimulation is crucial in predicting

superovulation response (Sevgi et al., 2019).

AMH plasma concentrations are positively correlated with antral follicle count (AFC) in cattle and can also be used as a marker of ovarian reserve (Center et al. 2018; Mossa et al. 2017). Rico et al. (2009) found that plasma AMH concentration was highly correlated with the numbers of 3 to 7 mm antral follicles detected before FSH treatment (r = 0.79, P<0.001) and the numbers of ovulations after treatment (r= 0.64, P<0.01). In addition, AMH was also positively and highly correlated with the number of corpus luteum (CL) and total embryos after superovulation in many studies (Hirayama et al. 2017; Souza et al. 2015; Sevgi et al. 2019; Monniaux et al. 2010). Center et al. (2018), classified cows into quartiles according to their plasma AMH levels and found that there was a 5-fold difference between AMH concentrations in Q1 (44.9 pg/mL) and Q4 (243.1 pg/mL) and a 2-fold difference (P < 0.01) in CL numbers between Q1 (12.0) and Q4 (25.6) (Table 2).

**Table 2.** The quartile categorization of plasma AMH concentrations and the relationship between superovulation response in beef cattle (Center et al., 2018).

| Item | Quartile of AMH concentration | | | | P-value |
|---|---|---|---|---|---|
| AMH, ng/ml | Q1<br>0.013 – 0.168 | Q2<br>0.169 – 0.263 | Q3<br>0.264 – 0.363 | Q4<br>0.364 – 0.898 | |
| No of donors/collections | 26 | 23 | 24 | 24 | 0.001 |
| No of follicles | 11.62±1.54 | 16.68±1.67 | 16.79±0.94 | 19.33±0.94[a] | 0.001 |
| No of CL | 11.62±1.54 | 13.68±1.67 | 17.58±1.60 | 20.54±1.60 | 0.001 |
| No of embryos | 9.77±1.76 | 9.36±1.91 | 15.50±1.83 | 20.13±1.83 | 0.001 |

AMH= anti-müllerian hormone, CL= corpus luteum

Batista et al. (2014) found a positive correlation between plasma AMH levels and the number of ovarian follicles detected by ultrasonography in Bos indicus (Nelore) and Bos taurus (Holstein) heifers. In another study carried out with Japanese Black heifers, a positive correlation reported between plasma AMH levels of heifers and the total number of follicles (r=0.647, P<0.01) and embryos (r=0.681, P<0.01). However, the researchers didn't find any correlation between AMH and the total number of transferable embryos in the same study (Fushimi et al. 2020).

In a recent study conducted using 46 Simental donor cows, researchers found a positive correlation between plasma AMH levels and the number of CL and total embryos (P<0,05) (Figure 5). Also they reported that every 200 pg/ml increase in serum AMH level leads to approximately 1 piece increase in corpus luteum (CL) number (r=0,68, P<0.05) (Sevgi et al. 2019). These results increase the interest in AMH as a reliable endocrine marker that provides accurate estimation to select the most suitable donor cows for MOET technology.

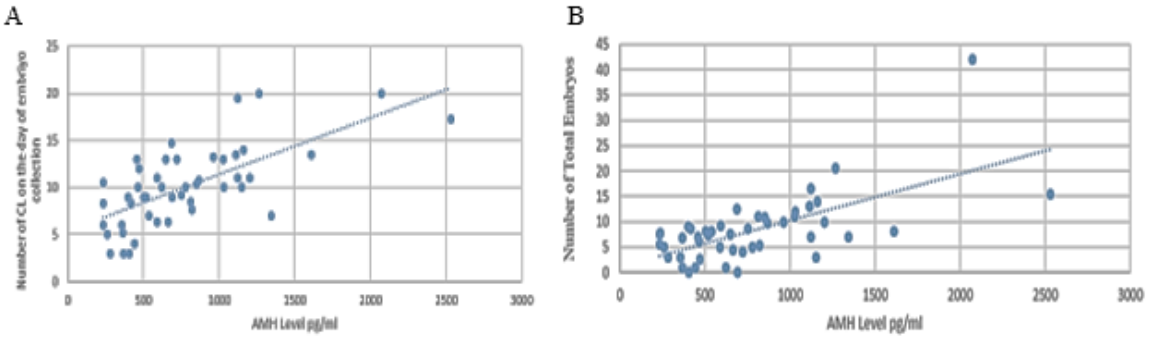


**Figure 5.** The relationship between plasma AMH levels and the number of the CL (A) and the number of total embryos (B) after superovulation therapy (Sevgi et al., 2019).

## 5. Genomic heritability of AMH

A meta-analysis of the literature reported that the heritability of the economically important female reproductive traits in dairy and beef cattle tends to be low (0.02 to 0.04) (Berry et al. 2014). There is currently few research articles on the Genome-Wide Association Study (GWAS) that identifies potential quantitative trait locus associated with phenotypic variation in AMH concentrations and on the genomic heritability of AMH in cattle (Table 3).

**Table 3.** Heritability of AMH and genomic regions associated with plasma AMH concentrations

| Literature | Genomic Heritability | Pedigree based heritability | Significant Genomic Regions (Position) | Material (n) |
|---|---|---|---|---|
| Nawaz et al. (2018) | 0.36 ± 0.03 | 0.43±0.07 | BTA11 (92.8 to 97.1 Mb) BTA20 (25.0 to 26.3 Mb) | Holstein heifers (n=2905) |
| Gobikrushanth et al. (2018) | 0.46 ± 0.31 | ---- | BTA11 (14-Mb) | Canadian Holstein cows (n=198) |
| Gobikrushanth et al. (2019) | 0.45 ± 0.05 | 0.40±0.06 (n=2628) | BTA7 (21.359 to 21.886 Mb) BTA11 (92.051 to 101.918 Mb) | Irish Holstein cows (n=1725) |
| Grigoletto et al. (2020) | 0.28 ± 0.07 | ---- | BTA11 (6 Mb) | Nellore cattle (n=944) |

Nawaz et al. (2018) carried out a study to estimate the genomic heritability of AMH from pedigree and genomic information and determine genomic regions associated with AMH production via genome-wide association studies (GWAS). To determine plasma AMH levels, 3259 Holstein heifers were used and 2905 of them were genotyped for SNP (single-nucleotide polymorphism) markers. Pedigree information of the last four generations was also evaluated for estimation of heritability of AMH. They reported the pedigree-based heritability of AMH as 0.43±0.07 and the genomic heritability of AMH as 0.36 ± 0.03 (Nawaz et al. 2018). In another study, the estimation of genomic heritability of AMH in Nellore cattle (n=944) was reported as 0.28 ± 0.07 (Grigoletto et al. 2020). Gobikrushanth et al. (2018) indicated a high (0.46 ± 0.31) heritability estimate for AMH in Holstein cows (n=198). These reports suggest that the heritability estimates of AMH were higher compared with the heritability of the most economically important female reproductive traits.

Nawaz et al. (2018) also reported significant genomic regions on BTA11 (92.8 to 97.1 Mb) and BTA20 (25.0 to 26.3 Mb). Through GWA analysis, they concluded that there were significant associations between AMH levels and the 11 SNP markers on chromosome 11 and 1 SNP marker on chromosome 20 (Figure 6). In another study, the strongest associations with the AMH were found in BTA11 (513 SNPs in the 14-Mb) (Gobikrushanth et al., 2018).

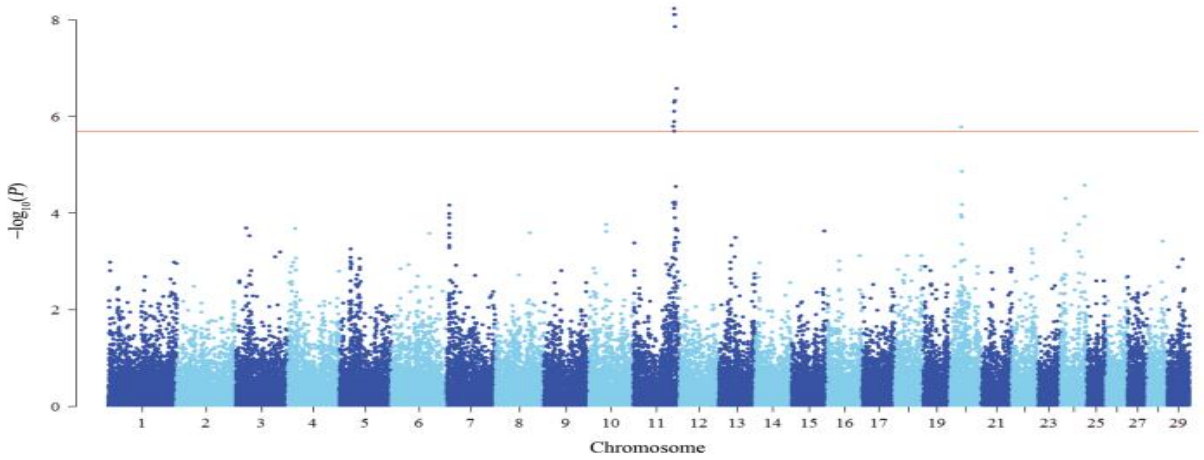


**Figure 6**. Manhattan plot of −log10 P-values for plasma AMH concentrations in dairy Holstein heifers (Nawaz et al., 2018).

## 6. Conclusions

Researches on the use of AMH in cattle have gained momentum in recent years. Evidence from many studies indicates that;

- Because of its stability, it is possible to measure plasma AMH concentration with a single sampling at any stage of the oestrous cycle.
- Plasma AMH concentration is positively and highly correlated with the number of corpus luteum (CL) and total embryos after superovulation.
- AMH concentration in dairy cattle is lower than in beef cattle.
- The increase in plasma AMH concentrations in females starting from the first month after birth and continues until puberty.
- The findings suggest that plasma AMH level is an inherited trait in cattle and can be improved through genomic selection.

Thanks to the intensive studies in recent years, considering its easy applicability and cost-benefit status, the AMH test has become a valuable and practical method to predict ovarian stimulation response in cattle

to be selected for embryo production and to increase the efficiency of embryo transfer technology.

**Author Contributions**

All tasks have been performed by single author.

**Conflict of Interest**

The author declares that there is no conflict of interest.

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