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The Pharma Innovation

**ISSN (E): 2277- 7695**
**ISSN (P): 2349-8242**
**NAAS Rating: 5.03**
TPI 2019; 8(11): 393-397

www.thepharmajournal.com
Received: 19-09-2019
Accepted: 26-10-2019

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# Effect of application of fly ash in conjunction with other nutrients and FYM on protein content and protein quality of rice (*Oryza sativa*)

**Sachidananda Muni**

**Abstract**
A field experiment was conducted in the year 2018 during the Kharif season at the Instructional Farm, Odisha University of Agriculture and Technology (OUAT), Bhubaneswar, Odisha to study the effect of application of fly ash (FA) in conjunction with other chemical fertilizers (CF) and farm yard manure (FYM) on the protein content and protein fractions of rice (*Oryza sativa*) cv. 'Swarna'. The treatments were applied in 5m × 3m size plots taking nine treatments replicated thrice in a randomized block design (RBD). The soil was acidic and the experimental FA was alkaline in nature. It was observed that the crude protein content of rice grains varied from 7.91% to 9.19% and that of true protein from 6.81% to 7.95%. The true protein content decreased by the application of only FA but increased significantly with the inclusion of NPK along with FYM. The amount of protein fraction albumin varied from 0.47% to 0.57% and that of globulin from 0.42% to 0.51%. The minor fraction prolamin varied from 0.09% to 0.15% and the major fraction glutelin from 4.94% to 5.71%. It is proved that cumulative application of only NPK fertilizers had no effect on the crude protein but in combination with FA and FYM reduced the amount of crude protein content and NPN along with the true protein content.

**Keywords:** True protein, albumin, globulin, prolamin, glutelin

## Introduction
FA (FA) is an obnoxious solid waste generated from coal based thermal power plants. It is produced in large quantities throughout the globe and India has a major share to it. It is a cause of concern to environmental hazards. Proper utilization of FA is a challenge to the scientists. It is chemically an amorphous ferro-alumino silicate and is an important pollutant in industrial areas. However it contains many other elements which may be essential to the plants and the soil health. FA produced in Odisha is mainly alkaline in nature. The alkaline FA has been identified as good soil amendment for reclaiming acid soils. It has also been reported that FA enhances the mineralization of organic sources. The soil in Bhubaneswar is mostly acidic and requires a liming agent. Hence alkaline FA may be used as a good liming agent for growing of crops.
Rice is the staple food of our country India and particularly Odisha. Hence FA may be used as a soil amending agent for growing rice. The quality of the rice grains grown need to be evaluated.

## Materials and Methods
Rice variety cv. Swarna was grown during Kharif season in 2018 in the Regional Research and Technology Transfer Station (RRTTS), Odisha University of Agriculture and Technology, Bhubaneswar, India taking nine numbers of different treatments in 5m × 3m plots replicated thrice in a randomized block design. Both of those were given nine treatments consisting of various doses of FA, nitrogen, phosphorous, potassium (NPK) fertilizer and FYM with only NPK serving as the control. The treatments were: (1) RD of 80 ,40 , 40 kg N , P , K $ha^{-1}$ (2) 20 t FA $ha^{-1}$ (3) 40 t FA $ha^{-1}$ (4) RD + 20 t FA $ha^{-1}$ (5) RD + 40 t FA $ha^{-1}$ (6) 50% RD + 20 t FA $ha^{-1}$ (7) 50% RD+ 20 t FA $ha^{-1}$ (8) 50% RD + 20 t FA $ha^{-1}$ + 10 t FYM $ha^{-1}$ (9) 50% RD + 40 t FA $ha^{-1}$ + 10 t FYM $ha^{-1}$. The RD with neither FA nor FYM served as the control. The averages of the data are taken as the results. 20 days old seedlings of the variety were transplanted.
For chemical estimations about 10 gram of grain samples were ground to pass through 100 mesh sieve.

Percentage of crude protein was calculated using a conversion factor of 5.95 (Arnon, 1949) [1]. The% of moisture in each sample was determined separately and the results were expressed on oven dry basis. Percentage of crude protein was calculated using a conversion factor of 5.95 (Thiex, 2009) [14].
NPN was estimated essentially according to the procedure given by Becker *et al*. (1940) [2]. Into a 150 mL of beaker, 0.5 gram of powdered grain sample was weighed and stirred with 20 mL of 0.8N (13.6%) trichloro acetic acid for one hour on a magnetic stirrer. The suspension was centrifuged for 10 minutes at 2000 r.p.m. The clear supernatant was taken for nitrogen determination by the auto analyser. Percentage of true protein was calculated by subtracting non-protein nitrogen (NPN) from total nitrogen and multiplying the results with 5.95.
The FA was obtained from the ICCL Power Plant, Chowdwar, Cuttack, India. The soil was acidic silty loam and the FA was alkaline. The doses were calculated to maintain a suitable pH nearly about 6.5.

**Protein Fractions:** Extraction of storage proteins was performed according to Ju *et al* (2001) [6]. 2.5 grams of previously powdered samples were taken in a 250 mL beaker and subjected to the following treatments for fractionation studies.
To the beaker 50 mL of 5% NaCl was added and was stirred for 3 hours on a magnetic stirrer. The contents of the beaker were then transferred to 50 mL centrifuge tube and centrifuged for 15 minutes. The clear supernatant was decanted into a 100 mL volumetric flask. The residue in the centrifuge tube was transferred to the same beaker used before for stirring. With another 20 mL of 5% NaCl it was stirred for the second time for one hour. The contents of the beaker were centrifuged again and the clear supernatant was decanted into the 100 mL volumetric flask containing the previous supernatant solution. The second step was repeated for another time and the final volume of the volumetric flask was made up to the mark with 5% NaCl solution. From this flask 10 mL of the solution was taken for estimation of nitrogen. The nitrogen estimated from this solution was multiplied with 5.95 and gave (albumin + globulin) fractions of protein. 10mL of the salt solution was taken from the volumetric flask in a dialysis tube and dialysed against distilled water for 36 hours inside a refrigerator with frequent changes of distilled water. After dialysis, the contents of the dialysis tube were transferred into a centrifuge tube and centrifuged for 15 minutes. The residue of the centrifuge tube was taken for nitrogen estimation. Nitrogen estimated from the residue was multiplied with 5.95 and gave the globulin fraction. Albumin fraction was calculated by subtracting the globulin content from the (albumin + globulin) determined previously.
The residue from the extraction with 5% NaCl was successively extracted with 70% ethanol and N/10 NaOH exactly in the same manner as described for extraction with 5% NaCl solution. The entire ethanol extract was taken for estimation of nitrogen. The volume of the N/10 NaOH extract was made upto 100 mL and 10 mL of this tube was taken for nitrogen estimation. The fractions extracted with 70% ethanol constituted prolamin and with N/10 NaOH glutelin.

## Results and Discussion

The properties of FA and the experimental soil has been given in Table 1 and 2 respectively.

**Table 1:** Properties of FA

| | | |
|---|---|---|
| **A. Physical Properties** | | |
| 1. | Bulk Density ($Mgm^{-3}$) | 0.847 |
| 2. | Water Holding Capacity (%) | 89.6 |
| 3. | Porosity (%) | 41.0 |
| **B. Chemical Properties** | | |
| 1. | pH (1:2) | 7.30 |
| 2. | EC ($dS\ m^{-1}$) | 0.73 |
| 3. | O.C (%) | 0.30 |
| 4. | N ($mg\ kg^{-1}$) (Alkaline $KMnO_4$) | 5.0 |
| 5. | P ($mg\ kg^{-1}$) (Olsen's method) | 30.0 |
| 6. | K ($mg\ kg^{-1}$) ($NH_4OAc$ extractable) | 180.0 |
| 7. | Ca ($mg\ kg^{-1}$) ($NH_4OAc$ extractable) | 900.0 |
| 8. | S ($mg\ kg^{-1}$) ($CaCl_2$ extractable) | 180.3 |
| 9. | Fe ($mg\ kg^{-1}$) (DTPA Extractable) | 9.46 |
| 10. | Mn ($mg\ kg^{-1}$) (DTPA Extractable) | 0.80 |
| 11. | Zn ($mg\ kg^{-1}$) (DTPA Extractable) | 9.46 |
| 12. | Cu ($mg\ kg^{-1}$) (DTPA Extractable) | 2.16 |
| 13. | Ni ($mg\ kg^{-1}$) (DTPA Extractable) | 1.98 |
| 14. | B ($mg\ kg^{-1}$) (Hot water soluble) | 0.90 |
| 15. | Cd ($mg\ kg^{-1}$) (DTPA Extractable) | 0.25 |

**Table 2:** Properties of the experimental Soil

| | | |
|---|---|---|
| **A. Physical Properties** | | |
| 1. | Bulk Density ($Mgm^{-3}$) | 1.42 |
| 2. | Water Holding Capacity (%) | 34.11 |
| 3. | Porosity (%) | 39.0 |
| 4. | CEC [$cmol(P^+)\ kg^{-1}$] | 8.65 |
| 5. | Sand (%) | 54.0 |
| 6. | Silt (%) | 29.4 |
| 7. | Clay (%) | 16.6 |
| 8. | Texture | Silty Loam |
| **B. Chemical Properties** | | |
| 9. | pH (1:2) | 5.32 |
| 10. | EC ($dS\ m^{-1}$) | 0.31 |
| 11. | O.C ($g\ kg^{-1}$) | 5.30 |
| **Macronutrients** | | |
| 12. | Available N ($kg\ ha^{-1}$) | 370.0 |
| 13. | Available P ($kg\ ha^{-1}$) | 19.6 |
| 14. | Available K ($kg\ ha^{-1}$) | 166.8 |
| 15. | Ca ($cmol(P^+)\ kg^{-1}$) | 5.05 |
| 16. | S ($mg\ kg^{-1}$) | 6.52 |
| **Micronutrients** | | |
| 17. | Fe ($mg\ kg^{-1}$) | 71.0 |
| 18. | Mn ($mg\ kg^{-1}$) | 30.6 |
| 19. | Zn ($mg\ kg^{-1}$) | 0.80 |
| 20. | Cu ($mg\ kg^{-1}$) | 0.54 |
| 21. | Ni ($mg\ kg^{-1}$) | 0.86 |
| 22. | B ($mg\ kg^{-1}$) | 0.72 |
| **Heavy Metal** | | |
| 23. | Cd ($mg\ kg^{-1}$) | 0.008 |

The crude protein content, NPN and true protein content along with their change over control has been given in Table-3 and represented in Fig.1.

### Crude protein

Table – 3 revealed that the crude protein content of rice grain varied from a minimum of 7.91% in $T_2$ receiving FA in lower dose to a maximum of 9.19% in $T_9$ receiving 50% RD of fertilizer integrated with FA in higher dose and FYM. Application of FA @ $20tha^{-1}$ and $40tha^{-1}$ registered a fall by 6.17% and 2.49% respectively over control. It might be due to the less availability of nitrogen in the FA treated plots. When 100% RD of NPK fertilizer was integrated with FA, there was an increase by 8.56% over FA and 3.79% over control. This

might be due to the synergistic effect of NPK fertilizer and FA. When the dose of fertilizer was reduced to 50%, a fall by 2.85% was recorded. $T_6$ receiving 50% the dose of NPK fertilizer integrated with FA in lower dose was at par with control receiving full dose of NPK fertilizer only. This proved that the reduction in the dose of fertilizer by 50% had been counter balanced by the combined application of fertilizer and FA. However, when FYM was included in the treatments, there was an increase by 7.47% over its exclusion and 8.36% over control. Increase in crude protein content of rice in FYM treated plots over fertilizer was reported (Subbiah *et al*, 2000) [12]. It is due to the more availability of nitrogen from FYM treated plots in conjunction with FA and NPK fertilizer which is utilized in the synthesis of amino acids and in turn protein (Despande *et al* 2010) [4]. Increase in the protein content of rice by the integrated application of FYM, NPK fertilizer and blue green algae. On an average, treatments receiving 40tha$^{-1}$ FA recorded an increase by 3.6% over 20tha$^{-1}$. The protein content of rice decreased with the increase of the dose of FA which might be due to the dilution effect (Nair *et al*, 1994) [8].

**Non-protein nitrogen (NPN)**

NPN content varied between the lowest of 0.17% in $T_2$ receiving FA @ 20tha$^{-1}$ and the highest of 0.27% in $T_6$ receiving half the dose of fertilizer along with FA in the lower dose. With FA there was a decrease by 5.55% over control. Treatments receiving FA in lower dose was at par with its higher dose. When 100% RD of NPK fertilizer was integrated with FA, there was an increase by 17.64% over FA and 11.11% over 100% RD of NPK (control). Reduction in the dose of fertilizer registered an increase by 23.07% over its higher dose. But when FYM was included in the treatments, there was a reduction by 17.30%. This indicated that more of nitrogen is utilized in the synthesis of true protein in those plots. On an average, treatments receiving FA @ 40tha$^{-1}$ was at par with its lower dose.

**Table 3:** Effect of different treatments on protein content of rice cv. Swarna

| Treatment | Crude Protein (%) | Change over control (%) | NPN (%) | True Protein (%) | Change over control (%) |
|---|---|---|---|---|---|
| $T_1$ | 8.43 | | 0.18 | 7.36 | |
| $T_2$ | 7.91 | -6.17 | 0.17 | 6.9 | -0.37 |
| $T_3$ | 8.22 | -2.49 | 0.17 | 7.21 | -0.12 |
| $T_4$ | 8.44 | +0.12 | 0.19 | 7.31 | -0.04 |
| $T_5$ | 9.06 | +7.47 | 0.21 | 7.82 | +0.37 |
| $T_6$ | 8.41 | -0.24 | 0.27 | 6.81 | -0.44 |
| $T_7$ | 8.59 | +1.90 | 0.25 | 7.11 | -0.2 |
| $T_8$ | 9.08 | +7.71 | 0.22 | 7.78 | +0.34 |
| $T_9$ | 9.19 | +9.02 | 0.21 | 7.95 | +0.48 |
| SE(±m) | 0.31 | | 0.01 | 0.29 | |
| CD(0.05) | 0.98 | | 0.04 | 0.83 | |

$T_1$: Recommended Dose (RD) (80 – 40 – 40) kgha$^{-1}$ N, $P_2O_5$, $K_2O$ $T_2$: 20 tha$^{-1}$ FA
$T_3$: 40 tha$^{-1}$ FA, $T_4$: RD + 20 tha$^{-1}$ FA, $T_5$: RD + 40 tha$^{-1}$ FA
$T_6$: 50% RD + 20 tha$^{-1}$ FA $T_7$: 50% RD + 40 tha$^{-1}$ FA
$T_8$: 50% RD + 20 tha$^{-1}$ FA+ 10 tha$^{-1}$ FYM $T_9$: 50% RD + 40 tha$^{-1}$ FA+ 10 tha$^{-1}$ FYM

**True protein**

True protein content varied from the lowest of 6.90% in $T_2$ receiving FA in lower dose to the highest of 7.97% in $T_9$ receiving 50% RD of fertilizer integrated with FA in higher dose and FYM. With FA in lower and higher doses, there was a fall by 5.73% and 1.64% over 100% RD of (control). When 100% RD of NPK fertilizer was blended with FA, there was an increase by 8.08% over FA and 4.09% over control. It might be due to the combined effect of NPK fertilizer and FA. But, when the dose of FA was reduced to 50%, there was a fall by 0.85% but recorded an increase by 3.21% over control receiving full dose of fertilizer only. This proved that the reduction in the dose of fertilizer had been counter balanced by the combined application of fertilizer and FA. However, when FYM was included, on an average there was a rise by 4.69% over its exclusion and registered a significant increase by 8.06% over control. Highest quantity of true protein in rice in FYM treated plots than others was reported (Stalin *et al* 2014) [13]. Treatments receiving FYM had more amount of crude protein, less NPN and consequently synthesis of more amount of amino acid and hence more amount of true protein. On an average, treatments receiving FA @ 40tha$^{-1}$ recorded an increase by 3.24% over its lower dose.

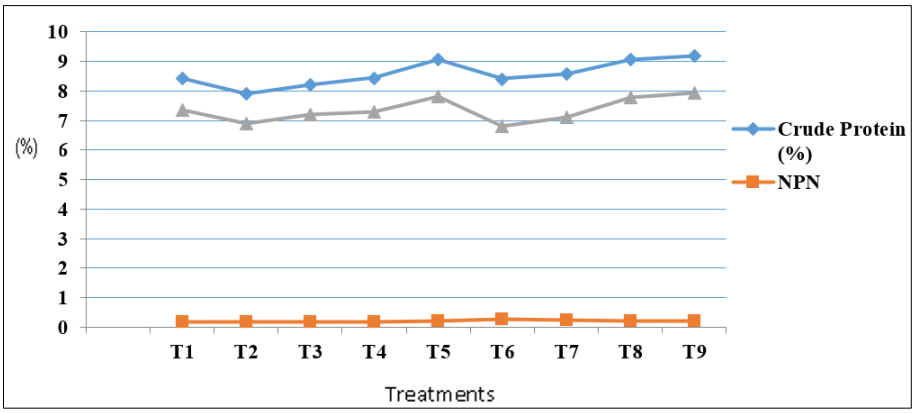


**Fig 1:** Crude protein, NPN and true protein content

The effect of application of different treatments on the protein fractions of Kharif rice cv. Swarna has been given in table 4 and protein fractions (g100gm$^{-1}$) of sample and protein (g100gm$^{-1}$) has been represented in Fig 2 and 3 respectively.

**Albumin**

Data presented in table 4 indicated that the albumin content of cv. Swarna varied from the minimum of 0.47% in $T_2$ to the maximum of 0.57% in $T_9$. With the application of FA at either dose, there was no effect over control. When 100% RD of NPK fertilizer was blended with FA, there was an increase in albumin fraction by 10.2% over FA and 5.88% over control. When the dose of fertilizer was reduced to 50%, there was a fall by 4.62%. $T_6$ was at par with $T_1$ (control) and $T_3$. This proved that the reduction in the dose of fertilizer had been counter balanced by the combined effect of fertilizer and FA @ 20tha$^{-1}$ and also by FA in higher dose. However when FYM was applied, there was an increase by 9.7% over its exclusion and 10.78% over control. This proved that the reduction in the dose of NPK fertilizer to 50% had been compensated by the synergistic effect of FA and FYM on the albumin fraction of rice protein. Albumin content of polished rice is reported to be about 5-10% (Nakase *et al* 2012) [9] and of protein is about 1-5% (Cagampang *et al*, 1966 and Houston *et al*, 1968) [3, 5]. Treatments receiving integrated doses of FA along with fertilizer with or without FYM except $T_6$ recorded significantly higher values over the lowest value of 0.48%. Treatments receiving FA @ 40tha$^{-1}$ registered an increase in albumin content by 3.35% over 20tha$^{-1}$.

**Globulin**

Globulin content varied from the minimum of 0.42% in $T_2$ to the maximum of 0.50% in $T_9$. Application of FA @ 20tha$^{-1}$ recorded a fall by 10.63% over control and but @40tha$^{-1}$ was at par. When 100% RD of NPK fertilizer was integrated with FA, there was an increase by 16.27% over FA and 6.38% over control. But when the dose of fertilizer was reduced to 50%, there was a fall by 5.0% which was at par with control. This proved that the reduction in the dose of fertilizer to 50% had been counter balanced by the blending effect of fertilizer and FA. However, when FYM was introduced, there was an increase by 3.15% over the treatments receiving same doses of nutrients but no FYM and control which indicated that the reduction in the dose of fertilizer had been counter balanced by the integrated use of FA in presence of FYM. Globulin content of polished rice is reported to be about 5-10%. Treatments receiving FA @ 40tha$^{-1}$ registered an increase in globulin content by 3.64% over its lower dose. The globulin content of rice protein is reported to be about 4-15% (Cagampang *et al*, 1966 and Houston *et al*, 1968) [3, 5].

**Table 4:** Effect on protein fractions, Effect of different treatments on protein fractions (g100gm$^{-1}$) of rice cv. Swarna

| Tr. No. | True Protein | Albumin (%) | Globulin (%) | Prolamin (%) | Glutelin (%) | Total | Unextracted |
|---|---|---|---|---|---|---|---|
| $T_1$ | 7.36 | 0.51 | 0.47 | 0.09 | 5.12 | 6.19 | 1.17 |
| $T_2$ | 6.9 | 0.47 | 0.42 | 0.10 | 4.94 | 5.93 | 0.97 |
| $T_3$ | 7.21 | 0.51 | 0.44 | 0.10 | 5.14 | 6.19 | 1.02 |
| $T_4$ | 7.31 | 0.53 | 0.49 | 0.12 | 5.35 | 6.49 | 0.82 |
| $T_5$ | 7.82 | 0.55 | 0.51 | 0.14 | 5.48 | 6.68 | 1.14 |
| $T_6$ | 6.81 | 0.50 | 0.46 | 0.15 | 5.21 | 6.32 | 0.49 |
| $T_7$ | 7.11 | 0.53 | 0.49 | 0.13 | 5.51 | 6.66 | 0.45 |
| $T_8$ | 7.78 | 0.56 | 0.48 | 0.15 | 5.56 | 6.75 | 1.03 |
| $T_9$ | 7.95 | 0.57 | 0.5 | 0.14 | 5.71 | 6.92 | 1.03 |
| SE(±m) | 0.29 | 0.02 | 0.02 | 0.01 | 0.20 | | |
| CD(0.05) | 0.83 | 0.04 | 0.03 | 0.02 | 0.55 | | |

$T_1$: Recommended Dose (RD) (80 – 40 – 40) kgha$^{-1}$ N, $P_2O_5$, $K_2O$, $T_2$: 20 tha$^{-1}$ FA
$T_3$: 40 tha$^{-1}$ FA, $T_4$: RD + 20 tha$^{-1}$ FA, $T_5$: RD + 40 tha$^{-1}$ FA
$T_6$: 50% RD + 20 tha$^{-1}$ FA $T_7$: 50% RD + 40 tha$^{-1}$ FA
$T_8$: 50% RD + 20 tha$^{-1}$ FA+ 10 tha$^{-1}$ FYM, $T_9$: 50% RD + 40 tha$^{-1}$ FA+ 10 tha$^{-1}$ FYM

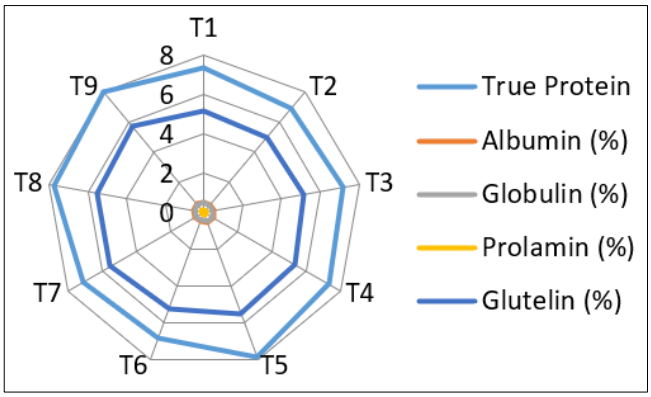


**Fig 3:** Protein fractions in g100gm$^{-1}$ sample

**Prolamin**

Prolamin content varied from the lowest of 0.09% in $T_1$ (control) to the highest of 0.15% in $T_6$ and $T_8$. With FA at either dose, an insignificant increase over control was registered. When 100% RD of NPK fertilizer was integrated with FA, a further increase by 30.0% over FA and 44.44% over control was registered. When the dose of fertilizer was reduced to 50%, there was a further increase by 7.69%. When FYM was included, there was an increase in prolamin fraction by 3.57% over its exclusion and 61.11% over control. $T_5$, $T_6$, $T_7$, $T_8$ and $T_9$ were at par indicating that the reduction in the dose of fertilizer to 50% had been counter balanced by the synergistic effect of 50% fertilizer and FA at either dose and with FYM over the synergistic effect of 100% fertilizer and FA in the higher dose. The prolamin content of polished rice is reported to be about 20%. All the treatments except those receiving FA at both the doses had significantly higher values over control. Treatments receiving FA in higher dose registered a decrease in prolamin fraction by 1.92% over the lower dose. The prolamin content of rice protein is about 2-8% (Cagampang *et al*, 1966 and Houston *et al*, 1968) [3, 5].

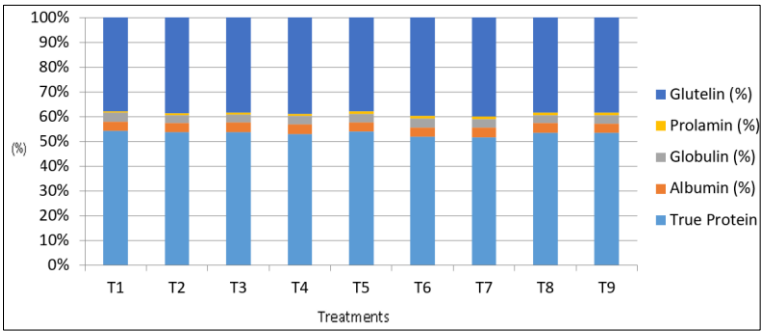


**Fig 4:** Protein fractions in g100gm$^{-1}$ protein

**Glutelin**

Glutelin content varied from the lowest of 4.94% in $T_2$ to the highest of 5.71% in $T_9$ (Fig.4.5.1.4). With the application of FA @ 20tha$^{-1}$, there was a decrease by 3.51% over control but FA in higher dose was at par. When 100% RD of NPK fertilizer was blended with FA, there was an increase by 7.44% over FA and 5.76% over control. But when the dose of fertilizer was reduced to 50%, a fall by 1.01% was registered. However, when FYM was included, there was an increase by 5.13% over its exclusion and 10.05% over control. FYM treated plots registered even higher values of glutelin fraction than the $T_4$ and $T_5$. This proved that the reduction in the dose of NPK fertilizer had been compensated by the synergistic effect of FA and FYM on the glutelin fraction of rice protein. Treatments receiving FA in higher dose registered an increase of glutelin content by 3.70% over its lower dose. The glutelin content of polished rice is reported to be about 60% (Nakase, 2012) [9] and that of rice protein is about 80% (Tecson *et al*, 1971, Padhyi *et al*, 1979 and Juliano 1985) [15, 10, 7]. Treatments receiving FA in the higher dose except the FA ($T_3$) registered a significant increase over the lowest value. The increase in protein content in milled rice is reflected mainly in the storage proteins, glutelin and prolamin located in protein bodies (Perez *et al* 2009) [11].

**Conclusion**

Proteins contents were higher in the treatment receiving full RD of NPK than FA. Increasing the dose of FA accumulated more protein but less NPN in all the treatments. Integration of 100% RD of NPK with FA further increased the protein content and it was more than the FA and control. Reduction in the dose of NPK to 50% reduced the amount of protein contents except NPN. Application of FYM further increased the protein content over its exclusion except NPN.

Except the minor fraction prolamin, all the protein fractions were insignificantly higher in 100% RD of NPK than FA treated plots. Integration of 100% RD of NPK with FA recorded higher values of protein fractions over FA and control but decreased slightly by the reduction in the dose of fertilizer. Treatments receiving FYM had higher values than its exclusion and control. Hence it is concluded that the blended application of RD of NPK fertilizer in lower dose with FYM has more amount of protein content and all the protein fractions except the minor fraction prolamin.

**Conflict of interest**

"The author declare no conflict of interests that could have appeared to influence the work reported in this paper."

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